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Rhodium-Catalyzed C-H Bond Activation for the Synthesis of Quinonoid Compounds: Significant Anti-Trypanosoma cruzi Activities and Electrochemical Studies of A-Ring Functionalized Quinones

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Abstract: Thirty four halogen and selenium-containing quinones, synthesized by rhodium-catalyzed C-H bond activation and palladium-catalyzed cross-coupling reactions, were evaluated against bloodstream trypomastigotes of T. cruzi. We have identified nine compounds with IC_{50}/24 h values of less than 2 µM. Electrochemical studies on A-ring functionalized naphthoquinones were also performed aiming to correlate redox properties with trypanocidal activity. For instance, (E)-5-styryl-1,4-naphthoquinone 60 and 5,8-diido-1,4-naphthoquinone 3, which are around fifty fold more active than the standard drug benznidazole, are potential derivatives for further investigation. These compounds represent powerful new agents useful in Chagas disease therapy.

Keywords: C-H functionalization, Quinones, Chagas disease, Trypanosoma cruzi, Electrochemistry.
1. Introduction

Chagas disease, caused by *Trypanosoma cruzi* (*T. cruzi*), is classified as a neglected tropical disease by the World Health Organization. This disease has high morbidity and mortality rates, affects 5-7 million people and displays a limited response to therapy [1]. It is transmitted to humans by triatomine vectors, blood transfusions, oral and congenital routes and less commonly, by organ transplantation, and laboratory accidents [2]. Chagas disease is characterized by two clinical phases: a short, acute phase defined by patent parasitaemia and a long, progressive chronic phase. The acute phase appears shortly after infection and is frequently asymptomatic. After 2 to 3 months, in most infected individuals, a host/parasite balance is achieved, leading to the chronic phase. Approximately two thirds of infected individuals remain in the indeterminate chronic form, but after 10 to 30 years, the other third will develop a symptomatic chronic disease with digestive and/or cardiac disturbances [3]. Two current major concerns are outbreaks of acute Chagas disease associated with the ingestion of contaminated food [4], and the disease’s emergence in non-endemic areas such as North America and Europe, due to the immigration of infected individuals [5].

*T. cruzi* is a hemoflagellate protozoan and its life cycle involves distinct forms during its passage through vertebrate and invertebrate hosts. The trypomastigote form ingested by the insect differentiates into the proliferative epimastigote form, which, on reaching the posterior intestine of the insect, differentiates into the metacyclic form. The latter, following invasion of vertebrate host cells, undergoes differentiation into the amastigote form, which, after several reproductive cycles, transforms into the trypomastigote form, responsible for the dissemination of the infection.

The current treatment for Chagas disease is restricted to two nitroheterocyclic drugs, benznidazole (Bz) and nifurtimox (Nif), and their efficacy varies according to the phase of the disease, with their effectiveness decreasing with advancement of the infection [6]. The major limitations of these drugs are their limited and variable curative activity in the established chronic form of the disease and their toxic effects, leading to treatment abandonment in several instances [7]. These drawbacks justify the urgent need to identify better drugs to treat chagasic patients. In this context, an intensive research program has focused upon the search for alternative natural, semi-synthetic and synthetic lead compounds [8].
Over the last few years, our group has dedicated efforts to the synthesis and identification of novel naphthoquinoidal compounds with potent antiparasitic activity [9]. In this context, we have modified the “redox centre” A- and C-rings of lapachones with the aim of preparing new bioactive compounds [9c]. For instance, a simple redox centre modification of β-lapachone led to a phenazine derivative with trypanocidal activity about two and six times higher than benznidazole and the original quinone, respectively [10]. Intrinsically related to this strategy, we have demonstrated that appendage of a triazole group to the C-ring of β-lapachone affords a derivative four times more active against the parasite than the naphthoquinoidal precursor [11]. Recently, Lim and co-workers [12] described the synthesis and evaluation of A-ring modified lapachones, which show activity against cancer cells (Scheme 1A); this study is of direct relevance to the strategies outlined herein.

Salomão and co-workers have reported the trypanocidal activity of C-2 and C-5 substituted naphthoquinones, as well as evidence for their mode of action [13]. As shown in Scheme 1B, in assays performed in the absence of blood and with incubation at 37°C several compounds presented IC₅₀ values of < 2 µM against T. cruzi. For instance, further assays with 5-acetoxy-1,4-naphthoquinone (IC₅₀ = 0.16 µM) revealed activity against the proliferative forms of T. cruzi, intracellular amastigotes and epimastigotes. In experiments with this latter parasite form, this compound led to mitochondrial swelling, vacuolization, and flagellar blebbing, as well as a remarkable decrease in the mitochondrial membrane potential and a discrete increase of ROS production. Such redox dependent effects are likely reliant on the acetyl group facilitating quinone reduction, as previously demonstrated by electrochemical analysis [13b]. These results show that A-ring substituted naphthoquinones can kill T. cruzi and highlight the importance of synthesizing and evaluating new quinoidal compounds against parasites.

Recently, our group has embarked on a research program aimed at addressing unmet synthetic challenges related to quinone modification. For instance, we have demonstrated that chiral squaramide catalyzed reaction of lawsone with Morita-Baylis-Hillman acetates of nitroalkenes leads to the asymmetric formation of pyranonaphthoquinones [14]. We have also developed C-H activation-based processes that achieve challenging bond formations and enable late stage functionalization of complex systems [15]. For example, we reported recently a method for the A-ring functionalization of naphthoquinones that is reliant on a rhodium-catalyzed C-5 selective C-H iodination reaction [16]. In this report, we outline the C-H functionalization enabled
synthesis of 30 quinoidal derivatives and their evaluation against *T. cruzi*. Four compounds were also prepared by C-C bond forming reactions achieved via palladium catalysis. Since the biological activity of quinones is intrinsically related to the quinoidal redox centre, we also describe the electrochemical behavior of nine representative compounds.

**Scheme 1.** Overview.
2. Results and Discussion

2.1 Chemistry

Naphthoquinone derivatives were the first class of compounds targeted for preparation. A-ring modifications were performed utilizing a carbonyl directed Rh-catalyzed C-H bond activation protocol reported recently by our group [16]; this methodology allowed C-5 selective iodination of several 1,4-naphthoquinones. In the simplest case, using 1,4-naphthoquinone (1) as substrate, the synthesis of mono-iodinated product 2 occurred in 69% yield, accompanied by a 5% yield of bis-iodinated by-product 3 (Scheme 1). For the purpose of clarity the numbering system shown in Scheme 1 is used during further discussion.

![Scheme 1. Iodinated naphthoquinoidal derivatives 2 and 3.](image)

Iodinated naphthoquinones containing electron donating and withdrawing groups were selected for subsequent biological evaluation. The use of hydroxyl A-ring-substituted substrates (e.g. 4) changed the regioselectivity of iodination to afford 5, where functionalization had occurred ortho to the hydroxyl group (C7) rather than at C5; this demonstrates that the carbonyl-directed pathway can be overridden by strong donor substituents. Control experiments showed that in the absence of the Rh-catalyst only traces of product were observed. Use of 250 mol% NIS enabled subsequent C5 iodination of 5 to provide 6 in 82% yield. Reaction of plumbagin 7 with 100 mol% NIS provided C7 iodination product 8 in 85% yield. Juglone (4) and plumbagin (7) were reacted with iodomethane and Ag2O in CHCl3 to afford 9 and 10 in 73% and 78% yield, respectively [17]. Rh-catalyzed C-H iodination of these substrates gave 11 and 12 in excellent yields. To prepare compounds containing methoxy groups in different A-ring positions, derivatives 5 and 8 were also methylated to afford 13 and 14 in 91% and 92% yield, respectively (Scheme 2).
Scheme 2. Iodinated A-ring substituted naphthoquinones 5, 6, 8 and 11-14.

Other substrates containing methoxy groups were obtained in good yields by oxidation of tetralones 15 and 16 with 2-iodo-benzoic acid and Oxone (substrates 17 and 18) [18]. When the C-H iodination methodology was applied to these substrates derivatives 19 and 20 were generated in good to excellent yields. The regioselectivity observed for the iodination of 19 is suggestive of secondary coordination of the methoxy group to the Rh-center, thereby facilitating functionalization at the most hindered A-ring position (Scheme 3). Consistent with this assertion, iodination of compound 21, in which the methoxy group of 19 has been replaced by a methyl substituent, occurred selectively at the less hindered position to generate 22 in 63% yield (Scheme 4).

Scheme 3. Preparation of iodinated derivatives 19 and 20 from tetralones.

In order to investigate the effects of other electron donating groups, iodination of 24, which contains an amino group, was pursued. Consistent with hydroxy containing systems 4 and 7, functionalization occurred at C-7 to afford 25 in 41% yield and with high selectivity. On other hand, iodination of 27, which contains an electron withdrawing ester at C-8, was selective for C-5, affording derivative 28 in 57% yield (Scheme 5). Note that Ivashkina and co-workers [19] have described the synthesis of 25 using I₂-HIO₃ but the product was obtained as mixture of C-5 and C-7 iodinated products.

Scheme 5. Iodinated compounds 25 and 28 prepared from 23 and 26.

Electron deficient naphthoquinones were also used as substrates, affording the targets in moderate to good yields. Interestingly, unsymmetrical substrate 29 delivered 30 (59% yield) as the sole product, indicating that the bromine substituent controls the relative directing ability of the two carbonyl groups for the alkylation event (Scheme 6).

Scheme 6. Iodinated naphthoquinoidal derivatives 30, 32 and 34.
We also sought to investigate the biological effects of substituents on other quinone-based systems. The evaluation of heteroatom substituted benzoquinoidal compounds as potential trypanocidal agents was particularly attractive because these can be accessed directly by our reported C-H functionalization protocol (Scheme 3) [20]. Rh-catalyzed reaction of 1,4-benzoquinone 35 with 1,3-diiodo-5,5-dimethylhydantoin (DIH) yielded derivative 36 in 95% yield. Exchanging the electrophile source to 1,3-dibromo-5,5-dimethylhydantoin (DBH) afforded 37 in 82% yield. C-H phenylselenation processes were also investigated. Reaction of 35 with 150 mol % N-(phenylseleno)phthalimide afforded a mixture of target 38 and bis-functionalization product 39. However, it was found that by using only 100 mol% N-(phenylseleno)phthalimide, 38 could be obtained in good yield and with high selectivity over 39 (4.5:1 selectivity). The latter compound could be accessed exclusively in 74% yield by using an excess (250 mol %) of N-(phenylseleno)phthalimide (Scheme 7).

Scheme 7. Chalcogenated 38 and 39 and iodinated derivatives 35 and 36.

Rh-catalyzed iodination of alkyl-substituted system 40 afforded target 41 in 66% yield and good regioselectivity. Here, iodination occurs at the site remote to the bulky tert-butyl group. A similar result was obtained for bromination of 40 to deliver 42 in 57% yield (Scheme 8).
Scheme 8. Synthesis of iodinated quinone 41 and brominated one 42 from 40.

The metal-catalyzed protocol was also applied to benzoquinone 46, containing two adjacent methyl groups, and this afforded 49 in 81% yield. Interestingly, attempted bromination of 46 lead to degradation. For benzoquinone 47, which contains an electron-donating methoxy-group, iodination occurred at the most electron rich position to afford 50 in 91% yield. Bis-iodination of bis-methoxy system 48 was achieved in high yield by using an excess of DIH to generate 51 in 76% yield (Scheme 9).

Scheme 9. Iodinated quinoidal derivatives.

Systems possessing electron-withdrawing substituents proved to be more challenging; for example, electron deficient substrate 52 reacted smoothly with DIH to provide 53 in 56% yield. Bromination of 52 provided 54 in only 45% yield (Scheme 10).

Scheme 10. Preparation of iodinated derivative 53 and brominated 54 from electron deficient compound 52.

As outlined in Scheme 11, application of the C-H functionalization conditions to 1,4-naphthoquinone 1 provided derivatives 55 and 56 in excellent yields and high selectivity. The use of [RhCpCl2]2 in combination with CuSO4 was required to achieve C-2 rather than C-5 selectivity (cf. Scheme 2). This aspect is notable because site-
selective C-H functionalization of naphthoquinones (C-2 versus C-5) is achieved under catalyst control (cf. Scheme 1).

![Scheme 1](image)

**Scheme 11.** C-2 substituted naphthoquinones 55 and 56.

Because iodination of 1,4-naphthoquinone was readily achieved, subsequent palladium-catalyzed C-C bond forming derivatizations also became accessible (Scheme 12), thereby providing an avenue for the synthesis of different classes of hybrid quinononoid-based compounds. Under highly optimized conditions,[21] Stille coupling of 2 afforded 57 in 75% yield. Heck alkenylations [22] of 2 with ethyl acrylate and styrene proceeded smoothly to provide 58 and 59, respectively. Several Sonogashira cross-coupling protocols were evaluated without success. To solve this problem, a previously reported procedure [23] was employed, wherein reaction of 2 with an alkynyl-copper(I) reagent provided Sonogashira-like product 60 in 64% yield.

![Scheme 12](image)

**Scheme 12.** Derivatization of 2 via palladium catalyzed C-C bond formatting reactions.

The structures of novel compounds 5, 6, 8, 13, 14, 25, 49 and 59 were determined by $^1$H, $^{13}$C and 2D NMR analysis in combination with either electrospray ionization or
electronic impact mass spectrometry. Additionally, compounds 5, 6 and 51 were analyzed by single crystal X-ray diffraction, as summarized below.

2.2. X-ray analysis

Compound 5 crystallizes in space group P2\(_1\)/c with four independent molecules in the asymmetric unit where two of them are superimposed with occupational disorder on some atoms. For clarity, only one molecule is shown in Figure 1. The molecule is perfectly planar (r.m.s. deviation = 0.0029 Å° for 14 non-hydrogen atoms). In the packing of the crystal, five C-H---O type interactions and three O-H---I type interactions link the molecules, forming chains that extend along the [b] axis and parallel to (4 0 4) plane.

Compound 6 crystallizes in space group P2\(_1\)/n with two molecules in the asymmetric unit. For clarity, only one molecule is shown in Figure 1. There are no significant conformational differences between them. The molecule is almost planar (r.m.s. deviation = 0.0051 Å° for 13 non-hydrogen atoms) except the I1 and I2 atoms that are out of the mean plane of the rings with deviations of -0.179(3) and 0.137(2) Å° respectively. In the crystal, molecules are linked by weak interactions C2-H2---O3 [d(H2···O3) = 2.56(2) Å, \(\angle\)C2-H2···O3 = 125(4)°], C18-H18---O2 [d(H18···O2) = 2.42(2) Å, \(\angle\)C18-H18···O2 = 170(3)°] and O-H---I [d(H···I) = 3.53(2)Å] forming chains parallel to the (3 -3 -3) and (3 3 -3) planes.

Finally, compound 51 crystallizes in space group Pnma. The molecule is symmetric where the asymmetric units are C1, C2, C3, C4, C5 I1, O1, O2 and O3. The I1, O1 and O3 atoms deviate from the mean plane of the ring by -0.154(2), -0.111(2) and -0.388(3) Å°, respectively. The C2-C3-O2-C5 torsion angle of the methoxy group is -158.2(2)°. In the crystal, the molecules are linked by I---O (methoxy group) teractions [d = 3.383(2) Å°] resulting in a highly symmetrical arrangement that extends along the [a] axis.

An Ortep-3 diagram of each molecule is shown in Figure 1 and Table 1 lists the main crystallographic data for 5, 6 and 51. Packing diagrams for all compounds are provided in the Supporting Information File.
**Figure 1.** A projection ORTEP-3 of 5, 6 and 51, showing the atom-numbering and displacement ellipsoids at the 50% probability level.

**Table 1.** Crystal data and structure refinement for compounds 5, 6 and 51.

<table>
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<th>Identification code</th>
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<th>Compound 6</th>
<th>Compound 51</th>
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<td><strong>Empirical formula</strong></td>
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<td>C₁₀H₄I₂O₃</td>
<td>C₈H₆I₂O₄</td>
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<tr>
<td><strong>Formula weight</strong></td>
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<td>425.9</td>
<td>419.9</td>
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<td>293(2) K</td>
<td>293(2) K</td>
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<tr>
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<td>0.71073 Å</td>
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<td>orthorhombic</td>
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<tr>
<td><strong>Space group</strong></td>
<td>P2₁/c</td>
<td>P2₁/n</td>
<td>Pnma</td>
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<tr>
<td><strong>Unit cell dimensions</strong></td>
<td>a = 7.7911(6) Å, α = 90°, b = 27.0192(4) Å, β = 93.1(5)°, c = 13.5802(12) Å, γ = 90°</td>
<td>a = 8.2431(2) Å, α = 90°, b = 15.1653(4) Å, β = 92.95(2)°, c = 16.8525(5) Å, γ = 90°</td>
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<tr>
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<td>2103.9(2) Å³</td>
<td>1698.7 (2) Å³</td>
</tr>
<tr>
<td><strong>Z</strong></td>
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<td>8</td>
<td>4</td>
</tr>
<tr>
<td><strong>Density (calculated)</strong></td>
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<td>2.69 Mg/m³</td>
<td>2.67 Mg/m³</td>
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<td>6.01 mm⁻¹</td>
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<td><strong>F(000)</strong></td>
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<td>1552</td>
<td>768</td>
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<td>1.8 to 27.1°</td>
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<td>-12≤h≤12, -22≤k≤22, -8≤l≤8</td>
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<td>3421 / 0 / 273</td>
<td>1241 /0 / 71</td>
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<td>1.21</td>
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<td><strong>Final R indices [I&gt;2sigma(I)]</strong></td>
<td>R1 = 0.074, wR2 = 0.15</td>
<td>R1 = 0.037, wR2 = 0.062</td>
<td>R1 = 0.014, wR2 = 0.034</td>
</tr>
<tr>
<td><strong>R indices (all data)</strong></td>
<td>R1 = 0.084, wR2 = 0.152</td>
<td>R1 = 0.064, wR2 = 0.069</td>
<td>R1 = 0.015, wR2 = 0.034</td>
</tr>
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</table>

**2.3. Biological Studies**
The potential of simple structural modifications for increasing antiparasitic activity of naphthoquinones is shown in Scheme 6. Juglone, a naturally occurring naphthoquinone, displays an IC$_{50}$/24 h value of 6.51 µM [13a]. We have previously reported that the addition of methyl or bromine groups to C-5 and/or C-7 leads to new naphthoquinones that are 4.7 and 5.2 times more active against T. cruzi than juglone, respectively. Remarkably, a 40 fold increase in activity is observed for the O-acetoxy derivative of juglone [13a] (Scheme 13).

Scheme 13. Simple modifications in juglone leading to compounds with potent trypanocidal activity. *IC$_{50}$/24 h values for the lytic activity on bloodstream trypomastigotes.

Considering these previous results and recent reports in the literature [24,25], we have accomplished modifications to the quinoidal system leading to A-, B-, or A- and B-ring substituted compounds, and these were assayed against T. cruzi. Note that direct functionalization by rhodium-catalyzed C-H bond activation allows the efficient and fast preparation of potent quinoidal compounds, as shown in Scheme 14.
Compounds were evaluated against bloodstream forms of *T. cruzi* (IC₅₀/24 h) and mammalian cells (LC₅₀/24 h), allowing the determination of the selectivity index (SI) derived from the ratio of LC₅₀/IC₅₀ (Table 2). From these 34 compounds, 22 are more active than benznidazole, the reference drug (IC₅₀/24 h = 9.68 ± 2.35 µM), with 2, 3, 13, 14, 19, 20, 21, 22, 25, 28, 51, 55, 56, 57 and 59 exhibiting IC₅₀/24h values lower than 2 µM, with an SI of between 7.5 and 62.5.

Compounds 2 and 3 show significant activity against the parasite with IC₅₀ values of 0.59 and 0.22 µM, respectively, low toxicity for the mammalian cell, and an SI higher than 19. Diiodinated naphthoquinone 3 was obtained as a by-product (vide supra) in poor yield (5%) and further optimization of its preparation will be necessary. In general, the presence of iodine and electron donating groups on the A-ring increases activity against *T. cruzi* and highlights the importance of our Rh-catalyzed C-H iodination methodology. Compounds 22 (IC₅₀ = 0.49 µM, SI 24.7) and 25 (IC₅₀ = 0.56 µM, SI 23.4), which combine either a methyl or amino group with an iodine substituent, show important activity, being around 20 times more active than benznidazole. As such, 22 and 25 are prototypes that warrant further study. Conversely, the presence of electron withdrawing groups on A-ring and, in some cases, a methyl group leads to decreased activity against *T. cruzi* [34 (7.26/SI 3.8), 12 (7.57/SI 6.9) and 11 (4.06/SI 6.1)].
Certain benzoquinones also show interesting trypanocidal activity, with an IC$_{50}$ value of less than 2 µM determined for compound 51. This compound is easily obtained from commercially available benzoquinone in just one-step. C-2 functionalization may be a good general strategy for preparing bioactive compounds, since naphthoquinones substituted at C-2 with iodine (5 IC$_{50} = 1.79$ µM, SI 8.2) and selenium (56, IC$_{50} = 1.13$ µM, SI 11.2) were 5.4 and 8.5 times more active than benznidazole, respectively.

Finally, the most active compound (E)-5-styryl-1,4-naphthoquinone 59 was prepared in two steps and is described here for the first time. The synthesis of 59 requires C-5 iodinated compound 2, which is prepared by Rh-catalyzed C-H functionalization. The C-I bond then enables Pd-catalyzed alkenylation with styrene to afford 59. This compound, with an IC$_{50}$/24h = 0.19 µM, is fifty fold more active than benznidazole, with an SI of 62.5. Although the studies described here are preliminary, it is worth noting that the combination of a practical synthetic route, high in vitro trypanocidal activity and low toxicity to mammalian cells, makes 59 a very attractive hybrid quinone in the search for better trypanocides.

**Table 2.** Activity of synthetic derivatives against bloodstream trypomastigotes of *T. cruzi* (Y strain) at 37 °C, cytotoxicity to mammalian cells and Selectivity Index (SI).

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC$_{50}$/24h (µM)</th>
<th>LC$_{50}$/24h (µM)</th>
<th>SI</th>
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<tr>
<td>2</td>
<td>0.59 ± 0.08</td>
<td>11.49 ± 0.54</td>
<td>19.5</td>
</tr>
<tr>
<td>3</td>
<td>0.22 ± 0.07</td>
<td>12.93 ± 1.43</td>
<td>28.8</td>
</tr>
<tr>
<td>5</td>
<td>42.0 ± 3.11</td>
<td>&gt; 100</td>
<td>&gt; 4.6</td>
</tr>
<tr>
<td>6</td>
<td>24.8 ± 4.49</td>
<td>&gt; 100</td>
<td>&gt; 5.1</td>
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<tr>
<td>8</td>
<td>0.99 ± 0.12</td>
<td>15.72 ± 3.49</td>
<td>15.8</td>
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<td>11</td>
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<td>13.59 ± 0.79</td>
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<td>12</td>
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<td>14</td>
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<td>6.1</td>
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<tr>
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<td>28</td>
<td>1.61 ± 0.44</td>
<td>13.94 ± 0.21</td>
<td>8.7</td>
</tr>
<tr>
<td>30</td>
<td>1.38 ± 0.57</td>
<td>12.15 ± 0.15</td>
<td>8.8</td>
</tr>
<tr>
<td>32</td>
<td>2.25 ± 0.48</td>
<td>11.82 ± 0.37</td>
<td>5.3</td>
</tr>
<tr>
<td>34</td>
<td>7.26 ± 1.41</td>
<td>27.83 ± 5.17</td>
<td>3.8</td>
</tr>
<tr>
<td>36</td>
<td>60.61 ± 6.86</td>
<td>86.02 ± 2.99</td>
<td>1.4</td>
</tr>
<tr>
<td>37</td>
<td>21.54 ± 5.83</td>
<td>34.35 ± 2.46</td>
<td>1.6</td>
</tr>
<tr>
<td>38</td>
<td>15.95 ± 3.52</td>
<td>13.78 ± 4.37</td>
<td>0.86</td>
</tr>
<tr>
<td>39</td>
<td>83.42 ± 9.08</td>
<td>&gt; 100</td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td>41</td>
<td>12.77 ± 1.61</td>
<td>35.93 ± 4.92</td>
<td>2.8</td>
</tr>
<tr>
<td>42</td>
<td>14.09 ± 2.32</td>
<td>42.30 ± 4.67</td>
<td>3.0</td>
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2.4. Electrochemistry Aspects

Electrochemistry is a useful tool for the design, development and characterization of redox-selective molecules and can provide information on their mechanism of action [26]. Motivated by this approach, we have successfully described the combined use of electrochemistry and biological evaluation to provide insights about the mechanism of action of 3-arylamino lapachone [27], as well as selenium [28] and triazole substituted [29] quinones. The ever-increasing interest in biologically active quinones has resulted in new strategies to decrease toxicity, modulate activity, and direct the compound to the target [30]. As such, it is pertinent to continue to explore their synthesis, biological activities and redox mechanisms through a combined theoretical and experimental approach.

Most quinones, including intermediary and reduced forms, exist inside hydrophobic cell membranes. Consequently, dimethylformamide (DMF) was chosen as an aprotic organic solvent for electrochemical studies, because it can mimic the nonpolar environment in the cell, where lipid peroxidation, one of the causes of membrane fragmentation, normally occurs [24]. We have studied the electrochemical properties of nine quinones. These compounds were selected based on their trypanocidal activity; both active and inactive variants have been studied to enable an understanding of the main qualitative features associated with activity.

As observed in Figure 2, in general there are two reducible systems (the quinone itself and the aryl iodide bond). The electrochemistry of quinones [31] and halogenated compounds, mainly aromatic ones (ArX), has been thoroughly investigated [32] on several electrodes. For quinones, in aprotic medium, general equations 1 to 3 are expected and the quinone/semiquinone/hydroquinone (Q/SQ*/H2Q) triad is an important component of many redox systems in biology [24,26].

<p>| | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>49</td>
<td>10.07 ± 3.26</td>
<td>15.40 ± 3.87</td>
<td>1.5</td>
</tr>
<tr>
<td>50</td>
<td>23.95 ± 7.08</td>
<td>32.62 ± 9.71</td>
<td>1.4</td>
</tr>
<tr>
<td>51</td>
<td>1.94 ± 0.51</td>
<td>27.71 ± 1.22</td>
<td>1.3</td>
</tr>
<tr>
<td>53</td>
<td>26.99 ± 4.30</td>
<td>&gt; 100</td>
<td>&gt; 4.7</td>
</tr>
<tr>
<td>54</td>
<td>15.40 ± 1.34</td>
<td>&gt; 100</td>
<td>&gt; 6.5</td>
</tr>
<tr>
<td>55</td>
<td>1.79 ± 0.37</td>
<td>14.72 ± 2.68</td>
<td>8.2</td>
</tr>
<tr>
<td>56</td>
<td>1.13 ± 0.22</td>
<td>12.67 ± 0.70</td>
<td>11.2</td>
</tr>
<tr>
<td>57</td>
<td>1.24 ± 0.31</td>
<td>12.67 ± 0.10</td>
<td>10.2</td>
</tr>
<tr>
<td>58</td>
<td>6.03 ± 1.19</td>
<td>12.65 ± 0.26</td>
<td>2.1</td>
</tr>
<tr>
<td>59</td>
<td>0.19 ± 0.08</td>
<td>11.87 ± 0.43</td>
<td>62.5</td>
</tr>
<tr>
<td>60</td>
<td>2.20 ± 0.33</td>
<td>12.49 ± 0.23</td>
<td>5.7</td>
</tr>
</tbody>
</table>
For halides, bi-electronic uptake in aprotic solvents causes rupture of the C-X bond, by either a concerted (Eq. (4)) or stepwise mechanism (Eqs. (5) and (6)), followed by reduction of the ensuing radical (Eq. (7)) to an anion. The latter is usually protonated by residual water to the respective C-H bond [30].

\[
\begin{align*}
Q + e^- & \rightleftharpoons SQ^{\cdot-} \\
SQ^{\cdot-} + e^- + 2H^+ & \rightleftharpoons H_2O \\
\text{Net} & \quad Q + 2e^- + 2H^+ \rightleftharpoons H_2O
\end{align*}
\]

\[(1) \quad (2) \quad (3)\]

The electrochemistry of iodinated quinones where the halogen is directly attached to the aromatic ring is less widely reported. In the present case, the reduction process of nine iodinated 1,4-naphthoquinones (1,4-NQ), 2, 3, 5, 6, 11, 19, 20, 22 and 55 was investigated on glassy carbon electrode (GCE), in anhydrous DMF with 0.1 M TBAPF₆ as the supporting electrolyte and electrochemical data were compared to trypanocidal activity. A detailed description of the voltammetric behavior of these novel compounds is out of the scope of the present paper. Here we will only consider some features relevant to the discussion of the correlation of redox potentials with trypanocidal activity. Several of the iodo-naphthoquinones analysed have additional A-ring substituents (-OH, -OCH₃, and -CH₃ groups) and two of them are bis-iodinated (3 and 6). All possess an aryl-iodide bond, except 56, which carries a vinyl iodide. Data on the main reduction peaks, with an emphasis to the first reduction step, Epc₁, appear in Table 2.

**Table 3.** Major electrochemical parameters of the iodinated quinones (c = 1 mmol L⁻¹), using cyclic voltammetry, on GCE, in DMF/TBAPF₆, 0.1 mol L⁻¹, v = 100 mV s⁻¹.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Epc₁ (V)</th>
<th>Epc₂ (V)</th>
<th>Epc₃ (V)</th>
<th>Epa₁' (V)</th>
<th>Epa₁ (V)</th>
<th>Epa₂' (V)</th>
<th>Epa₂ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-0.535</td>
<td>-1.212</td>
<td>-2.198</td>
<td>-</td>
<td>-0.470</td>
<td>-</td>
<td>-1.105</td>
</tr>
</tbody>
</table>
Considering the ease of the reduction related to Epc1 (Figure 2), the compounds can be ranked as shown below. Less negative potentials indicate higher electrophilicity: 

6 > 5 > 55 > 3 > 2 > 22 > 20 > 19 > 11 (Figure 2).

The CV profiles (Figure 2) and structural similarities allow the classification of the compounds into three main electrochemical classes.

The first class contains only one compound, 55, and is important as it allows comparison of the reduction of aromatic vs. vinylic C-I bonds. Aromatic variants suffer much easier reduction (see Epc1), while the ease of reduction of the quinone moiety is influenced by the electron withdrawing halogen at C2, being reduced at more positive potentials (-0.413 V) than similar C5-iodinated compound 2 (-0.535 V, see table 3).

The second class comprises compounds with substituents that can hydrogen bond (5 and 6). This feature results in easier reduction, presumably due to stabilization of the semiquinone (Eq. 1) through hydrogen-bonding. Reduction potentials for 5 and 6 are -0.216 V and -0.244 V, respectively; these values are much more positive than for all the other quinones. The electrochemical reduction of the C-I, leading to bond cleavage (Eqs. 4-7) occurs at highly negative potentials (Epc3, Table 2).

The third class of compounds, 2, 3, 22, 19, 11 and 20, has a different electrochemical profile. The first waves for all of them are related to the generation of the semiquinone (Eq. 1). With one exception, 22, all of them present an enhanced current at
the level of the second reduction wave \((Ipc_2/Ipc_1 > 1)\), with loss of reversibility \((Ipa/Ipc < 1)\). This is related to the reduction of the semiquinone together with the reduction of the C-I bonding. In the case of 3, where the current \((Ipc2)\) is the highest, the cleavage of both C-I bonds is expected [30] and observed.

Comparison of the Epc1 values with trypanocidal activity reveals a trend, with the two more electrophilic quinones (5 and 6) being less active than the others. As quinones are pro-drugs that need to be activated, being so easily reduced, it is possible that 5 and 6 suffer reduction fast, before reaching the biological target, leading, thus, to inactivity.
Figure 2. Several inversion potentials in the CV of iodinated quinones 2, 3, 5, 6, 11, 19, 20 and 22 (1 mM) in DMF + TBAPF₆ (0.1 M), glassy carbon electrode, cathodic direction, potential range: 0.5 V up to –2.5 V, v = 0.1 V s⁻¹.
3. Conclusions

The present work shows how a complementary combination of chemical, biological and electrochemical approaches can be used for the identification of new lead compounds for the treatment of Chagas disease. We have described potent trypanocidal naphthoquinones prepared in high efficiency by versatile synthetic methods. Compound 59, which was prepared in just two steps, has an IC$_{50}$/24h = 0.19 µM (fifty fold more active than benznidazole) and SI = 62.5 and is thus an important starting point for the development of a new drug against T. cruzi. In broader terms, the present study provides an efficient framework to prepare quinoidal compounds with potent activity against the etiological agent of Chagas disease.

4. Experimental Section

4.1. Chemistry

4.1.1. General procedures

1,4-Naphthoquinone and 1,4-benzoquinone were purchased from Alfa Aesar and Sigma Aldrich, purified via reduced pressure sublimation using a cold finger sublimation apparatus (50 °C, 0.9 mbar) and stored in a glovebox to prevent contact with moisture. All commercially available naphthoquinones, benzoquinones and further commercial chemicals were purchased from Sigma Aldrich, Alfa Aesar, Strem Chemicals and Santa Cruz Biotechnology. [RhCpCl$_2$)$_2$ was purchased from Sigma Aldrich.

Substrates 9 and 10, as well as derivatives 13 and 14 were obtained by reaction with iodomethane and Ag$_2$O in CHCl$_3$ [17]. Substrates 17 and 18 obtained by oxidation of corresponding tetralones with 2-iodobenzoic acid and Oxone [18]. Substrate 24 was synthesized by nitration of 1,4-naphthoquinone followed by reduction with SnCl$_2$/FeCl$_3$ [33]. Substrate 27 was synthesised following our latest report [16]. 2,3-Dimethyl-1,4-benzoquinone (46), 2-methoxy-1,4-benzoquinone (47) and 2,6-dimethoxy-1,4-benzoquinone (48) were prepared by oxidation of the corresponding hydroquinones (in case of 2,3-dimethyl-1,4-benzoquinone, the hydroxyl groups of the hydroquinone were methylated with dimethyl sulfate) with silica supported cerium ammonium nitrate [34]. 2,3-Dichloro-1,4-benzoquinone (52) was obtained by reaction of 1,4-benzoquinone (35) with SOCl$_2$/H$_2$SO$_4$ followed by dehydrogenation with Ag$_2$O [35].
Starting materials available from commercial suppliers were used as received, unless otherwise stated. All reagents requiring purification were purified using standard laboratory techniques, according to methods published by Perrin, Armarego, and Perrin (Pergamon Press, 1966). Catalytic reactions were run under an atmosphere of dry nitrogen or argon; glassware, syringes and needles were either flame dried immediately prior to use or placed in an oven (200 °C), for at least 2 h, and allowed to cool either in a desiccator or under an atmosphere of nitrogen or argon; liquid reagents, solutions or solvents were added via syringe through rubber septa; solid reagents were added inside a glovebox. Melting points were obtained on a Thomas Hoover apparatus and are uncorrected. Column chromatography was performed on silica gel (SiliaFlash G60 UltraPure 60-200 µm, 60 Å). Infrared spectra were recorded on an FTIR Spectrometer IR Prestige-21-Shimadzu. ¹H and ¹³C NMR were recorded at room temperature using a Bruker AVANCE DRX200 and DRX400 MHz instrument, in the solvents indicated. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). All assignments of NMR spectra were based on 2D NMR data (DEPT-135, COSY, HSQC and HMBC). Mass spectra were recorded using a Brüker Daltonics FT-ICRMS Apex 4e 7.0T FT-MS (ESI⁺ mode) and Shimadzu GCMS QP2010+ (EI⁺ mode). Infrared spectra were recorded on a Perkin Elmer Spectrum One FTIR spectrometer as thin films or solids compressed on a diamond plate. Data were processed employing Bruker Data Analysis software version 4.0. Compounds were named following IUPAC rules as applied by ChemBioDraw Ultra (version 12.0).

4.1.2. 5-Hydroxy-6-iodo-1,4-naphthoquinone (5). Yield: 88%; m.p. (°C) = 179.8-180.1; red crystals. IR (solid, cm⁻¹) ν: 3021 (w), 1698 (s), 1270 (m), 830 (m); HRMS (EI⁺): 299.9280 [M⁺]. Cald. for [C₁₀H₅IO₃]: 299.9283; ¹H NMR (400 MHz, CDCl₃) δ: 12.73 (s, O-H), 8.15 (d, J = 8.0 Hz, C₆-H), 7.33 (d, J = 8.0 Hz, C₅-H), 6.96 (s, C₂-H,C₃-H); ¹³C NMR (100 MHz, CDCl₃) δ: 190.0 (C₄), 183.8 (C₁), 160.1 (C₅), 146.1 (C₇), 139.8 (C₃), 138.0 (C₂), 131.5 (C₈a), 120.1 (C₈), 114.2 (C₄a), 94.9 (C₆-I).

4.1.3. 5-Hydroxy-6,8-diiodo1,4-naphthoquinone (6). Yield: 82%; m.p. (°C) = 164.3-165.8; purple crystals. IR (solid, cm⁻¹) ν: 3002 (w), 1613 (s), 1211 (m), 911 (m); HRMS (EI⁺): 425.8241 [M⁺]. Cald. for [C₁₀H₄I₂O₃]: 425.8250; ¹H NMR (500 MHz, CDCl₃) δ: 13.54 (s, O-H), 8.80 (s, C₇-H), 7.04 (d, J = 10.2 Hz, C₂-H), 6.96 (d, J = 10.2 Hz, C₃-H); ¹³C NMR (125 MHz, CDCl₃) δ: 189.2 (C₄), 182.0 (C₁), 161.7 (C₅), 158.5 (C₇), 140.4 (C₂), 136.5 (C₃), 130.0 (C₈a), 115.8 (C₄a), 96.0 (C₆-I), 83.0 (C₈-I).
4.1.4. 5-Hydroxy-6-iodo-2-methyl-1,4-naphthoquinone (8). Yield: 85%; m.p. (°C) = 125.1-125.9; red crystals. IR (solid, cm⁻¹) ν: 3048 (w), 1687 (s), 1198 (m), 854 (m); MS (EI⁺): 313.9 [M⁺]. Calcd. for [C₁₁H₁₇IO₃]: 313.9; ¹H NMR (400 MHz, CDCl₃) δ: 12.82 (s, 1H), 8.11 (d, J = 7.9 Hz, C7-H), 7.35 (d, J = 7.9 Hz, C8-H), 6.81 (s, C3-H), 2.19 (s, C9-H₃). ¹³C NMR (100 MHz, CDCl₃) δ: 189.9 (C4), 184.3 (C1), 159.9 (C5-CH₃), 150.0 (C2), 145.6 (C7), 134.8 (C3), 131.9 (C8a), 120.3 (C8), 114.4(C4a), 94.4 C6-I, 16.5 (C9).

4.1.5. 6-Iodo-5-methoxy-1,4-naphthoquinone (13). Yield: 91%; m.p. (°C) = 152.3-152.8; yellow powder. IR (solid, cm⁻¹) ν: 3014 (w), 1699 (s), 1199 (m), 867 (m); MS (EI⁺): 313.9 [M⁺]. Calcd. for [C₁₁H₁₇IO₃]: 313.9; ¹H NMR (400 MHz, CDCl₃) δ: 8.24 (d, J = 8.2 Hz, C7-H), 7.65 (d, J = 8.2 Hz, C8-H), 6.95 (d, J = 10.3 Hz, C2-H), 6.90 (d, J = 10.3 Hz, C3-H), 3.93 (s, C9-H₃). ¹³C NMR (100 MHz, CDCl₃) δ: 184.4 (C1), 183.3 (C4), 159.2 (C5), 144.7 (C7), 139.9 (C3), 136.9 (C2), 134.1 (C8a), 124.2 (C8), 124.1 (C4a), 104.2 (C6-I), 61.8 (C9).

4.1.6. 6-Iodo-5-methoxy-2-methyl-1,4-naphthoquinone (14). Yield: 92%; m.p. (°C) = 117.3-118.1; yellow powder. IR (solid, cm⁻¹) ν: 2985 (w), 1689 (s), 1321 (m), 902 (m); MS (EI⁺): 327.9 [M⁺]. Calcd. for [C₁₂H₁₈IO₃]: 327.9; ¹H NMR (400 MHz, CDCl₃) δ: 8.19 (d, J = 8.2 Hz, C7-H), 7.65 (d, J = 8.2 Hz, C8-H), 6.76 (s, C3-H), 3.91 (s, C10-H₂), 2.17 (s, C9-H₃). ¹³C NMR (100 MHz, CDCl₃) δ: 185.0 (C1), 183.3 (C4), 158.9 (C5), 146.3 (C2), 144.3 (C7), 136.9 (C3), 134.4 (C8a), 124.4 (C8), 124.3 (C4a), 103.8 (C6-I), 61.8 (C10), 15.9 (C9).

4.1.7. 5-Amino-6-iodo-1,4-naphthoquinone (25). Yield: 41%; m.p. (°C) = 181.2-182.9, purple powder. IR (solid, cm⁻¹) ν: 3078 (w), 1720 (s), 1380 (m), 923 (m); HRMS (EI⁺): 298.9433 [M⁺]. Calcd. for [C₁₀H₈NO₂]: 298.9443; ¹H NMR (400 MHz, CDCl₃) δ: 8.03 (d, J = 8.0 Hz, C7-H), 7.15 (d, J = 8.0 Hz, C8-H), 6.90 (d, J = 10.3 Hz, C3-H), 6.86 (d, J = 10.3 Hz, C2-H); ¹³C NMR (100 MHz, CDCl₃) δ: 186.65 (C4), 184.97 (C1), 149.21 (C5), 144.79 (C7), 140.05 (C3), 137.20 (C2), 132.92 (C8a), 117.45 (C8), 111.89 (C4a), 95.06 (C6-I).

4.1.8. 2,6-Diiodo-3,5-dimethoxy-1,4-benzoquinone (51). Yield: 91%; m.p. (°C) = 160.2-160.9; red crystals. IR (solid, cm⁻¹) ν: 2947 (w), 1667 (s), 1266 (s), 924 (s); HRMS (EI⁺): 261.9493 [M⁺]. Calcd. for [C₈H₆O₂]: 419.8355; ¹H NMR (500 MHz, CDCl₃) δ: 4.16 (s, C7-H₂O₂); ¹³C NMR (125 MHz, CDCl₃) δ: 175.2 (C1), 171.2 (C4), 160.4 (C2,C6), 98.1 (C3,C5), 61.6 (C7).

4.1.9. (E)-5-Styryl-naphthalene-1,4-dione (59). Yield: 51%; m.p.(°C): 140.8-141.9; red solid. IR (solid, cm⁻¹) ν: 2956 (w), 1717 (s), 1632 (s), 1278 (m), 1100 (m), 790
(m); HRMS(El⁺): 260.0822. Cald. for [C_{18}H_{12}O_{2}]⁺: 260.0837; ¹H NMR (500 MHz, CDCl₃) δ: 8.30 (d, J = 16.1 Hz, C9-H), 8.10 (dt, J = 7.6, 1.2 Hz, C8-H), 7.98 (dd, J = 7.9, 0.8 Hz, C6-H), 7.72 (t, J = 7.8 Hz, C7-H), 7.62 – 7.60 (m, 2H), 7.43 – 7.37 (m, 2H), 7.35 – 7.29 (m, 1H), 7.06 (d, J = 16.2 Hz, C10-H), 6.95 (d, J = 1.0 Hz, C2-H, C3-H). ¹³C NMR (125 MHz, CDCl₃) δ: 187.0 (C4), 185.2 (C1), 140.5 (C2), 140.2 (C5), 137.1 (C8a), 136.9 (C3), 133.5 (C10), 133.5 (C6), 133.3 (C7), 128.8 (C13, C15), 128.3 (C14), 127.9 (C4a), 127.7 (C9), 127.1 (C12, C16), 126.5 (C8).

4.2. Crystallographic data

X-ray diffraction data collection for three compounds were performed on an Enraf-Nonius Kappa-CCD diffractometer (95 mm CCD camera on κ-goniostat) using graphite monochromated MoKα radiation (0.71073 Å), at room temperature. Data collection was carried out using the COLLECT software [36] up to 50° in 2θ. Integration and scaling of the reflections, correction for Lorentz and polarization effects were performed with the HKL DENZO-SCALEPACK system of programs [37]. The structure of the compounds was solved by direct methods with SHELXS-97 [38]. The models were refined by full-matrix least squares on F² using the SHELXL-97 [39]. The program ORTEP-3 [40] was used for graphic representation and the program WINGX [41] to prepare materials for publication. All H atoms were located by geometric considerations (C-H = 0.93-0.97; O-H = 0.82 Å) and refined as riding with Uiso(H) = 1.5Ueq(C-methyl) or 1.2Ueq(other). Crystallographic data for the structures were deposited in the Cambridge Crystallographic Data Centre, with numbers CCDC 1526294, 1526293 and 1526364.

4.3. Animals

Albino Swiss mice were employed for the trypanocidal and cytotoxicity assays, in accordance to the guidelines of the Colégio Brasileiro de Experimentação Animal (COBEA), and these were performed under biosafety conditions. All animal experimentation procedures were approved by the Comissão de Ética em Experimentação Animal (CEUA/Fiocruz), license LW 16/13.
4.4. Trypanocidal Assay

The experiments were performed with the Y strain of *T. cruzi* [42]. Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO), with the final concentration of the latter in the experiments never exceeding 0.1%. Preliminary experiments showed that at concentrations of up to 0.5%, DMSO has no deleterious effect on the parasites [43]. Bloodstream trypomastigotes were obtained from infected Albino Swiss mice at the peak of parasitemia by differential centrifugation. The parasites were resuspended to a concentration of 10×10^6 cells/mL in DMES medium. This suspension (100 μL) was added to the same volume of each of the compounds, which had been previously prepared at twice the desired final concentrations. The incubation was performed in 96-well microplates (Nunc Inc., Rochester, USA) at 37°C for 24 h. Benznidazole (Lafepe, Brazil), the standard drug for treatment of chagasic patients, was used as control. Cell counts were performed in a Neubauer chamber, and the activity of the compounds corresponding to the concentration that led to 50% lysis of the parasites was expressed as the IC_{50}/24 h.

4.5. Cytotoxicity to mammalian cells

The cytotoxicity assays were performed using primary cultures of peritoneal macrophages obtained from Albino Swiss mice. For the experiments, 2.5×10^4 cells in 200 μL of RPMI-1640 medium (pH 7.2 plus 10% foetal bovine serum and 2 mM glutamine) were added to each well of a 96-well microtiter plate and incubated for 24 h at 37°C. The treatment of the cultures was performed in fresh supplemented medium (200 μL/well) for 24 h at 37°C. After this period, 110 μL of the medium was discarded and 10 μL of PrestoBlue (Invitrogen) was added to complete the final volume of 100 μL. Thus, the plate was incubated for 2 h and the measurement was performed at 560 and 590 nm, as recommended by the manufacturer. The results were expressed as the difference in the percentage of reduction between treated and untreated cells being the LC_{50} value, corresponding to the concentration that leads to damage of 50% of the mammalian cells [25].
4.6. Electrochemical studies

Cyclic voltammetry (CV) experiments were performed with a conventional three-electrode cell in an Autolab PGSTAT-30 potentiostat (Echo Chemie, Utrecht, the Netherlands) coupled to a PC microcomputer, using GPES 4.9 software. The working electrode was a glassy carbon (GC) BAS (d = 3 mm), the counter electrode was a Pt wire and the reference electrode an Ag|AgCl, Cl\(^-\) (saturated), all contained in a one-compartment electrochemical cell with a volumetric capacity of 5 mL. The GC electrode was cleaned up by polishing with alumina on a polishing felt (BAS polishing kit). The solvent used in aprotic media studies was extra dry N,N-dimethylformamide (99.8%) acquired from Acros Organics. In CV experiments, the scan rate varied from 10 to 1000 mV s\(^{-1}\). Electrochemical reduction and oxidation were performed in aprotic media (DMF + TBAPF\(_6\) 0.1 mol L\(^{-1}\)) at room temperature (25 ± 2 °C). Each compound (1 x 10\(^{-3}\) mol L\(^{-1}\)) was added to the supporting electrolyte and the solution was deoxygenated with argon before the measurements by cyclic voltammetry, in different potential intervals.

Appendix A. Supplementary data

Supplementary data related to this article can be found at DOI

Notes

The authors declare no competing financial interest.

Acknowledgements

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