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Design, structural and spectroscopic elucidation of new nitroaromatic carboxylic acids and semicarbazones for the *in vitro* screening of anti-leishmanial activity

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HIGHLIGHTS

- Four new nitroaromatic carboxylic acids and semicarbazones have been prepared and fully characterized.
- Compound (**3**) was the most efficient with an IC_{50} value of 3.0 μmol L^{-1}.
- Compound (**3**) might be effective as a veterinary anti-leishmania agent.
- Reduction of Ar–NO_2 into Ar–$NO_2^{\cdot -}$ is vital for the biocide activity of (**1**)–(**4**).

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ABSTRACT

In this paper we report the synthesis and characterization of four new nitroaromatic compounds, 2-{6-nitrobenzo[1,3]dioxol-5-(methyleneamino)}benzoic acid (**1**), 2-{[5-(2-nitrophenyl)furan-2-yl]methylene-amino}benzoic acid (**2**), 2-{(6-nitrobenzo[1,3]dioxol-5-yl)methylene}hydrazinecarboxamide (**3**) and 2-{[5-(2-nitrophenyl)furan-2-yl]methylene}hydrazinecarboxamide (**4**). Compounds (**1**)–(**4**) have been authenticated by infrared and NMR spectroscopy, and the structure of (**1**), (**2**) and (**4**) have been determined by X-ray diffraction. In addition, the *in vitro* ability of compounds (**1**)–(**4**) to inhibit the growth of *Leishmania infantum* has been evaluated. Comparisons of the redox potential of the compounds and leishmanicidal activity indicate that the presence of the electroactive nitro group is important for the biological activity. The inhibition activity of compound (**3**) is comparable to that of the reference drug, $SbCl_3$. Considering the important side effects and the low efficiency of $SbCl_3$ in the case of resistance, compound (**3**) deserves further attention as a promising anti-leishmanicidal drug for veterinary use.

Introduction

Leishmaniasis comprises a group of diseases with distinct clinical manifestations caused by different species of protozoa parasites. The negative impact of this disease on public health is evident when considering the growing expansion of endemic zones over recent years [1]. A recent review shows that over 98 countries and territories are endemic for leishmaniasis. It is estimated 0.2–0.4 million of new cases of visceral leishmaniasis and 0.7 to 1.2 million of cutaneous form of this disease to occur each year worldwide. More than 90% of global visceral leishmaniasis cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan [2]. *Leishmania infantum* is considered the main etiological agent of visceral leishmaniasis in southern Europe and canine leishmaniosis due to *L. infantum* is a major global zoonosis potentially fatal to human and dogs, which comprise the main reservoir of infection to humans [3,4].

Humans are infected by phlebotomine sandflies, which breed in forest areas, caves, or in the burrows of small rodents. The epidemiology of this infection depends on the characteristics of the parasite species, the local ecological characteristics of the

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transmission sites, current and past exposure of the human population to the parasite and human behavior [2]. Leishmaniasis appears as three major clinical forms in humans: (i) visceral, the most severe and life-threatening form; (ii) cutaneous, originating as nodules and ulcers that may persist for years; and (iii) mucocutaneous, causing permanent lesions in mouth, nose or genital mucosa [3].

Since the 1940s, treatment with antimony-based agents has been the accepted therapy for all forms of leishmaniasis [5]. However, during the past decade, anti-leishmanial therapy has become a bewildering subject, largely because of the complexity of the disease [6]. Pentamidine, amphotericin B and miltefosine have been introduced as second-line therapy. However, the resistance to antimony-based drugs has become a severe problem, and treatment with second-line drugs is generally expensive and frequently complicated by the occurrence of toxic side effects [7]. Consequently, there is a continuing need for new chemical substances with a leishmanicidal effect, for optimized delivery systems and newer cost effective drugs. The efficacy of the substances relies upon the clinical form of the *Leishmania* species and geographical region [8].

In this context, carboxylic acids and semicarbazones have long been the subject of pharmaceutical interest as a result of their potent biological activities [9,10]. Indeed, carboxylic acids and semicarbazones are classes of compounds capable to inhibit the growth of several pathogens [9–12]. Nitroaromatic compounds are exceptional for their range of activity, the relative lack of resistance and their interesting chemistry. The pharmacological effectiveness of nitro drugs normally correlates to their reduction potential. This indicates that part of their activity may derive from the nitro-bio-catalyzed production of superoxide (futile cycle) or is due to the action of their reduced metabolites (bioreductive alkylation) [13]. The most biologically relevant measure of nitro group reduction potential is the thermodynamically reversible addiction of the first electron. Although the values determined in aqueous solutions by cyclic voltammetry are not reversible reactions, and for nitroaromatic compounds may involve the addition of up to four electrons, the first is usually the most difficult [13].

Anti-leishmanials normally cross the cell membrane of the microorganism by passive diffusion and this process increases as the free radicals, from bio-reduction processes, destabilize the cell membrane. The literature shows that intracellular concentration of nitro compounds, in the leishmania, affects not only the amount of free radicals, but the damage caused to the microbe by oxidative stress [14]. In the last decade nitroimidazoles have been synthesized, nifurtimox, metronidazole, benznidazole and megazole [15]. Many research groups have focused in the study of new promising drugs. Even though, new nitro-possessing compounds are still being investigated and required to overcome bio-resistance and side effects [16–18].

In spite of the individual biocide potentiality of carboxylic acids, semicarbazone and nitroaromatic-possessing compounds, it has not been fully described the leishmanicidal ability of derivatives containing two or more of these groups. Little informations are found in the literature concerning higher leishmanicidal activity due to synergistic property of molecular hybrids. A new compound that possesses nitro groups and a CF_3 fragment has been tested. During the reduction cycle of its $-NO_2$ group, other generated reactive species disrupt the mitochondrial function of the parasite [19].

In fact, our group has been interested in the syntheses of novel prototypes of new bioactive compounds [20–28]. We describe in this manuscript the synthesis, characterization and evaluation of the *in vitro* activity of two carboxylic acids and two semicarbazones nitro substituted derivatives against *L. infantum*.

Experimental

Chemistry

Materials and instruments

All starting materials were purchased from Aldrich, Merck or Synth and used as received. NMR spectra were recorded at 400 MHz using a Bruker DPX-400 spectrometer equipped with an 89 mm wide-bore magnet. The infrared spectra were recorded with samples pressed as KBr pellets on a Perkin–Elmer GX FT-IR spectrometer in the range of 4000 and 400 cm^{-1}. All melting points were determined on a Mettler FP90 apparatus with heating cell FP82HT and digital temperature controller, along with Microscope CH-2. Carbon, hydrogen and nitrogen analysis were performed on a Perkin–Elmer PE-2400 CHN-analysis using tin sample-tubes. Intensity data for the X-ray study were collected at 120 and 150 K on a Xcalibur, Atlas, Gemini, Kα/Mo radiation (λ = 0.71073 Å) and Kα/Cu radiation (λ = 1.54184 Å). Data collection, reduction and cell refinement were performed using the CrysAlisRED program [29]. The structures were solved employing the SUPERFLIP [30] and refined with SHELXL-97 [31]. Further details are given in Table 1. All hydrogen atoms were refined with anisotropic atomic displacement parameters. The hydrogen atom named H2, for compound (**1**) and H2A, for compound (**2**), involved in a strong hydrogen bond, were added in the structure in idealized positions and further refined according to the riding model [32]. The program ORTEP-3 for Windows [33] was used in the preparation of Fig. 1. The electrochemical experiments were conducted at room temperature and degassing was performed by purging an inert gas (nitrogen). Cyclic voltammetry were carried in DMF containing 0.1 mol L^{-1} tetrabutylammonium tetrafluoroborate [$(Bu_4N)BF_4$]. These measurements were performed by using an electrochemical analyzer from Bioanalytical Systems, model 100BW. The working electrode was a Palsens, the counter electrode was a platinum coil and the reference electrode was Ag/AgCl.

*Synthesis of 2-{6-nitrobenzo[1,3]dioxol-5-(methyleneamino)}benzoic acid (**1**)*

To a round-bottom-flask charged with 2-aminobenzoic acid (2.06 g, 15.0 mmol) dissolved in methanol (20 mL) was slowly dropped a hot methanol solution of 6-nitropiperonal (2.99 g, 15.0 mmol). The reaction mixtures were heated under reflux and stirred for 5 h. After cooling to room temperature, the yellow solids obtained were filtered and washed with ethyl ether. Yellow crystals suitable for X-ray diffraction experiments were obtained after a slow evaporation of a chloroform/toluene solution. Yield 84%. Mp. 204.6–205.2 °C. Elemental analysis: Anal. Calc. for $C_{15}H_{10}O_6N_2$: C, 57.32; H, 3.21; N, 8.92. Found: C, 57.49; H, 3.17; N, 8.92%. IR (cm^{-1}): 1710 ν_{as}(COO), 1432 ν_s(COO), 1602 ν(C=N), 1526 $\nu_{as}(NO_2)$, 1332 $\nu_s(NO_2)$. ^{1}H NMR (DMSO, 400 MHz): δ 10.08 (s, 1H, H8), 8.69 (s, 1H, H1, H2), 7.88–7.44 (m, 2H, H10, H14), 7.32 (s, 1H, H3), 7.21 (t, *J* = 7 Hz, 1H, H5), 6.97–6.65 (m, 1H, H6), 6.49 (t, *J* = 8 Hz, 1H, H4), 6.33 (s, 2H, H12A, H12B). ^{13}C NMR (DMSO, 100 MHz): δ 188.2 (C8), 169.6 (C1), 151.9 (C7), 151.5 (C11), 151.3 (C13), 145.7 (C15), 133.7 (C5), 131.2 (C3), 127.5 (C9), 116.3 (C4), 114.6 (C6), 109.6 (C2), 107.0 (C10), 105.0 (C14), 104.4 (C12).

*Synthesis of 2-{[5-(2-nitrophenyl)furan-2-yl]methyleneamino}benzoic acid (**2**)*

Prepared accordingly using 2-aminobenzoic acid (2.06 g, 15.0 mmol) dissolved in methanol (20 mL) and 5-(2-nitrophenyl-furfural) (3.29 g, 15.0 mmol). Yield 73%. Mp. 219.6–220.9 °C. Elemental analysis: Anal. Calc. for $C_{18}H_{12}O_5N_2$: C, 64.28; H, 3.59; N, 8.33. Found: C, 64.05; H, 3.53; N, 8.32%. IR (cm^{-1}): 1700 ν_{as}(COO),