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Short Report

Synthesis and *in vitro* Evaluation of New Benzenesulfonamides as Antileishmanial Agents

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Este artigo descreve a síntese e a atividade antileishmania de novos derivados pirazolil benzenossulfonamídicos. Estes foram elucidados por métodos espectrométricos. Alguns compostos mostraram uma atividade *in vitro* significativa contra *Leishmania amazonensis*, destacando-se o derivado **1e**. Nenhum dos derivados pirazolil benzenossulfonamídicos mostraram qualquer toxicidade em macrófagos murinos.

This paper describes the synthesis and the antileishmanial activity of new pyrazolyl benzenesulfonamide derivatives. These were elucidated by spectrometric methods. Some compounds showed a significant *in vitro* activity against *Leishmania amazonensis*, highlighting the derivative **1e**. These pyrazolyl benzenesulfonamide derivatives did not show any toxicity in murine macrophage.

Keywords: pyrazolyl benzenesulfonamides, synthesis, antileishmanial activity

Introduction

Leishmania species are the casual agents of several clinical manifestations called leishmaniasis, which are transmitted to human and others mammals by the bite of an infected female *Phlebotomine* sandfly. Depending on the causative species, it can be manifested as cutaneous, mucocutaneous, diffuse cutaneous, and visceral leishmaniasis.[1] More than 2 million new cases of leishmaniasis occur each year, with approximately 350 million persons at risk of infection.[2,3] However, the chemotherapy for leishmaniasis is generally ineffective mainly due to the emergence of drug-resistant strains and toxicity of the therapeutic agents. The pentavalent antimonials meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) are the drugs of first choice since the 50s. These compounds are widely used as primary therapy whereas other drugs, such as amphotericin B, pentamidine and paromomycin have been also employed as second line drugs.[4] Another effective medicine is

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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 not indicated in pregnant women and shows severe gastrointestinal side effects.[5,6] Azole antifungals ketoconazole, miconazole and itraconazole have been used to treat cutaneous leishmaniasis with variable success rates.[7]

There is a great interest in the synthesis of pyrazole derivatives, since they show a wide range of pharmacological properties including antimicrobial, anti-inflammatory, analgesic, antipyretic and antileishmania.[8-11] Our research group reported the synthesis of various pyrazole derivatives that showed antileishmanial activity, among them we can highlight 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles,[12] 1*H*-pyrazole-4-carbohydrazides,[13,14] 1-aryl-1*H*-pyrazole-4-carboximidamides[15] and 4-(1*H*-pyrazol-1-yl)benzenesulfonamides.[16] The sulfonamide group, in particular benzenesulfonamide, has proven importance in medicinal chemistry. A considerable number of sulfonamides are well known as antibacterial,[17] anti-inflammatory[18] and anticancer[19] agents.

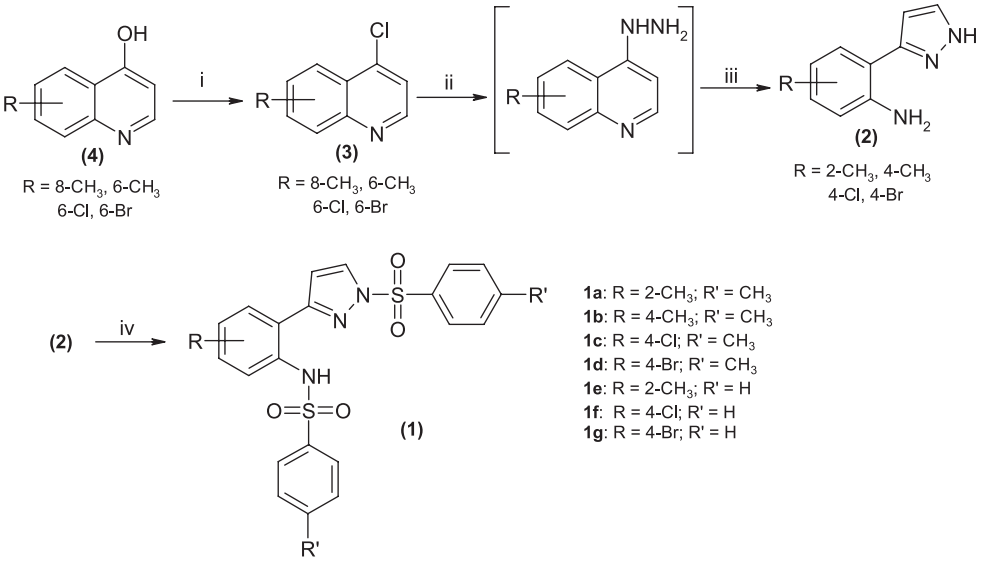
Based on the above report and in the continuation of our studies on chemotherapy of leishmaniasis, herein we described synthesis of some new pyrazolyl benzenesulfonamic derivatives, as well as the evaluation of their antileishmanial activity and cytotoxicity.

Results and Discussion

The synthesis of sulfonamide derivatives (**1**) was performed by reaction between 2-(1*H*-pyrazol-3-yl)anilines (**2**) and corresponding sulfonyl chlorides. The sequence is outlined in Scheme 1. The 4-hydroxyquinolines (**4**) can be readily made via the Gould-Jacobs method.[20-22] The compounds (**4**) were easily chlorinated in refluxing phosphorus oxychloride at 120 ºC over a period of 24 h, affording the 4-chloroquinolines (**3**).[20,22] We previously described the preparation of 2-(1*H*-pyrazol-3-yl)anilines (**2**) in mild conditions and excellent yields from 4-chloroquinolines (**3**).[22] 4-chloroquinolines react with an excess of hydrazine in diethyleneglycol initally at 90-100 ºC for one hour. Nucleophilic aromatic substitution of the chlorine atom in **3** by hydrazine occur in this temperature range to generate intermediate 4-hydrazinoquinolines, which on raising the temperature to 130-140 ºC, react further over a period of six hours to favor the rearrangement, affording the products **2**.[22] The preparation of new pyrazolyl benzenesulfonamide derivatives (**1a**-**g**) was performed through a substitution reaction between derivatives 2-(1*H*-pyrazol-3-yl)anilines (**2**) and excess of sulfonyl chloride. Benzenesulfonyl chlorides are electrophilic reagents that react readily with primary and secondary amines, such as NH of pyrazole and NH_2 of the benzene ring in **2**.[23]

All products were obtained with high purity levels and excellent yields, ranging from 91-82%, as shown in Table 1. These compounds were generally identified by ^{1}H nuclear magnetic resonance (^{1}H NMR), ^{13}C NMR, Fourier transform infrared absorption (FT-IR) spectroscopies and high-resolution mass spectrometry (HR-MS). In particular, the structure of **1e** was obtained by X-ray diffraction.

The atom arrangements and atom numbering scheme for molecule **1e** are shown in Figure 1. The bond angles



Scheme 1. Reactions conditions: (i) $POCl_3$, reflux, 24 h; (ii) NH_2NH_2, diethyleneglycol, 90-100 ºC, 1 h; (iii) NH_2NH_2, diethyleneglycol, 130-140 ºC, 6 h; (iv) sulfonyl chlorides, triethylamine, THF, reflux, 3 h.

Table 1. Yields of compounds **1a-g**

Compound[a,b]	R / R'	Yield / %[c]
1a	2-CH_3 / CH_3	82
1b	4-CH_3 / CH_3	84
1c	4-Cl / CH_3	91
1d	4-Br / CH_3	88
1e	2-CH_3 / H	81
1f	4-Cl / H	84
1g	4-Br / H	89

[a]Identified by ^{1}H NMR, ^{13}C NMR, FT-IR and HR-MS; [b]all compounds were recrystallized from ethanol; [c]isolated yields.

and bond lengths are all in the expected regions. The angle between the best planes through the pyrazole and aminoaryl rings in **1e** is 20.53 (0.11), compared to that in the recently published 3-(2'-amino-3'-methylphenyl) pyrazole[22] of 12º. A strong intramolecular hydrogen bond, N(3)–H(3)---N(1), is present in **1e**, while a series of weak C–H---O intermolecular interactions generate chains and dimers.

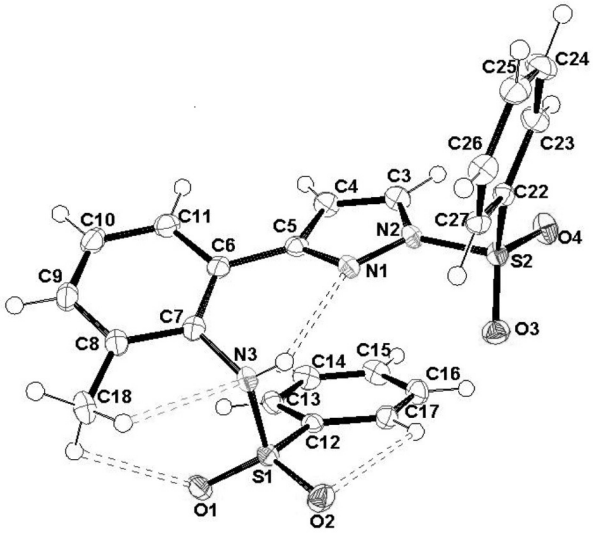


Figure 1. Atom arrangement and atom numbering scheme for molecule **1e**. Probability ellipsoids are drawn at the 50% level. Hydrogens on nitrogens are drawn as spheres of arbitrary radii.

In order to correlate the infectivity of parasite and susceptibility to drugs, sulfonamide derivatives were evaluated in different growth stages of *Leishmania amazonensis.* Also, the toxicity of those compounds to the host cell was evaluated and did not show any toxicity in murine macrophage at 320 μg mL^{-1} of concentration.

Variations in the efficacy of drugs for the treatment of leishmaniasis are frequently a consequence of the immune status of the patient, the pharmacokinetic properties of the drug and the intrinsic differences in drug sensitivities of each *Leishmania* species.[13] Data from literature report that those different sensitivities occur among species and even in developmental growth stages.[24] The life cycle of *Leishmania spp.* includes sequential development of invertebrate-stage promastigotes from a noninfective to an infective stage. Promastigotes obtained during the stationary phase of growth within the fly or culture is more infective for a vertebrate host than during their logarithmic phase. Thus, the putative metacyclics in the stationary phase showed increased infectivity compared with log phase cells.[25] Metacyclogenesis consists in a life cycle differentiation process known to occur naturally in the developmental stage of *Leishmania* parasites.[26] During this process, besides the structural modifications, several proteins with functions in protein synthesis, protein metabolism, protein folding and messenger RNA (mRNA) processing are down-regulated[25,27] while several other surface macromolecules are over expressed, such as a lipophosphoglycan (LPG) and a protease (GP63), which are related to the infective capacity of those forms.[26] *L. amazonensis* reach the higher amount of metacyclic forms (about 73%) at the 3rd day of growth, while at 24 h in culture none of those forms are detected, using lysis complement test.[28] Based in our data, we can suggest that the highest susceptibility of logarithmic forms would be associated with the lack of metacyclic forms, which are the infective ones.

Against axenic amastigotes, only three compounds (**1d**, **1e** and **1g**) showed good activity superior to that obtained with ketoconazole. The difference encountered for IC_{50} values between promastigotes and amastigotes would be explained by the differences in the culture conditions (temperature, pH and fetal calf serum concentration), resulting in low drug availability, since some amount of drug could be linked to the serum albumin. However, we cannot explain the good result obtained for compound **1d**, assayed at the same conditions. Alternatively, the overall activity profile of the tested compounds **1** could be compared with the antileishmanial properties shown by previously described sulfonamides.[16] Thus, we found that there is significant difference in the antipromastigote activity by addition of the sulfonamide group.

The most active sulfonamide compound against *L. amazonensis* promastigotes was the compound **1e**. This compound was also effective against axenic amastigote, as well as compound **1d**. Also, the derivatives **1d** and **1e** showed an activity about 50-52% higher against axenic amastigotes, when compared to ketoconazole. Furthermore, none of the compounds showed toxicity to murine macrophage at 320 μg mL^{-1} of concentration.

Table 2. IC_{50} (compound concentration required to kill parasites by 50% ± standard deviation) of pyrazolyl benzenesulphonamide compounds against *L. amazonensis*. All assays were performed three times, in triplicate

Compound	IC_{50} (24 h) / μM		
	Promastigotes		Axenic amastigote
	Early log phase	Late log phase	
1a	352.0	> 838.7	> 838.7
1b	755.3	838.7	263.9
1c	25.8	159.0	138.9
1d	201.6	290.0	43.0
1e	6.7	41.3	56.0
1f	6.4	47.34	446.3
1g	60.0	77.4	83.6
Ketoconazole	12.5	29.8	164.1

Conclusions

Our results seem promising since pyrazolyl benzenesulfonamide derivative **1e** showed to be active *in vitro* against *L. amazonensis* promastigotes and axenic amastigotes. Furthermore, the *in vitro* experiment with macrophage demonstrated that, in a concentration equivalent to IC_{50}, the compound is not hazardous to the host cells, afact that is a stimulus to continue our studies with this class of compounds.

Experimental

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained at 300.00 and 75.00 MHz, respectively, on a Varian Unityplus instrument equipped with a 5 mm probe, using tetramethylsilane as the internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Samples were dissolved in the solvents specified, $CDCl_3$ or DMSO-d_6. Fourier transform infrared (FT-IR) absorption spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrophotometer by reflectance in KBr. The experimental analysis of electrospray ionization high-resolution mass spectrometry (ESI/MS) were performed in a high-resolution Micromass apparatus, model Q-TOF. Melting points (m.p.) were determined with a Fisatom model 430 D apparatus.

Preparation of pyrazolyl benzenesulfonamides derivatives (**1a**-**g**): 0.9 mmol of **2**, 7.0 mL of THF, 200 mg of triethylamine and 2.0 mmol of the sulfonyl chloride were stirred under reflux for 3 hours. Later, the solvent was evaporated and the mixture was dropped in a beaker with ice and water. The formed crystals were recrystallized from ethanol.

4-Methyl-*N*-(2-methyl-6-{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrazol-3-yl}phenyl)benzenesulfonamide, **1a**: R = 2-CH_3; R' = CH_3

m.p.: 149 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3275.8, 1335.9, 1163.7, 1595.3, 1524.8, 1380.4, 1304.3, 1115.4, 1089.9, 1036.1; ^1H NMR ($CDCl_3$, 300.00 MHz): δ 5.87 (d, 1H, *J* 2.7 Hz, H4), 7.80 (d, 1H, *J* 2.7 Hz, H5), 7.02 (d, 1H, *J* 7.8 Hz, H4'), 7.15 (t, 1H, *J* 7.8 Hz, H5'), 7.30 (d, 1H, *J* 7.8 Hz, H6'), 8.00 (dd, 2H, *J* 8.4, 1.8 Hz, H2'' and H6''), 6.71 (dd, 2H, *J* 8.4, 1.8 Hz, H3'' and H5''), 7.46 (d, 2H, *J* 8.1 Hz, H2''' and H6'''), 6.78 (d, 2H, *J* 8.1 Hz, H3''' and H5'''), 2.66 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 8.79 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 154.9 (C3), 106.6 (C4), 132.3 (C5), 132.4 (C1'), 139.6 (C2'), 127.5 (C3'), 126.9 (C4'), 126.3 (C5'), 128.3 (C6'), 146.5 (C1''), 130.3 (C2'' and C6''), 126.7 (C3'' and C5''), 133.2 (C4''), 142.6 (C1'''), 128.4 (C2''' and C6'''), 126.2 (C3''' and C5'''), 134.4 (C4'''), 21.6 (CH_3), 21.2 (CH_3), 19.7 (CH_3); ESI-HRMS ($M + H^+$) calcd.: 482.5972; found: 482.1410.

4-Methyl-*N*-(4-methyl-2-{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrazol-3-yl}phenyl)benzenesulfonamide, **1b**: R = 4-Me; R' = CH_3

m.p.: 163 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3144.0, 1339.5, 1161.1, 1594.8, 1520.8, 1506.8, 1455.3, 1400.1, 1382.6, 1319.1; ^1H NMR ($CDCl_3$, 300.00 MHz): δ 6.50 (d, 1H, *J* 2.8 Hz, H4), 8.08 (d, 1H, *J* 2.8 Hz, H5), 7.58 (d, 1H, *J* 8.3 Hz, H3') 7.07 (dd, 1H, *J* 8.3, 2.0 Hz, H4'), 7.19 (d, 1H, *J* 2.0 Hz, H6'), 8.08 (dd, 1H, *J* 8.6, 1.8 Hz, H2'' and H6''), 7.36 (dd, 2H, *J* 8.6, 1.8 Hz, H3'' and H5''), 7.45 (dd, 2H, *J* 8.5, 1.8 Hz, H2''' and H6'''), 6.98 (dd, 2H, *J* 8.5, 1.8 Hz, H3''' and H5'''), 2.43 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 10.11 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 155.3 (C3), 106.4 (C4), 131.1 (C5), 119.7 (C1'), 130.6 (C2'), 121.4 (C3'), 128.9 (C4'), 132.9 (C5'), 133.6 (C6'), 143.0 (C1''), 130.4 (C2'' and C6''), 128.6 (C3'' and C5''), 136.0 (C4''), 146.4 (C1'''), 128.9 (C2''' and C6'''), 126.9 (C3''' and C5'''), 21.6 (CH_3), 21.1 (CH_3), 20.5 (CH_3); ESI-HRMS ($M + H^+$) calcd.: 482.5972; found: 482.0807.

N-(4-Chloro-2-{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrazol-3-yl}phenyl)-4-methylbenzenesulfonamide, **1c**: R = 4-Cl; R' = CH_3

m.p.: 144 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3143.4, 1341.2, 1165.7, 1595.2, 1516.9, 1493.5, 1466.4, 1384.6, 1314.6, 1089.0; ^1H NMR ($CDCl_3$, 300.00 MHz): δ 6.52 (d, 1H, *J* 2.7 Hz, H4), 8.12 (d, 1H, *J* 2.7 Hz, H5), 7.65 (d, 1H, *J* 9.0 Hz, H3') 7.22 (dd, 1H, *J* 9.0, 2.4 Hz, H4'), 7.38 (d, 1H, *J* 2.4 Hz, H6'), 8.08 (d, 2H, *J* 8.1 Hz, H2'' and H6''), 7.40 (d, 2H, *J* 8.1 Hz, H3'' and H5''), 7.46 (d, 2H, *J* 8.1 Hz, H2''' and H6'''), 7.03 (d, 2H, *J* 8.1 Hz, H3''' and

H5'''), 2.44 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 10.27 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 153.9 (C3), 106.4 (C4), 134.6 (C5), 122.4 (C1'), 135.8 (C2'), 126.9 (C3'), 129.2 (C4'), 121.2 (C5'), 131.4 (C6'), 146.7 (C1''), 131.4 (C2'' and C6''), 130.5 (C3'' and C5''), 116.6 (C4''), 143.4 (C1'''), 130.7 (C2''' and C6'''), 128.5 (C3''' and C5'''), 132.7 (C4'''), 21.6 (CH_3), 21.3 (CH_3); ESI-HRMS ($M + H^+$) calcd.: 503.0154; found: 503.0162.

N-(4-Bromo-2-{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrazol-3-yl}phenyl)-4-methylbenzenesulfonamide, **1d**: R = 4-Br; R' = CH_3

m.p.: 128 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3143.2, 1340.3, 1164.0, 1594.6, 1517.7, 1492.5, 1386.7, 1314.7, 1088.6; ^{1}H NMR ($CDCl_3$, 300.00 MHz): δ 6.53 (d, 1H, *J* 2.7 Hz, H4), 8,13 (d, 1H, *J* 2.7 Hz, H5), 7.59 (d, 1H, *J* 8.7 Hz, H3'), 7.35 (dd, 1H, *J* 8.7, 2.4 Hz, H4'), 7.53 (d, 1H, *J* 2.4 Hz, H6'), 8.08 (d, 2H, *J* 8.4 Hz, H2'' and H6''), 7.42 (d, 2H, *J* 8.4 Hz, H3'' and H5''), 7.46 (d, 2H, *J* 8.1 Hz, H2''' and H6'''), 7.04 (d, 2H, *J* 8.1 Hz, H3''' and H5'''), 2.44 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 10.29 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 154.0 (C3), 106.3 (C4), 134.1 (C5), 121.0 (C1'), 135.8 (C2'), 122.3 (C3'), 129.2 (C4'), 127.8 (C5'), 131.4 (C6'), 143.6 (C1''), 130.5 (C2'' and C6''), 129.1 (C3'' and C5''), 132.7 (C4''), 146.7 (C1'''), 129.6 (C2''' and C6'''), 126.9 (C3''' and C5'''), 21.6 (CH_3), 21.3 (CH_3); ESI-HRMS ($M + H^+$) calcd.: 547.4667; found: 547.4677.

N-{2-Methyl-6-[1-(phenylsulfonyl)-1*H*-pyrazol-3-yl]phenyl} benzenesulfonamide, **1e**: R = 2-Me; R' = H

m.p.: 138 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3146.5, 1344.9, 1170.9, 1584.2, 1524.1, 1481.3, 1449.2, 1427.6, 1386.1, 1092.2; ^{1}H NMR ($CDCl_3$, 300.00 MHz): δ 5.88 (d, 1H, *J* 3.0 Hz, H4), 7.80 (d, 1H, *J* 3.0 Hz, H5), 7.05-7.01 (m, 1H, H4'), 7.17 (t, 1H, *J* 7.8 Hz, H5'), 7.32 (d, 1H, *J* 7.8 Hz, H6'), 8.15-8.12 (m, 2H, H2'' and H6''), 6.99-6.96 (m, 3H, H3'', H4'' and H5''), 7.76-7.66 (m, 2H, H2''' and H6'''), 6.84-6.80 (m, 3H, H3''', H4''' and H5'''), 2.67 (s, 3H, CH_3), 8.85 (s, 1H, CH_3); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 155.0 (C3), 106.9 (C4), 135.0 (C5), 127.9 (C1'), 139.6 (C2'), 132.4 (C3'), 126.7 (C4'), 126.3 (C5'), 128.2 (C6'), 137.2 (C1''), 131.8 (C2'' and C6''), 129.7 (C3'' and C5''), 127.0 (C4''), 136.3 (C1'''), 132.2 (C2''' and C6'''), 130.7 (C3''' and C5'''), 127.4 (C4'''), 19.7 (CH_3); ESI-HRMS ($M + H^+$) calcd.: 454.5440; found: 454.0550.

N-{4-Chloro-2-[1-(phenylsulfonyl)-1*H*-pyrazol-3-yl]phenyl} benzenesulfonamide, **1f**: R = 4-Cl; R' = H

m.p.: 154 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3134.1, 1336.0, 1166.8, 1584.2, 1524.8, 1497.0, 1449.2, 1397.4, 1091.3; ^{1}H NMR ($CDCl_3$, 300.00 MHz): δ 6.51 (d, 1H, *J* 3.0 Hz, H4), 8.13 (d, 1H, *J* 3.0 Hz, H5), 7.85 (d, 1H, *J* 8.4 Hz, H3'), 7.24 (dd, 1H, *J* 8.4, 1.5 Hz, H4'), 7.41 (d, 1H, *J* 1.5 Hz, H6'), 8.22-8.18 (m, 2H, H2'' and H6''), 7.50-7.44 (m, 3H, H3'', H4'' and H5''), 7.75-7.59 (m, 2H, H2''' and H6'''), 7.50-7.44 (m, 3H, H3''', H4''' and H5'''), 10.25 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 154.1 (C3), 106.6 (C4), 135.2 (C5), 121.2 (C1'), 138.7 (C2'), 122.8 (C3'), 129.9 (C4'), 126.8 (C5'), 132.6 (C6'), 135.8 (C1''), 132.6 (C2'' and C6''), 131.4 (C3'' and C5''), 127.9 (C4''), 136.7 (C1'''), 133.5 (C2''' and C6'''), 131.6 (C3''' and C5'''), 128.7 (C4'''); ESI-HRMS ($M + H^+$) calcd.: 474.9622; found: 474.9761.

N-{4-Bromo-2-[1-(phenylsulfonyl)-1*H*-pyrazol-3-yl]phenyl} benzenesulfonamide, **1g**: R = 4-Br; R' = H

m.p.: 156 ºC; FT-IR (KBr) ν_{max}/cm^{-1}: 3133.1, 1335.2, 1164.0, 1583.8, 1525.1, 1493.9, 1448.8, 1379.2, 1090.8; ^{1}H NMR ($CDCl_3$, 300.00 MHz): δ 6.52 (d, 1H, *J* 3.0 Hz, H4), 8.13 (d, 1H, *J* 3.0 Hz, H5), 7.60 (d, 1H, *J* 8.4 Hz, H3'), 7.40-7.37 (m, 1H, H4'), 7.53 (d, 1H, *J* 2.4 Hz, H6'), 8.22-8.19 (m, 2H, H2'' and H6''), 7.52-7.48 (m, 3H, H3'', H4'' and H5''), 7.74-7.68 (m, 2H, H2''' and H6'''), 7.26-7.24 (m, 3H, H3''', H4''' and H5'''), 10.29 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.00 MHz): δ 154.0 (C3), 106.6 (C4), 135.1 (C5), 116.9 (C1'), 138.5 (C2'), 122.8 (C3'), 129.8 (C4'), 121.4 (C5'), 132.6 (C6'), 134.4 (C1''), 132.6 (C2'' and C6''), 130.8 (C3'' and C5''), 126.8 (C4''), 135.8 (C1'''), 132.7 (C2''' and C6'''), 131.6 (C3''' and C5'''), 128.5 (C4'''); ESI-HRMS ($M + H^+$) calcd.: 519.4135; found: 519.9426.

Biological and cytotoxity assays

Parasites

Promastigotes (early log/24 h and late log/72 h growth phases) of *L. amazonensis* (MHOM/BR/77/LTB0016 strain), were grown at 26 °C in Schneider's medium at pH 7.2 supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 100 UI mL^{-1} penicillin, 100 µg mL^{-1} streptomycin and 1 mmol L^{-1} L-glutamine. Promastigote metacyclogenesis rate from early to late logarithmic growth curve was evaluated through complement lysis test. *L. amazonensis* axenic amastigotes were cultured at 32 ºC in Schneider's insect medium pH 5.5 supplemented with 20% (v/v) FCS, 0.5 mmol L^{-1} Hepes, 60 UI mL^1 and 60 µg mL^1 streptomycin.[28]

Drug assay

Parasites (promastigotes and axenic amastigotes), after having been harvested from the medium were counted in Neubauer's chamber and adjusted to a concentration of 4×10^6 parasites mL^{-1}. Drugs were added to parasite

cultures for screening, in a concentration range from 320 to 0.15 μg mL^{-1} solubilized in dimethyl sulfoxide (DMSO) and the final concentration of the solvent in the experiments never exceeding 1.6%, considered not hazardous for the parasite.[29] Ketoconazole (Sigma-Aldrich) was used as reference drug. After 24 h of incubation at specific temperatures (26 °C for promastigotes and 32 °C for axenic amastigotes), the number of surviving parasites was counted in Neubauer's chamber and the percentage of growth inhibition was calculated comparing to the controls (parasites without drug). The IC_{50}/24 h values were calculated by Origin 5.0 software (Microcal Software, Inc.) with a specific toolbox for estimating curves.

Cytotoxic assay

The cytotoxic effect of the pyrazolyl benzenesulfonamide compounds was assayed on mice peritoneal macrophages. The cells were isolated from peritoneal cavity of BALB/c mice with cold Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with 1 mmol L^{-1} L-glutamine, 1 mol L^{-1} HEPES, penicillin G (10^5 IU L^{-1}) and streptomycin sulfate (0.10 g L^{-1}). The 2×10^6 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 removed and the adhered macrophages were washed twice with RPMI. Compounds were added to the cell culture at concentration of the respective IC_{50}/24 h for axenic amastigotes of *L. amazonensis* and incubated for 48 h. After that, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) was added and after 2 h the reaction was interrupted with DMSO.[30] The results were read in a MicroQuant spectrophotometer (Biotek-Instrument Inc., Winooski, VT) at a wavelength of 570 nm.

Statistical analyses

Each experiment was done three to four times, in triplicate. Significance was determined using a non-paired Student *t*-test and Mann-Whitney analyses. Differences were considered to be significant when $p < 0.05$.

Crystallography

Data were obtained at 120(2) K with Mo-Kα radiation by means of the Bruker-Nonius 95mm CCD camera on k-goniostatr of the EPSRC crystallographic service, based at the University of Southampton. Data collection was carried out under the control of the program COLLECT[31] and data reduction and unit cell refinement were achieved with the COLLECT[31] and DENZO[32] programs. Correction for absorption was achieved in each case by a semi-empirical method based upon the variation of equivalent reflections with the program SADABS 2007/2.[33] The ORTEP-3 for Windows[34] program was used in the preparation of the Figures. SHELXL-97[35] and PLATON[36] were used in the calculation of molecular geometry. The structures were solved by direct methods using SHELXL-97 and fully refined by means of the program SHELXL-97. Difference map peaks provided positions for the hydrogen atoms of the NH groups for which the coordinates, along with isotropic displacement parameters, were fully refined. All other hydrogen atoms were placed in calculated positions. Crystal data and structure refinement details are listed in Table 3.

Table 3. Crystal data and structure refinement details

Empirical formula	$C_{22}H_{19}N_3O_4S_2$
Formula weight / (g mol^{-1})	453.52
Temperature / K	120(2)
Wavelength / Å	0.71073
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	
a / Å	13.0172(4)
b / Å	8.1423(3)
c / Å	19.3454(5)
β / degree	94.278(2)
Volume / $Å^3$	2044.71(11)
Z	4
Density (calculated) / (Mg m^{-3})	1.473
Absorption coefficient / mm^{-1}	0.297
F(000)	944
Crystal size / mm	0.36 × 0.32 × 0.12
Theta range for data collection / degree	1.57 to 27.60
Index ranges	$-16 \le h \le 16$; $-10 \le k \le 10$; $-25 \le l \le 25$
Reflections collected	27701
Independent reflections	4688 [R(int) = 0.0471]
Reflections observed (> 2 sigma)	3508
Absorption correction	multi-scan
Max. and min. transmission	0.829460 and 0.829460
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4688 / 3 / 290
Goodness-of-fit on F^2	1.100
Final R indices [I > 2 sigma(I)]	$R_1 = 0.039$ $wR_2 = 0.097$
R indices (all data)	$R_1 = 0.064$ $wR_2 = 0.119$
Largest diff. peak and hole / (e $Å^{-3}$)	0.28 and –0.55
CCDC No.	708840

Supplementary Information

Supplementary data (FT-IR, ^1H NMR, ^{13}C NMR, HR-MS and crystallographic data) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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