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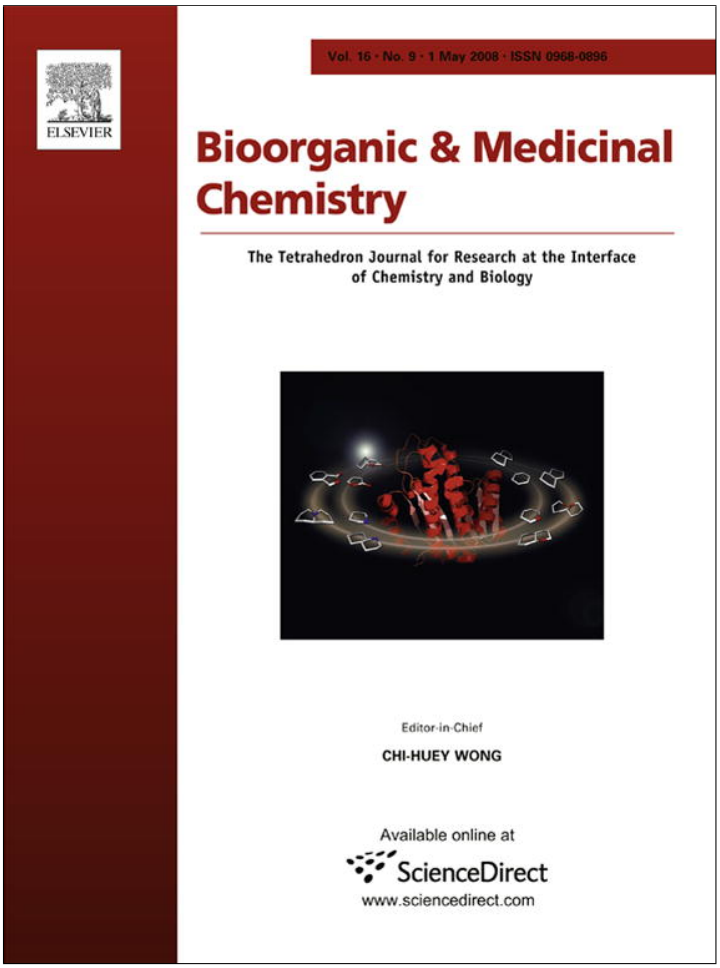

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Synthesis and anti-*Trypanosoma cruzi* activity of derivatives from nor-lapachones and lapachones

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Abstract—New naphthoquinone derivatives were synthesized and assayed against bloodstream trypomastigote forms of *Trypanosoma cruzi*, the etiological agent of Chagas' disease. The compounds were rationalized based on hybrid drugs and appear as important compounds against this parasite. From nor-lapachol were prepared five substituted *ortho*-naphthofuranquinones, a non-substituted *para*-naphthofuranquinone, a new oxyrane and an azide and from α-lapachone a new non-substituted *para*-naphthofuranquinone. Other five substituted *ortho*-naphthofuranquinones recently designed as cytotoxic, were also evaluated. The most active compounds were the ortho naphthofuranquinones 3-(4-methoxyphenylamino)-2,3-dihydro-2,2-dimethylnaphtho[1,2-*b*]furan-4,5-dione and 3-(3-nitrophenylamino)-2,3-dihydro-2,2-dimethylnaphtho[1,2-*b*]furan-4,5-dione with trypanocidal activity higher than that of benznidazole, the standard drug. The compounds were rationalized based on hybrid drugs and appear as important compounds against *T. cruzi*. The trypanocidal activity of these substances endowed with redox properties representing a good starting point for a medicinal chemistry program aiming the chemotherapy of Chagas' disease.

1. Introduction

Chagas' disease (American trypanosomiasis), caused by the protozoa *Trypanosoma cruzi*, is endemic in 21 countries of the Americas.[1] Acute infections are usually asymptomatic, but the ensuing chronic *T. cruzi* infections have been associated with high ratios of morbidity and mortality.[2] Most human infections in the Western Hemisphere occur through contact with infected blood-sucking insects of the triatomine species. However, economic hardship, political problems, or both, have spurred migration from endemic countries to developed ones such as Australia, Canada, Spain, and the United States.[3] The life cycle of *T. cruzi* involves obligatory passage through vertebrate and invertebrate hosts, in a series of different developmental forms. The bloodstream trypomastigote ingested by the insect differentiates into the proliferative epimastigote, which differentiates into metacyclic trypomastigote on the posterior intestine of the insect. The metacyclic form invades the vertebrate host cell, differentiates into the proliferative amastigote form, which transforms into the trypomastigote form responsible for the dissemination of the infection. When *T. cruzi* is transmitted to man through the feces of triatomines, at bite sites or in mucosa, through blood transfusion or orally through contaminated food, it invades the bloodstream and lymphatic system and becomes established in the muscle and cardiac tissue, the digestive system and phagocytic cells.[4]

At present, the only accepted drugs for the treatment of Chagas' disease are nifurtimox (Lampit®) and

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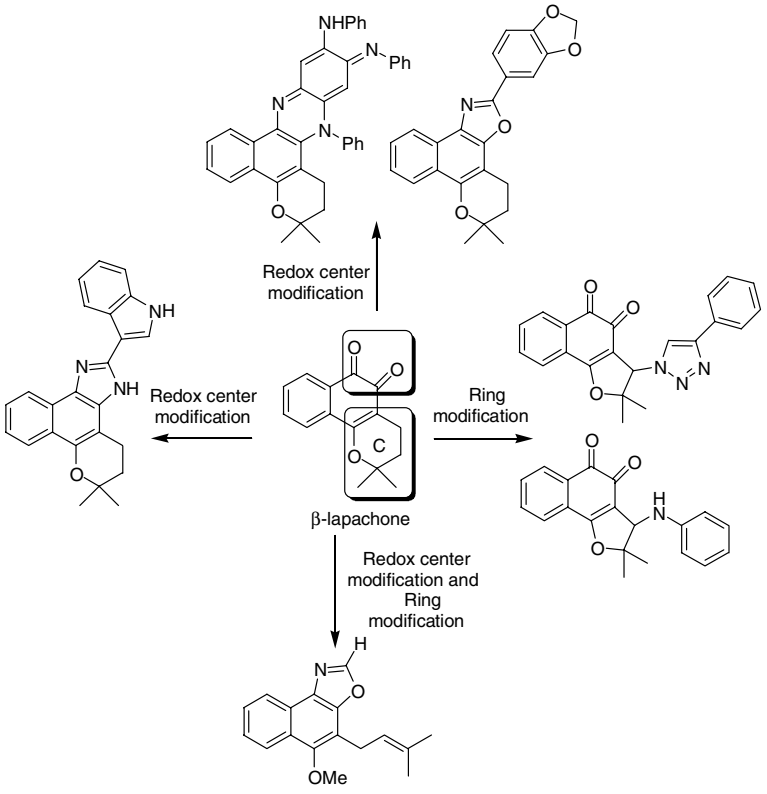
benznidazole (Rochagan® or Radanil®), effective for acute infections, but controversy on their use for chronic patients is due to undesirable side effects, frequently forcing the abandonment of the treatment, poor indices of apparent cure, and a lack of consensus about criteria for parasitological cure.[5,6]

In this context, an intensive effort has been devoted to finding new prototype compounds among natural and synthetic sources. Compounds having a quinone core have promising biological activity. In this regard, naphthoquinones have a broad distribution in the plant kingdom and are involved in several oxidative processes such as photosynthesis and electron transfer reactions.[7] The 1,2- and 1,4-naphthoquinones are considered privileged structures in medicinal chemistry, with the easiness of reduction–oxidation of the quinoidal moiety being the basis for their participation in electron transport and oxidative-phosphorylation processes. Taking into account that molecular hybridization[8a] is a powerful approach for the design of new compounds based on the recognition of the pharmacophoric sub-unities in the molecular structure, we have been searching for new quinone derivatives as potential anti-*T. cruzi* compounds exploring the electrophilicity of 1,2-quinoidal carbonyls by reactions with different heteroatom nucleophilic centers[7] (Scheme 1). From the heartwood of *Tabebuia* trees (Bigoniaceae) we have extracted lapachol, and prepared about 60 derivatives which were screened for their trypanocidal effect, using the infective bloodstream form of *T. cruzi*.[8–12] Among these compounds, three naphthoimidazoles derived from β-lapachone were the most active against the three evolutive forms of the parasite, and by ultrastructural, flow cytometry and biochemical studies, it was shown that they interfere with the energetic metabolism and DNA fragmentation.[13,14] Our group also reported the synthesis and trypanocidal activity of new naphthofuranquinones obtained from nor-lapachol, 2-hydroxy-3-allyl-naphthoquinone,[15] of heterocyclic oxyranes,[16,17] and [1,2,3]-triazole derivatives of nor-β-lapachone.[18] Giving continuity to our studies on the chemistry of naphthoquinones and their activity against *T. cruzi*, we now focus on new *ortho*- and *para*-naphthoquinone derivatives.

2. Results and discussion

2.1. Chemistry

Lapachol (**1**) (2-hydroxy-3-(3′-methyl-2-butenyl)-1,4-naphthoquinone) was extracted from the heartwood of *Tabebuia sp.* (*Tecoma*) and purified by a series of recrystallizations. From this quinone, nor-lapachol (**2**) (2-hydroxy-3-(2-methyl-propenyl)-[1,4]-naphthoquinone) was obtained by Hooker oxidation.[19] The treatment of **2** with HCl/AcOH produced nor-α-lapachone (**3**) (2,3-dihydro-2,2-dimethyl[2,3-*b*]furan-4,9-dione) which was transformed into 3-bromo-nor-α-lapachone (**4**) (3-bromo-2,3-dihydro-2,2-dimethyl[2,3-*b*]furan-4,9-dione) as previously described by us.[20] Reaction of **4** with sodium azide in CH_2Cl_2 and with aniline gave, respectively, the corresponding azide and arylamino derivatives **10** and **11**. This azidonaphthoquinone **10**



Scheme 1. Examples of trypanocidal compounds obtained from β-lapachone by ring and redox center modifications.

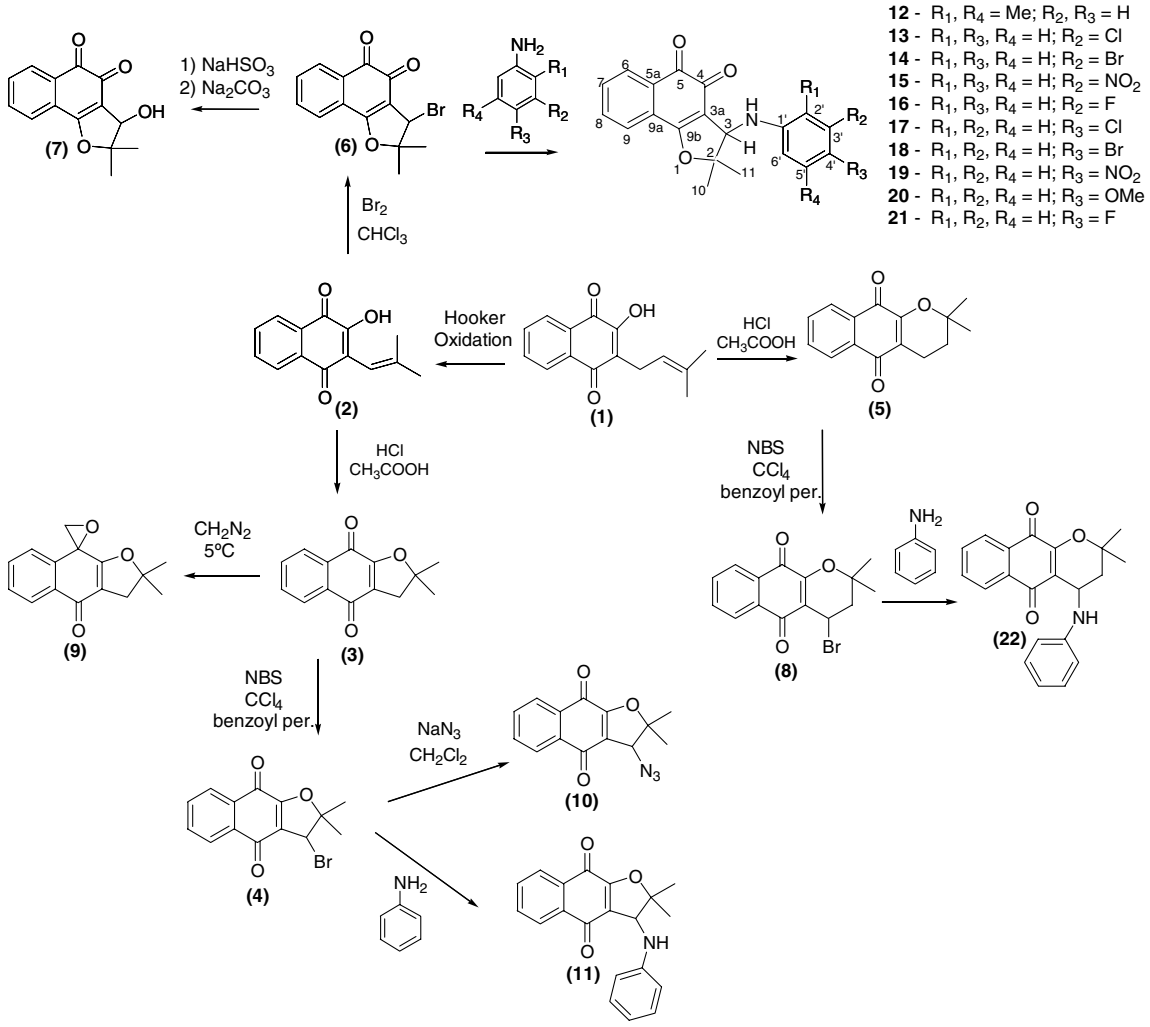
is a chemically versatile compound, which can be transformed into triazolequinones. Also, reduction of the azido group originates the corresponding amine that can be oxidized to its nitro derivative, which presents a broad reactivity.

The naphthoquinone **5** (2,2-dimethyl-3,4-dihydro-2H-5,10-dioxy-naphtho[2,3-*b*]pyrane) was obtained from **1**, through cyclization of its isoprenyl lateral chain in the presence of HCl/AcOH, by nucleophilic attack of oxygen atom of the hydroxyl group. By reacting **5** with NBS in the presence of benzoyl peroxide, and CCl_4 as solvent, (**8**) (4-bromo-3,4-dihydro-2,2-dimethyl-2H-benzo[*g*]chromene-5,10-dione) was obtained[20] and its subsequent reaction with aniline gave the new *para*-arylaminonaphthoquinone **22** (2,2-dimethyl-4-phenylamino-3,4-dihydro-2H-benzo[g]chromene-5,10-dione). The reaction of **2** with bromine in $CHCl_3$ originated (**6**) (3-bromo-2,3-dihydro-2,2-dimethylnaphtho[1,2-*b*]furan-4,5-dione), the starting material for the synthesis of the naphthoquinone (**7**) (2,3-dihydro-3-hydroxy-2,2-dimethylnaphtho[1,2-*b*]furan-4,5-dione) and of the *ortho*-arylaminonaphthofuranquinones **12**–**21**. The synthesis of **12**–**15** and **21** was previously reported by our group.[24]

The first step involves in situ preparation of **6**, which then reacts with the desired arylamine (Scheme 2). The new oxyran **9** (2,2-dimethyl-3-hydro-4-oxanaphtho[2,3-b]furane-9-spiro-2′-oxyrane) was synthesized by addition of an ethereal solution of freshly prepared diazomethane to **3**. The reaction of naphthoquinones leading to oxyrans has been previously described and their structures were unequivocally established.[21–23]

The formation of arylamino *ortho*-naphthofuranquinones **12**–**21** possibly occurs through a carbenium ion, since the alternative reaction, direct displacement, is less favorable due to spacial steric hindrance caused by the two methyl groups.[20] The *para*-naphthofuranquinone (**11**)[24] and the *para*-naphthopyranquinone (**22**), described herein for the first time, were obtained in good yields by a methodology similar to that used for the synthesis of *ortho*-naphthofuranquinones.

The structures of the synthesized compounds (Scheme 2) were confirmed by spectroscopic techniques, such as ^{1}H and ^{13}C NMR, infrared, and electron-impact mass spectra. X-ray crystallography study of the fluoro derivative **21** was performed.



Scheme 2. Synthetic route for preparing the naphthoquinones assayed against trypomastigote forms of *T. cruzi*.

The structure of **21** was solved by direct methods and refined through the interactive blocked-matrix least square calculations. The refinement was conducted until all atomic parameters shifts were smaller than their standard deviations. All H atoms were located by geometric considerations placed (C–H = 0.93–0.98 Å) and refined as riding with $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}$(methyl C). In the final difference Fourier map there are no peaks greater than 0.57 $Å^{-3}$. An Ortep3 diagram of the molecule is given in Figure 1 and Table 1 displays the main crystal data and structure refinement for the compound.

Bond lengths and angles are in good agreement with the expected values reported in the literature.[25] The atoms of the naphthoquinonic ring are coplanar and the largest deviation [0.044(3) Å] from the least-square plane is exhibited by atom C3. Atoms O1 and C12 of the furane ring lie in the mean least-square plane of the naphthoquinonic ring with deviations of 0.045(2) and 0.035(2) Å, respectively, while atom C11 is 0.105(2) Å out of that plane (Fig. 2), and this attribute is a conformation of the pure envelope to furane ring. The packing parameters calculated for this conformation were: $q^2 = 1.629(2)$ Å and $\phi^2 = 254.5(8)°$.[26]

The dihedral angle between the least-square plane calculated through the atoms [C13–C18] of benzene ring and that of the naphthoquinonic ring is 74.07(1)°. In the crystalline packing, molecules are held together by strong N–H···O interaction where N1–H1 = 0.96,H1···;$O2^i$ = 2.13 Å and N1–H1·$O2^i$ = 159° ($i = 1/2 + x, 1/2 - y, z$), forming chains propagated in the **a** direction (Fig. 3).

Table 1. Crystal data and structure refinement of the fluoro derivative **21**

Crystal data	
$C_{20}H_{15}FNO_3$	$Z = 8$
$M_r = 336.33$	$D_x = 1.324$ Mg m^{-3}
Orthorhombic, *Pcab*	Mo $K\alpha$
$a = 8.3124$ (2) Å	$\mu = 0.10$ mm^{-1}
$b = 12.2195$ (4) Å	$T = 293$ (2) K
$c = 33.2167$ (11) Å	Prism, colorless
$V = 3373.93$ (18) $Å^3$	$0.15 \times 0.10 \times 0.09$ mm
Data collection	
KappaCCD diffractometer	3823 independent reflections
CCD rotation images,thick slice scans	2047 reflections with $I > 2\sigma(I)$
Absorption correction: none	$R_{int} = 0.054$
17805 measured reflections	$\theta_{max} = 27.5°$
Refinement	
Refinement on F^2	$(\Delta/\sigma)_{max} < 0.001$
$R[F^2 > 2\sigma(F^2)] = 0.075$	$\Delta\rho_{max} = 0.57$ e $Å^{-3}$
$wR(F^2) = 0.225$	$\Delta\rho_{min} = -0.18$ e$Å^{-3}$
$S = 1.05$	Extinction correction: none
3823 Reflections	H atoms treated by constrained refinement
226 Parameters	$w = 1/[\sigma^2(F_o^2) + (0.1028P)^2 + 0.9409P]$ where $P = (F_o^2 + 2F_c^2)/3$

2.2. Trypanocidal activity

The naphthoquinone **7** which presents important activity against methicillin-resistant bacterial strains[27] was assayed for the first time against *T. cruzi* and its activity was only twofold lower than that of benznidazole. While nor-α-lapachone (**3**) was inactive against the parasite, its derivative the azide **10** presented an IC_{50}/24 h = 179.3 ± 12.0 μM (Table 2). In the same experimental conditions, the corresponding IC_{50} value for benznidazole is 103.6 ± 0.6 μM,[18] and for crystal violet, 536.0 ± 3.0 μM.[8b]

The synthesis of oxyrans from naphthoquinones was initially reported by Pinto et al.,[21] and more recently the epoxide obtained from α-lapachone (**5**) was shown to inhibit the proliferation of *T. cruzi* epimastigotes.[17] In this context, the oxyran **9** was synthesized from nor-α-lapachone (**3**), but when assayed against trypomastigote form was inactive.

Comparing the non-substituted arylamino derivatives **11** and **22**, we observed the good activity of the *para*-naphthoquinone **22**, while **11** was inactive against the parasite. Also, **22** was more active than the original quinone, α-lapachone (**5**), possibly due to the insertion of the arylamino group, increasing the lipophilicity of the molecule, which could be associated with a better penetration through the plasma membrane.

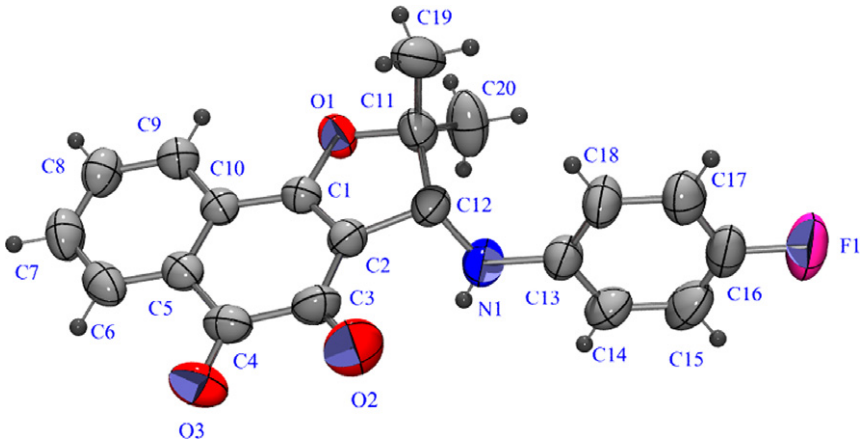


Figure 1. An ellipsoid plot Ortep3 of **21** showing the atom labeling and 50% probability displacement ellipsoids.

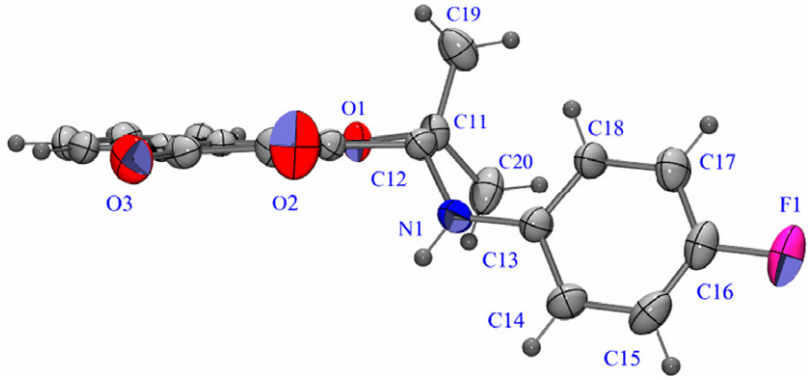


Figure 2. Projection down direction of the naphthoquinonic ring of **21**.

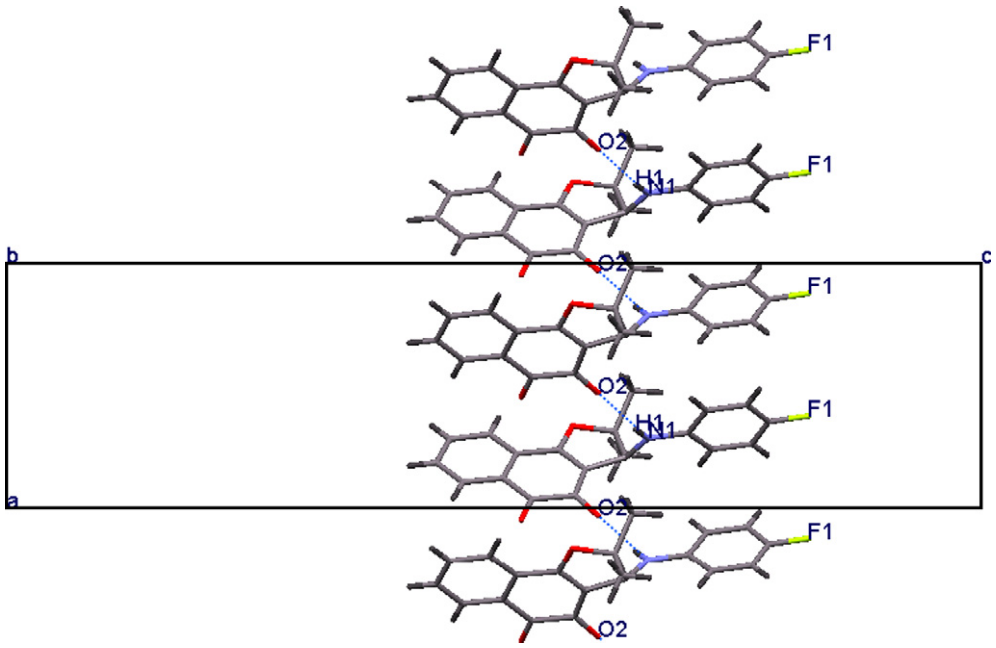


Figure 3. View of the crystal packing showing in dotted lines the N1–H1···O2 interactions of compound **21**.

In this paper, we also presented the synthesis of the arylamines **16**–**20** substituted by electron-withdrawing or electron-donating groups and the **12**–**21** were also evaluated against *T. cruzi* (Table 2). The compounds **15** and **20** were both more active than benznidazole, and were followed by **14**, **13**, and **17** (Fig. 4), while the others presented IC_{50} values higher than 800 μM. These two latter arylamines, **13** and **17**, were more active than **1** (lapachol), the original quinone. In the same experimental condition, the IC_{50}/1 day value for crystal violet is 536.0 ± 3.0 μM.[8b] A NO_2 group is present both in **15** and in **19**, but the latter compound is much less active, suggesting that the position of this group affects the electronic distribution, mainly at position 4 of the aromatic ring, decreasing the electronic density of the nitrogen, which may affect the redox potential of the quinone center.

Approaches on the planning of new trypanocidal compounds based on molecular hybridization were previously accomplished by our group in order to prepare new naphthoquinoidal [1,2,3]-triazoles.[18] These compounds proved to be more active against the *T. cruzi* than their original precursor nor-β-lapachone, and such activity was especially dependent on structural features and on the substituent position on the furanic ring.[18]

3. Conclusions

Plants containing naphthoquinones are still employed in folk medicine,[28] and their molecular structure endows them with redox properties that could interfere in biological oxidative processes. Naphthofuranquinones display a wide variety of biological activities, being in some instances more active than their pyranic counterparts. Due to the already described lytic effect upon bloodstream trypomastigotes of *T. cruzi*,[15] new derivatives were prepared and assayed against the parasite.

The substituted arylamino quinones and substituted naphthoquinones appear as interesting new prototype compounds and experiments are currently underway in

Table 2. Activity of the compounds against Y strain trypomastigote forms of *T. cruzi*

Compounds	IC_{50}/24 h[a] (μM)
1	410.8 ± 53.2[b]
2	1281.0 ± 167.0[b]
3	>4800[b]
4	—[c]
5	>4800[b]
6	—
7	212.3 ± 10.6
8	—
9	>4000
10	179.3 ± 12.0
11	>4000
12	1756.1 ± 91.8
13	332.8 ± 23.3
14	140.8 ± 11.9
15	86.3 ± 4.6
16	>4000
17	384.4 ± 52.5
18	952.5 ± 71.1
19	857.3 ± 96.4
20	88.2 ± 6.7
21	2517.9 ± 169.8
22	342.0 ± 42.8
Benznidazole	103.6 ± 0.6

[a] Mean ± SD of at least three independent experiments.
[b] Ref. 8.
[c] In situ preparation.

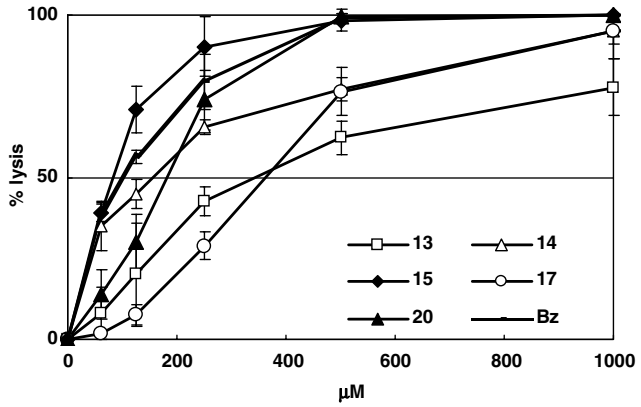


Figure 4. Graphic representation of the effect of the most active arylamines naphthoquinones and of benznidazole against bloodstream trypomastigotes of *Trypanosoma cruzi*.

our laboratories to investigate their mode of action, especially free-radicals generation, possibly opening new perspectives to the development of more potent and selective trypanocidal drugs.

4. Experimental protocols

4.1. General procedures

Melting points were obtained on a Reichert micro hot-stage and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica-gel (Acros Organics 0.035–0.070 mm, pore diameter ca 6 nm). Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrometer. ^{1}H and ^{13}C NMR were recorded at room temperature using a Varian Unity Plus 300 instrument, in the solvents indicated, with TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. electron-impact mass spectra (70 eV) were obtained using a MAT8500 instrument and a VG Autospec apparatus (Micromass, Manchester, UK). The main fragments were described as a relation between atomic mass units and the charge (m/z) and the relative abundance in percentage of the base-peak intensity.

4.2. Synthesis of 3-azido-2,2-dimethyl-2,3-dihydro-naphtho[2,3-*b*]furan-4,9-dione (10)

3-Bromo-nor-α-lapachone (**4**) (307 mg, 1 mmol) in dichloromethane (25 mL), was treated with an excess of sodium azide (195 mg, 3 mmol) at room temperature. The reactional system was stirred for 24 h, filtered and purified by column chromatography over silica-gel, using as eluent a gradient mixture of hexane/ethyl acetate (9/1 to 7/3) with increasing polarity. Compound **10** was obtained as an orange solid (123 mg, 0.45 mmol, 45% yield, m.p. 115–116 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.16–8.10 (2H, m), 7.80–7.69 (2H, m), 4.84 (1H, s), 1.63 (3H, s), 1.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.1 (C=O), 159.9 (C=O), 134.5 (CH), 133.2 (CH), 132.6 (C_0), 131.5 (C_0), 126.5 (CH), 126.2 (CH), 120.7 (C_0), 93.9 (C_0), 68.2 (CH), 29.5 (CH_2), 26.9 (CH_3), 21.7 (CH_3); IR ν_{max}(cm^{-1}, KBr) 2977–2851 (CH_2, CH_3), 1654 (C=O), 1602 (C=O), (2105) N_3; EI HRMS (m/z) 269.08000 Calcd for $C_{14}H_{11}N_3O_3$: 269.08004; (m/z) (%) 227.0 (100.00), 199.0 (17.00), 104.0 (14.10), 76.0 (11.20).

4.3. Synthesis of 2,2-dimethyl-3-hydro-4-oxanaphtho[2,3-*b*]furane-9-spiro-2′-oxyrane (9)

The naphthoquinone **3** (228 mg, 1 mmol) and an ethereal solution of freshly prepared diazomethane (excess) were kept at 5°C for 48 h, and then the solvent was evaporated under reduced pressure and the residue obtained was purified by column chromatography over silica-gel, using as eluent a gradient mixture of hexane/ethyl acetate (9/1 to 7/3) with increasing polarity. Compound **9** was obtained as a yellow solid (145 mg, 0.6 mmol, 60% yield, m.p. 120–122 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.05–7.94 (2H, m), 7.74–7.71 (2H, m), 2.86 (1H, dd, J = 12.9, 0.9 Hz), 2.36 (1H, dd, J = 5.6, 0.9 Hz), 2.0–1.96 (2H, m), 1.43 (3H, s), 1.27 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 192.8 (C=O), 191.9 (C_0), 134.0 (CH), 133.9 (CH), 132.7 (C_0), 132.3 (C_0), 127.1 (CH), 94.4 (C_0), 75.6 (C_0), 47.3 (CH_2), 41.7 (CH_2), 41.4 (CH_2), 27.5 (CH_3), 27.2 (CH_3); IR ν_{max}(cm^{-1}, KBr) 2932–2969 (CH_2, CH_3), 1682 (C=O); EI HRMS (m/z) 242.09430. Calcd for $C_{15}H_{14}O_3$: 242.09429; (m/z) (%) 227.0 (100.00), 242.0 (47.00), 76.0 (35.10), 104.0 (29.00).

4.4. General procedures for preparation of 16–20

To a solution of nor-lapachol (**2**) (228 mg, 1 mmol) in 25 mL of chloroform, 2 mL of bromine (6 mg, 38 mmol)

was added. The bromo intermediate **6** (3-bromo-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione) precipitated immediately as an orange solid. Over this mixture, an excess of the appropriate arylamine was added and, then, the mixture was stirred for 30 min. After addition of 50 mL of water, the organic phase was separated and washed with 10% HCl (3 × 50 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The arylamino derivative was purified by column chromatography over silica-gel, using as eluent a gradient mixture of hexane/ethyl acetate (9/1 to 7/3) with increasing polarity.

4.4.1. 3-(3-Fluoro-phenylamino)-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (16). Using an excess of 3-fluoro-phenylamine, **16** was obtained as a red solid (253 mg, 0.75 mmol, 75% yield, m.p. 221–225 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10 (1H, ddd, J = 7.5, 1.5, 0.7 Hz), 7.74–7.61 (3H, m), 7.10 (1H, m), 6.42 (1H, m), 6.38 (1H, dd, J = 8.0, 2.1), 6.26 (1H, dt, J = 11.2, 2.4 Hz), 4.78 (1H, s), 1.68 (3H, s), 1.58 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 180.7 (C═O), 175.2 (C═O), 169.5 (C_0), 163.8 (d, J = 243 Hz), 148.9 (C_0), 134.5 (CH), 132.5 (CH), 131.1 (C_0), 130.3 (d, J = 16.0 Hz), 129.4 (CH), 127.2 (C_0), 114.7 (C_0), 124.9 (CH), 108 (d, J = 2.3 Hz), 104.5 (d, J = 23.8 Hz), 99.8 (d, J = 24.9 Hz), 96.5 (C_0), 61.5 (CH), 27.2 (CH_3), 21.6 (CH_3); IR ν_{max}(cm^{-1}, KBr) 3364 (R_2NH), 1641 (C═O), 1611 (C═O); EI HRMS (*m*/*z*) 337.11140. Calcd for $C_{20}H_{16}FNO_3$: 337.11142; (*m*/*z*) (%) 227.0 (100.00), 337.0 (15.00), 199.0 (10.10).

4.4.2. 3-(4-Chloro-phenylamino)-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (17). Using an excess of 4-chloro-phenylamine, **17** was obtained as a red solid (317 mg, 0.9 mmol, 90% yield, m.p. 210–214 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.1 (H_6 or H_9, ddd, J = 7.9, 2.2, 0.7 Hz), 7.72–7.60 (H_6 or H_9; H_7 and H_8, m), 7.13 ($H_{3'}$ and $H_{5'}$, dd, J = 6.7, 2.1 Hz), 6.5 ($H_{2'}$ and $H_{6'}$, dd, J = 6.7, 2.1 Hz), 4.75 (H_3, d, J = 5.7 Hz), 1.66 (H_{10} or H_{11}, s), 1.56 (H_{10} or H_{11}, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 180.8 (C═O), 175.3 (C═O), 169.6 (C_0), 145.8 (C_0), 134.6 (CH), 132.6 (CH), 131.1 (C_0), 129.5 (CH), 129.1 (CH), 127.2 (C_0), 125.0 (CH), 122.6 (C_0), 114.6 (C_0), 114.1 (CH), 96.6 (C_0), 61.7 (CH), 27.3 (CH_3), 21.7 (CH_3); IR ν_{max} (cm^{-1}, KBr) 3365 (R_2NH), 1689 (C═O), 1647 (C═O); MS (70 eV, *m*/*z*) (%) 39.05 (4.10), 41.05 (4.62), 43.05 (7.02), 50.05 (3.17), 51.05 (3.15), 75.15 (6.05), 76.10 (6.77), 77.10 (5.37), 102.15 (4.06), 110.95 (4.08), 126.85 (7.32), 152.10 (6.06), 171.10 (5.81), 199.10 (12.48), 209.20 (6.73), 227.15 (100.00), 228.25 (15.41).

4.4.3. 3-(4-Bromo-phenylamino)-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (18). Using an excess of 4-bromo-phenylamine, **18** was obtained as a red solid (270 mg, 0.67 mmol, 67% yield, m.p. 207–210 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25 ($H_{3'}$ and $H_{5'}$, dd, J = 6.7, 2.1 Hz), 6.46 ($H_{2'}$ and $H_{6'}$, dd, J = 6.7, 2.1 Hz), 7.72–7.60 (H_6 or H_9; H_7 and H_8, m), 8.1 (H_6 or H_9, ddd, J = 7.9, 2.2, 0.7 Hz), 4.75 (H_3, d, J = 5.7 Hz), 1.66 (H_{10} or H_{11}, s), 1.56 (H_{10} or H_{11}, s); NMR (75 MHz, $CDCl_3$) δ: 181.3 (C═O), 175.6 (C═O), 169.9 (C_0), 146.5 (C_0), 131.4 (C_0), 127.5 (C_0), 115.1 (C_0), 110.0 (C_0), 96.9 (C_0), 134.8 (CH), 132.8 (CH), 132.2 (CH), 129.7 (CH), 125.3 (CH), 114.9 (CH), 61.9 (CH), 27.6 (CH_3), 21.9 (CH_3); IR ν_{max}(cm^{-1}, KBr) 3365 (R_2NH), 1689 (C═O), 1646 (C═O); MS (70 eV, *m*/*z*) (%) 43.05 (6.58), 75.10 (4.53), 76.10 (8.78), 77.10 (5.21), 104.10 (4.14), 128.10 (7.04), 142.95 (4.33), 156.95 (5.82), 171.00 (6.44), 199.20 (10.96), 209.20 (6.31), 227.15 (100.00), 228.20 (17.60), 397.05 (4.47).

4.4.4. 3-(4-Nitro-phenylamino)-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (19). Using an excess of 4-nitro-phenylamine, **19** was obtained as a red solid (254 mg, 0.70 mmol, 70% yield, m.p. 248–250 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.03 ($H_{3'}$and $H_{5'}$, d, J = 9.0 Hz), 6.78 ($H_{2'}$ and $H_{6'}$, d, J = 9.0 Hz), 7.84–7.71 (H_6 or H_9; H_7 and H_8, m), 7.42 (H_6 or H_9, d, J = 9.0 Hz), 4.96 (H_3, d, J = 8.3 Hz), 1.65 (H_{10} or H_{11}, s), 1.45 (H_{10} or H_{11}, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 180.7 (C═O), 175.0 (C═O), 168.4 (C_0), 154.0 (C_0), 136.6 (C_0), 131.2 (C_0), 127.2 (C_0), 114.8 (C_0), 95.8 (C_0), 133.0 (CH), 129.0 (CH), 135.2 (CH), 126.5 (CH), 124.8 (CH), 112,5 (CH), 59.4 (CH), 26.8 (CH_3), 21.5 (CH_3); IR ν_{max}(cm^{-1}, KBr) 3343 (R_2NH), 1644 (C═O), 1599 (C═O); MS (70 eV, *m*/*z*) (%) 39.00 (5.13), 43.05 (7.68), 50.05 (4.34), 76.10 (10.16), 77.10 (5.83), 104.10 (4.18), 114.95 (5.35), 128.10 (7.89), 171.15 (4.43), 199.20 (10.14), 203.20 (4.53), 209.20 (5.45), 227.20 (100.00), 228.25 (15.72), 364.20 (4.86).

4.4.5. 3-(4-Methoxy-phenylamino)-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (20). Using an excess of 4-methoxy-phenylamine, **20** was obtained as a brown solid (218 mg, 0.6 mmol, 60% yield, m.p. 190–194 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.48 ($H_{3'}$ and $H_{5'}$, d, J = 9.0 Hz), 6.54 ($H_{2'}$ and $H_{6'}$, d, J = 8.3 Hz), 7.73–7.60 (H_6 or H_9; H_7 and H_8, m), 7.42 (H_6 or H_9, dd, J = 8.1, 1.3 Hz), 4.71 (H_3, s), 1.65 (H_{10} or H_{11}, s), 1.59 (H_{10} or H_{11}, s), 3.74 (CH_3, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 180.9 (C═O), 175.4 (C═O), 168.4 (C_0), 152.3 (C_0), 141.5 (C_0), 131.3 (C_0), 127.4 (C_0), 115.4 (C_0), 96.8 (C_0), 134.5 (CH), 132.4 (CH), 129.4 (CH), 125.0 (CH), 114.8 (CH), 114,3 (CH), 55.7 (CH), 62.5 (CH_3), 27.3 (CH_3), 21.7 (CH_3); IR ν_{max}(cm^{-1}, KBr) 3357 (R_2NH), 1693 (C═O), 1641 (C═O) MS (70 eV, *m*/*z*) (%) 39.05 (4.95), 41.05 (6.23), 43.05 (9.49), 76.10 (6.45), 77.10 (8.85), 104.05 (4.24), 108.10 (8.58), 122.10 (4.80), 123.10 (6.17), 128.05 (9.06), 153.10 (5.10), 157.10 (5.37), 171.05 (7.01), 199.15 (15.28), 209.10 (11.03), 227.10 (100.00), 228.20 (17.13), 306.15 (0.53),349.25 (21.04).

4.5. 2,2-Dimethyl-4-phenylamino-3,4-dihydro-2H-benzo[*g*]chromene-5,10-dione (22)

The bromo derivative **8** (307 mg, 1 mmol) obtained from α-lapachone (**5**)[20] was dissolved in 5 mL of aniline (5.1 g, 58 mmol) and the mixture was left under stirring for 30 min. After addition of 50 mL of water, the organic phase was extracted with dichloromethane, washed with 10% HCl (3 × 50 mL), dried over sodium

sulfate filtered, concentrated under reduced pressure and was purified by column chromatography over silica-gel, using as eluent a gradient mixture of hexane/ethyl acetate (9/1 to 7/3) with increasing polarity. Compound **22** was obtained as a brown solid (233 mg, 0.7 mmol, 70% yield, m.p. 175–178 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.31 (1H, dd, J = 14.2, 4.1 Hz), 2.06 (1H, dd, J = 14.4, 5.6 Hz), 4.72 (1H, dd, J = 5.6, 4.3 Hz), 7.28–7.23 (2H, m), 6.89–6.81 (3H, m), 8.13-8.07 (2H, m), 7.77–7.68 (2H, m), 1.55 (3H, s), 1.50 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 183.4 (C═O), 179.8 (C═O), 155.1 (C_0), 146.6 (C_0), 134.1 (C_0), 132.9 (CH), 132.0 (C_0), 130.7 (C_0), 129.2 (CH), 126.2 (CH), 126.0 (CH), 118.8 (C_0), 118.3 (CH), 113.8 (CH), 79.0 (C_0), 43.3 (CH), 37.4 (CH_2), 18.6 (CH_3), 26.1 (CH_3); IR ν_{max}(cm^{-1}, KBr) 3381 (R_2NH), 1686 (C═O), 1607 (C═O); EI HRMS (*m*/*z*) 333.13650. Calcd for $C_{15}H_{14}O_3$: 333.13649; (*m*/*z*) (%) 333.0 (100.00), 241.0 (53.20), 223.0 (50.00), 93.0 (41.50).

5. Trypanocidal activity

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 up to 0.5%, DMSO has no deleterious effect on the parasites. Bloodstream trypomastigotes from the Y strain[29] were obtained at the peak of parasitaemia from infected albino mice, isolated by differential centrifugation and resuspended in Dulbecco's modified Eagle's medium (DMEM) to a parasite concentration of 10^7 cells/mL in the presence of 10% of mouse blood. This suspension (100 μL) was added in the same volume of each compound previously prepared at twice the desired final concentrations. Cell counts were performed in Neubauer chamber and the trypanocidal activity was expressed as IC_{50}, corresponding to the concentration that leads to lysis of 50% of the parasites.

6. X-ray crystallographic analysis

X-ray data collection for compound **21** was accomplished on an Enraf-Nonius KappaCCD area-detector diffractometer The programs used in crystallographic study were: Data collection was made using the COLLECT program.[30] Integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs.[31] The structure of **21** was solved by direct methods with SHELXS-97.[32] The models were refined by full-matrix least squares on F^2 with SHELXL-97.[32] The programs SHELXL-97 and ORTEP-3[33] were used within WinGX[34] to prepare materials for publication. Crystallographic data for compound **21** have been deposited with the Cambridge Crystallographic Data Center as Supplementary Publication No. CCDC 667698. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CH21EZ, UK (fax: +44 1223 336 033 or e-mail: deposit@ccdc.cam.ac.uk).

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References and notes

1. Moncayo, A.; Ortiz-Yanine, M. I. *Ann. Trop. Med. Parasitol.* **2006**, *100*, 663–677.
2. Schmunis, G. A. *Mem. Inst. Oswaldo Cruz* **2007**, *102*(Suppl. I), 75–85.
3. Teixeira, A. R.; Nitz, N.; Guimaro, M. C.; Gomes, C.; Santos-Buch, C. A. *Postgrad. Med. J.* **2006**, *82*, 788–798.
4. Coura, J. R. *Mem. Inst. Oswaldo Cruz* **2007**, *102*(Suppl. I), 113–122.
5. Coura, J. R.; De Castro, S. L. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 3–24.
6. Jannin, J.; Villa, L. *Mem. Inst. Oswaldo Cruz* **2007**, *102*(Suppl. I), 95–97.
7. Pinto, A. V.; Menna-Barreto, R. F. S.; De Castro, S. L. In *Recent Progress in Medicinal Plants*; Govil, J. N., Ed.; Phytomedicines; Studium Press: Houston, Texas, 2006; Vol. 16, pp 112–127.
8. (a) Viegas, C., Jr.; Danuello, A. C.; Bolzani, V. S.; Barreiro, E. J.; Fraga, C. A. M. *Curr. Med. Chem.* **2007**, *14*, 1829–1852;; (b) Pinto, A. V.; Neves-Pinto, C.; Pinto, M. C. F. R.; Santa-Rita, R. M.; Pezzella, C.; De Castro, S. L. *Arzneim-Forsch* **1997**, *47*, 74–79.
9. Neves-Pinto, C.; Dantas, A. P.; Moura, K. C. G.; Emery, F. S.; Polequevitch, P. F.; Pinto, M. C. F. R.; De Castro, S. L.; Pinto, A. V. *Arzneim-Forsch* **2000**, *50 II*, 1120–1128.
10. Neves-Pinto, C.; Malta, V. R.; Pinto, M. C. F. R.; Santos, R. H.; De Castro, S. L.; Pinto, A. V. *J. Med. Chem.* **2002**, *45*, 2112–2115.
11. Moura, K. C.; Emery, F. S.; Neves-Pinto, C.; Pinto, M. C. F. R.; Dantas, A. P.; Salomão, K.; De Castro, S. L.; Pinto, A. V. *J. Braz. Chem. Soc.* **2001**, *12*, 325–338.
12. Moura, K. C.; Salomão, K.; Menna-Barreto, R. F. S.; Emery, F. S.; Pinto, M. C. F. R.; Pinto, A. V.; De Castro, S. L. *Eur. J. Med. Chem.* **2004**, *39*, 639–645.
13. Menna-Barreto, R. F. S.; Henriques-Pons, A.; Pinto, A. V.; Morgado-Diaz, J. A.; Soares, M. J.; De Castro, S. L. *J. Antimicrob. Chemother.* **2005**, *56*, 1034–1041.
14. Menna-Barreto, R. F. S.; Corrêa, J. R.; Pinto, A. V.; Soares, M. J.; De Castro, S. L. *Parasitol. Res.* **2007**, *101*, 895–905.
15. Silva, R. S. F.; Costa, E. M.; Trindade, U. L. T.; Teixeira, D. V.; Pinto, M. C. F. R.; Santos, G. L.; Malta, V. R. S.; De Simone, C. A.; Pinto, A. V.; De Castro, S. L. *Eur. J. Med. Chem.* **2006**, *41*, 526–530.
16. Ferreira, V. F.; Jorqueira, A.; Souza, A. M. T.; Silva, M. N.; Souza, M. C. B. V.; Gouvêia, R. M.; Rodrigues, C. R.; Pinto, A. V.; Castro, H. C.; Santos, D. O.; Araújo, H. P.; Bourguignon, S. C. *Bioorg. Med. Chem.* **2006**, *14*, 5459–5466.
17. Jorqueira, A.; Gouvêa, R. M.; Ferreira, V. F.; da Silva, M. N.; de Souza, M. C. B. V.; Zuma, A. A.; Cavalcanti, D. F. B.; Araújo, H. P.; Santos, D. O.; Bourguignon, S. C. *Parasitol. Res.* **2006**, *99*, 429–433.
18. da Silva Júnior, E. N.; Souza, M. C. B. V.; Pinto, A. V.; Pinto, M. C. F. R.; Ferreira, V. F.; Menna-Barreto, R. F. S.; Silva, R. S. F.; Teixeira, D. V.; de Simone, C. A.; De

Castro, S. L. *Eur. J. Med. Chem.* **2007**. doi:10.1016/j.ejmech.2007.10.015, Oct 22 Epub ahead of print.
19. Fieser, L. F.; Fieser, M. *J. Am. Chem. Soc.* **1948**, *70*, 3215–3222.
20. Pinto, A. V.; Pinto, M. C. F. R.; de Oliveira, C. G. T. *An. Acad. Bras. Cienc.* **1982**, *54*, 107–114.
21. Pinto, A. V.; Pinto, M. C. R.; Gilbert, B.; Pellegrino, J.; Mello, R. T. *Trans. R. Soc. Trop. Med. Hyg.* **1977**, *71*, 133–135.
22. Ferreira, V. F.; Pinto, A. V.; Pinto, M. C. R. F.; Silva, M. N.; Skakle, J. M. S.; Souza, M. C. B. V.; Wardell, S. M. S. V. *Acta Crystallogr.* **2002**, *C58*, 560–562.
23. Silva, M. N.; de Souza, M. C. B. V.; Ferreira, V. F.; Pinto, A. V.; Pinto, M. C. R. F.; Wardell, S. M. S. V.; Wardell, J. L. *Arkivoc* **2003**, *10*, 156.
24. da Silva Júnior, E. N.; Souza, M. C. B. V.; Pinto, A. V.; Pinto, M. C. F. R.; Goulart, M. O. F.; Barros, F. W. A.; Pessoa, C.; Costa-Lotufo, L.; Montenegro, R. C.; Moraes, M. O.; Ferreira, V. F. *Bioorg. Med. Chem.* **2007**, *15*, 7035–7041.
25. Allen, F. H. *Acta Cryst.* **2002**, *B58*, 380–388.
26. Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354–1358.
27. Pereira, E. M.; Machado, T. B.; Leal, I. C. R.; Jesus, D. M.; Damaso, C. R. A.; Pinto, A. V.; Giambiagi-deMarval, M.; Kuste, R. M.; dos Santos, K. R. N. *Ann. Clin. Microbiol. Antimicrob.* **2006**, *5*, 1–7.
28. Arenas, P. *J. Ethnopharmacol.* **1987**, *21*, 279–295.
29. Silva, L. H. P.; Nussenszweig, V. *Folia Clin. Biol.* **1953**, *20*, 191–208.
30. Enraf-Nonius, COLLECT. Nonius BV, Delft, The Netherlands, 2000.
31. Sheldrick, G. M. *SHELXS-97 and SHELXL-97*; Univ. of Göttingen: Germany, 1997.
32. Farrugia, L. J. J. *App. Cryst.* **1997**, *30*, 565–566.
33. Farrugia, L. J. J. *App. Cryst.* **1999**, *32*, 837–838.
34. Otwinowski, Z.; Minor, W. H. K. L. In *Methods in Enzymology*; Carter, C. W., Sweet, R. M., Eds.; Academic Press: New York, 1997; pp 307–326.