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Short communication

Synthesis, and biological evaluation of new 1,3,4-thiadiazolium-2-phenylamine derivatives against *Leishmania amazonensis* promastigotes and amastigotes

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Abstract

1,3,4-Thiadiazolium-2-aminide, which is a class of mesoionic compounds, were tested against promastigote and amastigote forms of *Leishmania amazonensis*. Parasites were assayed with or without the drugs in axenic media, using pentamidine isethionate as a reference drug. The very promising results showed us the most active compounds were the 4′- and 3′-methoxy derivatives against promastigote forms, while the highest activity against the amastigote forms was obtained with the 4′-fluor and 3′-bromo derivatives.

Keywords: antileishmanial activity; mesoionic compounds; 1,3,4-thiadiazolium-2-aminide

1. Introduction

Parasitic protozoan diseases constitute the world's most widely spread human health problem. It is estimated that 3 billion individuals suffer from one or more parasitic infections, and the greatest cause of mortality is attributed to the trypanosomatid and apicomplexan parasites [1]. The combination of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp., results in 20 million disease cases. *Leishmania* is a parasitic protozoa (Kinetoplastida: Trypanosomatidae) which causes different diseases in human being, including: cutaneous, mucocutaneous and visceral leishmanisasis [2,3].

As a part of our research program on chemotherapy against diseases caused by trypanosomatids, we decided to assay different salts of mesoionic derivatives, never used before in these parasites, but already tested by us against cancer cells, with promising results [4,5], in order to reach high activity and low toxicity.

In the searching of new series of anti-*Leishmania* drugs we synthesised and assayed some salts of mesoionic derivatives of the 1,3,4-thiadiazolium-2-aminide class (**1**). In this work we report the synthesis and the in vitro anti-*Leishmania* activity of a mesoionic compounds 14 salts series, 4-phenyl-5-(4′-X- or 3′-Y-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides (**2**), where X = H, 4′-OCH_3, 4′-CH_3, 4′-NO_2, 4′-F, 4′-Cl, 4′-Br, 4′-CN, 4′-OCH_2CH_3, 4′-OH, 3′-OCH_3, 3′-NO_2, 3′-Cl, and 3′-Br (Fig. 1).

2. Chemistry

Mesoionic systems have provided numerous compounds with useful and wide-ranging biological activities, including antibacterial, antifungal, and antitumoural activities [6–10]. These compounds have an interesting structural feature and it is derived from their betaine-like character involving well separated regions with positive and negative charges. They possess a pentatomic heterocyclic ring associated with a sextet of p and π electrons, supporting a positive charge counterbalanced by formal negative charge on the atom of α

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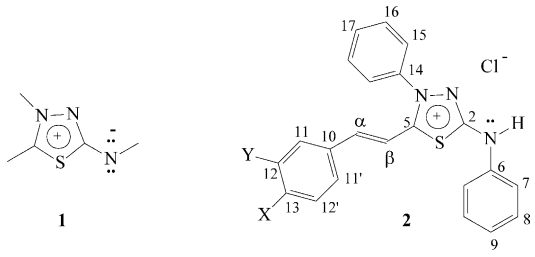


Fig. 1. Chemical structure of the mesoionic salt derivatives, 4-phenyl-5-(3′-Y- or 4′-X-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides (**2**). The numeration of structure is only for chemical shifts attribution.

side chain. The association of these characteristics with the little polyhetero-atomic system suggests a high probability of strong interactions with the biomolecules such as DNA and/or proteins [8].

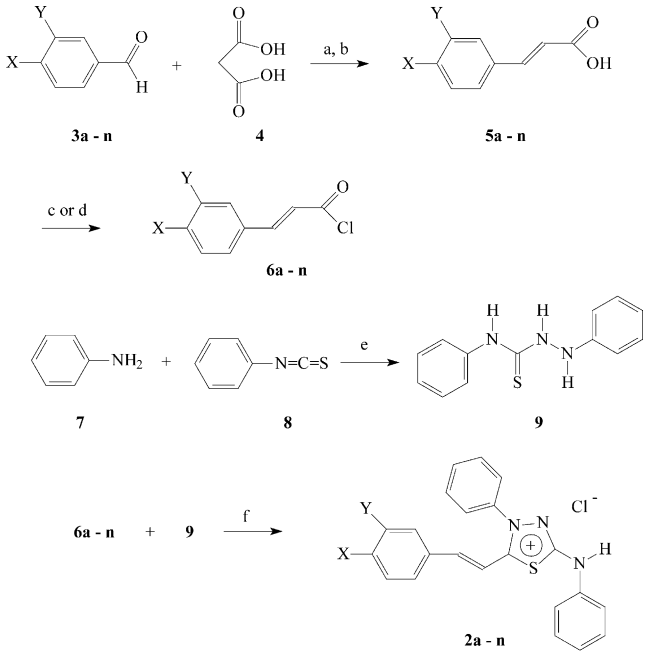
3. Pharmacology

The chemotherapy on the leishmaniasis treatment is still based in the work of Gaspar Viana, who started it in 1912 by treating the disease with trivalent antimonials, which was very toxic to patients. Pentavalent antimonials (SbV) in the stibogluconate (Pentostam) form or meglumine antimonate (Glucantime) are the drugs chosen for the treatment of all leishmaniasis forms [11,12]. However, to reach the parasites inside the macrophages, the pentavalent antimonials are reduced into the highly toxic trivalent derivative. Pentamidine isethionate and Amphotericin are also used, despite the high toxicity with several side effects, including renal and cardiac problems, associated with all those drugs [13,14].

A number of anti-tripanosomatids compounds have been found to be effective against experimental tumours. It is already known that some common metabolic pathways, such as the glycolysis, may be altered by chemotherapic agents, including arsenical drugs, which are effective against both trypanosomatids and tumours [15–17]. The urgency for more selective and less toxic drugs has led us to an evaluation of chemical therapy based on many similarities between tumour and trypanosomatidal cells, because of their fast replication [18].

4. Results and discussion

The 4-phenyl-5-[4′-X or 3′-Y-cinnamoyl]-1,3,4-thiadiazolium-2-phenylamine chlorides (**2a**–**n**), were synthesised following our previous procedure [19], where **2h**, **2i**, **2m** and **2n** are new compounds, as showed in Fig. 2. The 4′-X- and 3′-Y-cinnamic acids (**5a**–**n**) were prepared using Knövenaegel–Hans condensation reactions [20], in high yields and the corresponding chloride acids (**6a**–**n**) were easily obtained by treatment with $SOCl_2$ in excess or with dry benzene added to a mixture (1:3 v/v) of respective appropriate substituted cinnamic acid at room temperature. The 1,4-diphenylthiosemicarbazide (**9**) was prepared by a known method [21]. Thus, the dehydroacylation reaction between the appropriate 4′-X- or 3′-Y-cinnamoyl chloride and **9**, in dry 1,4-dioxane provided the target compounds (**2a**–**n**) in moderate to high yields (68–90%) and high purity, after one recrystallization in ethanol:dichloromethane. The mesoionic salt derivatives were fully characterised by IR, ^{1}H- and ^{13}C-NMR spectroscopies.



Reagents: a, Piperidine, pyridine; b, HCl, Δ; c, $SOCl_2$, d, benzene, $SOCl_2$; e, toluene, Δ; f, 1,4-dioxane.

Fig. 2. Reaction conditions for synthesis of mesoionic compound salts.

The energy minimisation of selected conformations of **2a**–**n** was done by the PCModel program. Minimal energy conformations were used for matrix construction and utilised in semi-empirical calculations using the AM1 Hamiltonian, MOPAC 6.0 software package [22], allowing full geometry optimisation and the most stable conformations were deduced from calculations. The geometry results were employed for the determination of theoretical parameters like HOMO and LUMO energies (of the highest occupied and lowest unoccupied molecular frontier orbital, respectively) and dipole moments for each molecule. The results of theoretical calculations predicted the low-energy conformation shown in perspective form in Fig. 3, corresponding to a model with cinnamoyl moiety rotates ~60°, and the phenyl group, attached to the exocyclic N-atom, rotate

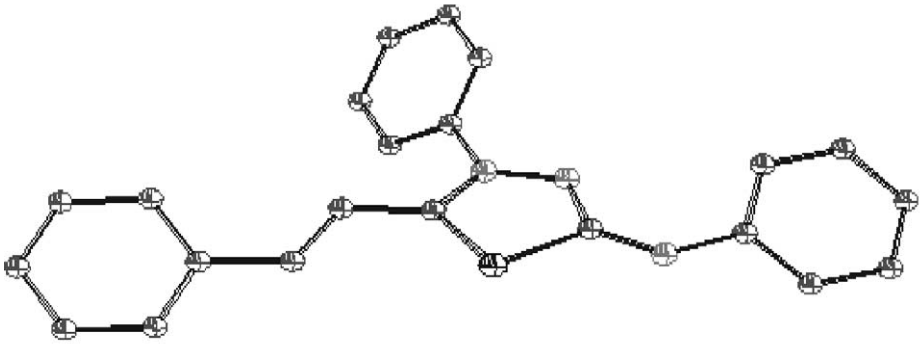


Fig. 3. An ORTEP view of the 4-phenyl-5-(cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine (**2a**) molecule.

$\sim 20^{\circ}$ related to the pentatomic heterocyclic ring (coplanar with its attached phenyl group).

4.1. Anti-leishmanial assays

The IC_{50} were obtained by a direct counting of the parasites in a Neubauer chamber (Table 1). In general, all the compounds were more effective against the promastigote forms of the parasite. At least five mesoionic salts [**2a** (H), **2c** (4′-OCH_3), **2k** (3′-OCH_3), **2m** (3′-Cl), and **2n** (3′-Br)] showed IC_{50} lower than 0.52 μM, and the **2c** and **2k** were more actives than the Pentamidine ($IC_{50} = 0.46$ μM), which was used as reference drug. Concerning the amastigote forms at least ten derivatives [**2a** (H), **2c** (4′-OCH_3), **2d** (4′-NO_2), **2e** (4′-F), **2g** (4′-CN), **2h** (4′-Br), **2i** (4′-EtO), **2j** (4′-OH), **2k** (3′-OCH_3), and **2n** (3′-Br)] were more effective than Pentamidine ($IC_{50} = 118$ μM). An enormous difference in the sensitivity was observed between both evolutive stages for all derivatives tested, as described to other compounds of different chemical classes [23–25].

Axenic amastigotes showed less sensibility than the promastigotes to all assayed compounds. This high difference between both evolutive stages of *Leishmania* parasite could suggest that different mechanisms should be involved in the anti-leishmanial results.

The promastigotes surface of all *Leishmania* species present, among others molecules, the lipophosphoglycan (LPG) which is associated to several biological functions of the parasite, including the increase of infectivity and the action as a scavenger of free oxygen radicals inside the macrophage. Concerning the amastigotes, the existence of LPG has been a conflicting report subject. However, a definitive study of Turco and Sacks [26] has demonstrated an acid-labile phosphosaccharide repeated unit, comparing to the promastigote LPG, in the amastigote the structure is noticeably different, in an overall anionic charge.

Table 1
IC_{50} values of mesoionic salt derivatives against *L. amazonensis*

Compound	X	Y	Promastigotes IC_{50} [a]		Amastigotes IC_{50} [a]	
			μg mL^{-1}	μM	μg mL^{-1}	μM
2a	H	H	0.18±0.01	0.47±0.03	40.97±4.68	104.54±11.95
2b	CH_3	H	0.40±0.02	0.98±0.05	116.69±1.17	287.46±20.15
2c	OCH_3	H	0.07±0.01	0.17±0.01	10.10±2.06	23.93±4.88
2d	NO_2	H	0.44±0.05	1.00±0.12	23.12±2.58	52.92±5.92
2e	F	H	0.38±0.03	0.92±0.06	2.20±0.12	5.37±0.28
2f	Cl	H	0.64±0.09	1.51±0.22	79.46±7.72	186.34±18.11
2g	CN	H	11.59±3.54	27.80±8.48	33.29±2.62	79.85±6.28
2h	Br	H	0.41±0.05	0.87±0.10	15.66±1.12	33.26±2.38
2i	OCH_2CH_3	H	0.65±0.09	1.49±0.20	33.43±2.02	76.68±4.64
2j	OH	H	3.09±0.01	7.58±0.03	46.18±2.18	113.21±5.33
2k	H	OCH_3	0.02±0.01	0.04±0.01	17.67±1.19	41.88±2.83
2l	H	NO_2	0.69±0.13	1.58±0.29	84.49±6.24	193.38±14.27
2m	H	Cl	0.21±0.01	0.48±0.05	75.94±6.56	178.11±15.39
2n	H	Br	0.25±0.03	0.52±0.05	2.58±0.17	5.48±0.04
Pentamidine	–	–	0.28±0.05	0.46±0.08	67.00±4.33	118.00±7.31

[a] Compound concentration required to kill promastigote or amastigote forms of *L. amazonensis* by 50%±S.D. All assays were performed in triplicate.

The mesoionic derivatives are very sensitive to pH changes while added in a salt form yielding the respective mesoionics, easily characterised by marked colour change. The promastigotes were grown in the pH neutral affording the structural change described above. On the other hand, in the amastigote cultures (pH 5.5), these compounds maintain their hydrochloride form, as heterocyclic cations. We could suggest an association between this fact and the highest effectiveness of the mesoionic on the extracellular stage (promastigote), compared to the intracellular one (amastigote).

Furthermore, the increase in serum concentration in the amastigotes culture, would allow the interaction of the assayed compounds in the cationic form with the serum protein, resulting in a lower drug disponibility. This fact is also valid for Pentamidine, which will be protonated at this acidic pH.

The theoretical electronic parameters were correlated against the IC_{50} values and no significant correlations were observed.

However, when the IC_{90} values were correlated with the ^{13}C-NMR chemical shifts of heterocyclic ring's C2 carbon atom, a discreet correlation was observed (correlation coefficient = 0.70). The electronic effects influence the charge distribution of the heterocyclic ring [10] which showed no obvious correlation with the anti-leishmanial activity. On the other hand, the combination of the electronic and lipophilic parameters may be required for biological activity versus structure relationships. The very active compounds, considering the IC_{90} values, **2n** (3′-Br) and **2k** (3′-OCH_3) derivatives ($IC_{90} = 1.7$ μM), showed a very different hydrophobic (Hansch hydrophobicity constants [27], $\pi_{Br} = 0.86$ and $\pi_{OCH_3} = -0.02$) and electronic effects (Hammett substituent constants [27], $\sigma_m = 0.39$ and 0.12, respectively), and their combination resulted in a similar biological activity.

The present results of mesoionic derivatives activity against *Leishmania amazonensis* are very promising, deserving further studies with other species of leishmania and action mechanisms. It will be suitable to synthesise new derivatives of the same mesoionic class searching for a better activity.

5. Experimental protocols

Materials used were obtained from commercial supplies and used without purification, unless otherwise noted. Melting points were obtained with a Koffler hot-stage apparatus and were not corrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 MHz instrument, employing tetramethylsilane as the internal reference at room temperature. The chemical shifts (δ) are reported in ppm and the coupling constants (J) in hertz. IR spectra were recorded on a Perkin–Elmer 5987A spectrometer in KBr pellets. Microanalyses were performed on a Perkin–Elmer Model 2400 instrument, and all values were within ±0.4% of the calculated compositions. The correlation analysis was performed with Origin 6.0 software (Microcal Software).

5.1. Chemistry

5.1.1. 4′-X- and 3′-Y-cinnamic acids **5a–n**

The preparation of these compounds was carried out by published procedures [28]. The purity of the compounds was checked by means of TLC using silica gel plate with fluorescent indicator and CH_3Cl:methanol (9:1) as eluent, melting point and ^{1}H-NMR.

5.1.2. General procedure for the preparation of 4-phenyl-5-(4′-X- or 3′-Y-cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides **2a–n**

These compounds were synthesised by the coupling of the corresponding freshly prepared 4′- or 3′-substituted cinnamoyl chlorides (10 mmol) which were added to a stirred solution of 1,4-diphenylthiosemicarbazide (10 mmol) in dry 1,4-dioxane (20 mL) at room temperature. After 24–48 h standing, the products were separated by vacuum filtration, washed with dry 1,4-dioxane and recrystallized from ethanol:dichloromethane (1:1 v/v) to afford yellow crystals. In this way, the following compounds were prepared:

2a (X = H): Yield 68%; m.p. 266–267 °C; IR (KBr, cm^{-1}) 3430, 3035, 2669, 1607, 1565, 1535, 1489, 1449, 1327, 955; ^{1}H-NMR (DMSO-d_6) δ 12.55 (s, NH), 8.01 (d, Hα, $J = 16.1$), 7.83–7.43 (m, H_{arom}), 7.07 (d, Hβ, $J = 16.3$); ^{13}C-NMR (DMSO-d_6) δ 163.94 (C5), 159.24 (C2), 147.84 (Cα), 137.04 (C14), 134.32 (C10), 133.88 (C17), 131.94 (C13), 130.50 (C11), 130.22 (C16), 129.59 (C8), 129.27 (C12), 126.18 (C15), 124.23 (C9), 118.73 (C7), 111.62 (Cβ).

2b (X = CH_3): Yield 73%; m.p. 196–197 °C; IR (KBr, cm^{-1}) 3423, 3026, 2663, 1609, 1566, 1539, 1511, 1449, 1326, 1175, 938; ^{1}H-NMR (DMSO-d_6) δ 11.31 (s, NH), 7.15 (d, Hα, $J = 16.2$), 6.17 (d, Hβ, $J = 16.2$); ^{13}C-NMR (DMSO-d_6) δ 162.02 (C5), 158.36 (C2), 149.24 (Cα), 143.64 (C13), 137.73 (C14), 136.33 (C6), 131.77 (C17), 130.24 (C10), 130.03 (C11), 129.76 (C12), 128.84 (C8), 128.66 (C16), 125.18 (C15), 124.21 (C9), 118.46 (C7), 108.63 (Cβ), 21.07 (CH_3).

2c (X = OCH_3): Yield 82%; m.p. 195–196 °C; IR (KBr, cm^{-1}) 3410, 3027, 2969, 2693, 1600, 1511, 1450, 1565, 1310, 1253, 1111, 1025, 953; ^{1}H-NMR (DMSO-d_6) δ 11.81 (s, NH), 7.13 (d, Hα, $J = 16.0$), 7.01–6.98 (m, H_{arom}), 6.76 (d, H11 and 11′, $J = 7.8$), 6.55 (d, H12 and 12′, $J = 8.4$), 6.23 (d, Hβ, $J = 15.4$), 2.92 (s, CH_3O); ^{13}C-NMR (DMSO-d_6) δ 163.28 (C13), 162.54 (C5), 158.44 (C2), 148.34 (Cα),138.66 (C14), 137.05 (C6), 135.22 (C10), 131.58 (C17), 130.19 (C16), 129.48 (C8), 126.57

(C11), 216.21 (C15), 123.35 (C9), 118.49 (C7), 114.83 (C12), 108.83 (Cβ), 55.66 (OCH_3).

2d (X = NO_2): Yield 89%; m.p. 232–233 °C; IR (KBr, cm^{-1}) 3410, 3044, 2720, 1615, 1565, 1522, 1450, 1341, 956; ^{1}H-NMR (DMSO-d_6) δ 12.35 (s, NH), 8.396 (d, H12, 12′, $J = 8.2$), 8.26 (d, Hα, $J = 16.6$), 8.151 (d, H11, 11′, $J = 8.3$), 8.00–7.87 (m, H_{arom}), 7.41 (d, Hβ, $J = 16.2$); ^{13}C-NMR (DMSO-d_6) δ 162.05 (C5), 159.68 (C2), 149.59 (C13), 144.67 (Cα), 139.99 (C10), 138.54 (C14), 137.05 (C6), 131.90 (C17), 130.25 (C16), 130.15 (C8), 129.56 (C11), 126.23 (C15), 124.32 (C9), 124.20 (C12), 118.71 (C7), 115.52 (Cβ).

2e (X = F): Yield 85%; m.p. 251–253 °C; IR (KBr, cm^{-1}) 3442, 3007, 2669, 1618, 1570, 1512, 1448, 1315, 1230; ^{1}H-NMR (DMSO-d_6) δ 12.33 (s, NH), 8.13 (d, Hα, $J = 16.2$), 7.69 (d, H12, 12′, $J = 8.4$, ^{1}H × ^{1}H-COSY H11, 11′), 7.54 (d, H11, 11′, $J = 8.4$), 7.18 (d, Hβ, $J = 16.2$); ^{13}C-NMR (DMSO-d_6) δ 164.83 (C5), 159.04 (C2), 144.58 (Cα), 138.69 (C14), 136.96 (C6), 131.80 ($^1J_{C-F} = 251.8$, C10), 131.67 (C17), 130.57 ($^3J_{C-F}$ 3.8, C11, C11′), 130.16 (C8), 129.51 (C16), 126.15 (C12), 124.12 (C9), 118.62 (C7), 116.47 ($^2J_{C-F}$ 21.9, C12), 111.39 (Cβ).

2f (X = Cl): Yield 83%; m.p. 264–265°C; IR (KBr, cm^{-1}) 3423, 3046, 2653, 1615, 1566, 1538, 1497, 1450, 1326; ^{1}H-NMR (DMSO-d_6) δ 12.61 (s, NH), 8.11 (d, Hα, $J = 16.5$, ^{1}H × ^{1}H-COSY Hβ), 7.91 (H12, 12′, $J = 8.7$, ^{1}H × ^{1}H-COSY H11, 11′), 7.65 (H11, 11′, $J = 8.7$), 7.22 (d, Hβ, $J = 16.2$); ^{13}C-NMR (DMSO-d_6) δ 159.13 (C2), 146.2 (Cα), 136.97 (C6), 136.28 (C13), 132.76 (C17), 131.62 (C10), 130.69 (C8), 130.1 (C16), 129.45 (C12), 129.21 (C11), 126.08 (C15), 124.03 (C9), 118.67 (C7), 112.24 (Cβ).

2g (X = CN): Yield 82%; m.p. 185 °C; IR (KBr, cm^{-1}) 3404, 2932, 2224, 1623, 1564, 1537, 1502 and 1420, 1310; ^{13}C-NMR (DMSO-d_6) δ 161.0 (C5), 145.4 (Cα), 137.0 (C6), 131.5 (C17), 128.5 (C8), 129.6 (C16), 132.9 (C12), 128.7 (C11), 124.9 (C15), 124.4 (C9), 118.4 (C7), 113.2 (Cβ).

2h (X = Br): Yield 70%; m.p. 283–285 °C; ^{1}H-NMR (DMSO-d_6) δ 12.58 (s, NH), 8.00 (d, Hα, $J = 16.0$, ^{1}H × ^{1}H-COSY Hβ), 7.86–7.17 (m, H_{arom}), 7.10 (d, Hβ, $J = 15.9$); ^{13}C-NMR (DMSO-d_6) δ 162.63 (C5), 159.18 (C2), 146.42 (Cα), 136.95 (C6), 124.16 (C13), 131.68 (C17), 133.04 (C10), 129.45 (C8), 130.16 (C16), 132.12 (C12), 130.90 (C11), 126.11 (C15), 125.46 (C9), 118.58 (C7), 112.13 (Cβ).

2i (X = OCH_2CH_3): Yield 85%; m.p. 148–150 °C; ^{1}H-NMR (DMSO-d_6) δ 12.22 (s, NH), 7.96 (d, Hα, $J = 15.9$, ^{1}H × ^{1}H-COSY Hβ), 7.85–6.97 (m, H_{arom}), 6.90 (d, Hβ, $J = 15.9$), 4.07 (q, H_3C-CH_2-O, $J = 7.0$), 1.31 (t, H_3C-CH_2-O, $J = 6.9$); ^{13}C-NMR (DMSO-d_6) δ 161.93 (C5), 158.54 (C2), 148.08 (Cα), 138.65 (C6), 163.38 (C13), 131.54 (C17), 137.07 (C10), 128.94 (C8), 130.24 (C16), 113.10 (C12), 127.95 (C11), 125.19 (C15), 124.79 (C9), 119.84 (C7), 108.57 (Cβ), 63.68 (CH_2-O), 14.53 (CH_3-CH_2-O).

2j (X = OH): Yield 65%; m.p. 205 °C; IR (KBr, cm^{-1}) 3050, 1600 and 1500, 1570, 1620; ^{1}H-NMR (DMSO-d_6) δ 12.35 (s, NH), 7.86 (d, Hα, $J = 15.5$), 7.80–7.57 (m, H_{arom}), 7.40 (m, H11, 11′), 7.12 (m, H12, 12′), 6.80 (d, Hβ, $J = 15.5$); ^{13}C-NMR ($CDCl_3/CD_3OD$) δ 162.58 (C5), 159.00 (C13), 157.94 (C2), 149.93 (Cα),139.49 (C6), 138.07 (C14), 131.67 (C17), 131.45 (C11), 130.18 (C8), 129.00 (C16), 125.42 (C15), 124.24 (C9), 124.00 (C14), 118.85 (C7), 116.42 (C12), 105.86 (Cβ).

2k (Y = OCH_3):Yield 68%; m.p. 284–285 °C; ^{1}H-NMR (DMSO-d_6) δ 12.38 (s, NH), 7.92 (d, Hα, $J = 16.1$), 7.86–7.31 (m, H_{arom}), 7.06 (d, Hβ, $J = 16.0$), 3.76 (s, CH_3O); ^{13}C-NMR (DMSO-d_6) δ 162.82 (C12′), 159.66 (C5), 159.15 (C2), 147.96 (Cα), 137.02 (C6), 138.55 (C14), 135.22 (C10), 121.12 (C11), 131.74 (C17), 130.19 (C16), 117.62 (C11′), 126.16 (C15), 129.54 (C8), 124.22 (C9), 114.49 (C13), 118.64 (C7), 118.89 (Cβ), 55.38 (CH_3O).

2l (Y = NO_2): Yield 73%; m.p. 185 °C; IR (KBr, cm^{-1}) 3050, 1620, 1570, 1335; δ ^{13}C-NMR ($CDCl_3$/CD_3OD) δ 145.17 (Cα), 137.4 (C6), 136.3 (C14), 134.5 (C10), 133.42 (C11), 131.58 (C17), 129.75 (C12), 128.69 (C16), 125.21 (C11′), 124.96 (C15), 124.63 (C8), 124.36 (C9), 122.7 (C13), 118.44 (C7), 112.79 (Cβ).

2m (Y = Cl): Yield 72%; m.p. 261–263 °C; ^{1}H-NMR (DMSO-d_6) δ 12.79 (s, NH), 7.98 (d, Hα, $J = 16.4$), 7.92–7.36 (m, H_{arom}), 7.19 (d, Hβ, $J = 15.5$); ^{13}C-NMR ($CDCl_3/CD_3OD$) δ 162.45 (C5), 159.12 (C2), 145.94 (Cα), 137.02 (C6), 138.55 (C14), 131.71 (C17), 128.52 (C11′), 126.16 (C13), 129.43 (C8), 130.15 (C16), 127.73 (C15), 124.09 (C9), 118.55 (C7), 133.91 (C12′), 130.94 (C12), 113.21 (Cβ).

2n (Y = Br): Yield 80%; m.p. 263–264 °C; ^{1}H-NMR (DMSO-d_6) δ 12.84 (s, NH), 7.98 (d, Hα, $J = 16.1$), 7.87–7.27 (m, H_{arom}), 7.19 (d, Hβ, $J = 16.2$); ^{13}C-NMR ($CDCl_3/CD_3OD$) δ 162.46 (C5), 159.13 (C2), 145.90 (Cα), 137.05 (C6), 138.59 (C14), 131.73 (C17), 131.29 (C11′), 134.17 (C13), 128.00 (C11), 129.43 (C8), 130.19 (C16), 126.28 (C15), 122.46 (C9), 118.56 (C7), 124.09 (C12′), 130.19 (C12), 113.17 (Cβ).

5.2. Pharmacology

5.2.1. Anti-leishmanial assay

L.amazonensis (MHOM/BR/77/LTB0016 strain) promastigotes were grown at 25 °C in Schneider's Drosophila medium [29] supplemented with 10% (v/v) heat-inactivated foetal calf serum (FCS). The axenic amastigotes were also cultivated in Schneider's medium with 20% FCS, pH 5.5 and in a temperature of 32 °C [30–33]. Parasites (promastigotes/amastigotes) were harvested from the medium, resuspended in fresh medium, counted in Neubauer chamber and adjusted to a concentration of 4×10^6 parasites mL^{-1}, for the drug assay.

The mesoionic compounds were added for screening (from 320 to 0.15 μg mL^{-1} for promastigote and 320–10 μg mL^{-1} for amastigote) solubilised in DMSO (the highest concentration used was 1.6% v/v, not hazardous to the parasite). After 24 h of incubation the parasites were counted 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 concentration causing 50% inhibition (IC_{50}) was obtained from the drug concentration–response curve, and the results were expressed as the mean ± standard deviation determined from three independent experiments.

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