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Short communication

Synthesis of naphthofuranquinones with activity against *Trypanosoma cruzi*

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Abstract

Four new naphthofuranquinones, obtained from 2-hydroxy-3-allyl-naphthoquinone (**1**) and nor-lapachol (**2**), have their structures established by physical and X-ray analysis and their activity evaluated against *Trypanosoma cruzi*. Compounds **3** and **4** were obtained by addition of iodine to **1** followed by cyclization generating a furan ring. Compound **5** was obtained through the acid-catalyzed reaction by dissolution of **1** in sulfuric acid. Compound **6** was synthesized by addition of bromine and aniline to **2**. The IC_{50}/24 h for **3**–**6** in assays with *T. cruzi* trypomastigotes was between 157 and 640 μM, while the value for crystal violet was 536.0 ± 3.0 μM. Compounds **3**–**5** also inhibited epimastigote proliferation. The trypanocidal activity of the new naphthofuranquinones endowed with redox properties reinforces a rational approach in the chemotherapy of Chagas' disease.

Keywords: *Trypanosoma cruzi*; Chagas' disease; Naphthoquinones; β-Lapachone; Naphthoimidazoles; Chemotherapy

1. Introduction

The flagellate protozoan *Trypanosoma cruzi* is the causative agent of Chagas' disease, which affects about 16–18 million people in Latin America [1,2]. At present, the only available therapeutic agent for treatment of this disease is the nitroheterocycle benznidazole, which presents severe side effects and is not effective for chronic phase patients. To prevent transmission of the disease through blood transfusion, the World Health Organization recommended the use of crystal violet in blood banks in endemic areas [3], what is generally not well accepted by the assisted population. In this context, an intense research program has been focused upon the search for alternative drugs to both benznidazole and crystal violet [4]. As part of a program on the chemistry of naturally occurring quinones, we have assayed a series of naphthoquinones isolated from the Brazilian flora and their semi-synthetic derivatives against *T. cruzi*. The most active among the 60 assayed compounds were naphthoimidazoles with an aromatic group linked to the imidazole ring [5–8]. In the present work, we prepared new naphthofuranquinones from 2-hydroxy-3-allyl-naphthoquinone (**1**) and nor-lapachol (**2**) and assayed their activity against *T. cruzi*.

2. Chemistry

2-Hydroxy-3-allyl-1,4-naphthoquinone (**1**, C-allyl lawsone) was prepared by alkylation with allyl bromide of the sodium salt of 2-hydroxy-1,4-naphthoquinone (lawsone). 2-Hydroxy-3-(2′-methyl-1-propenyl)-1,4-naphthoquinone (**2**, nor-lapachol) was obtained from lapachol by Hooker oxidation and through its cyclization reaction, using sulfuric acid (Scheme 1). Naphthoquinones **3** and **4** were synthesized by the reaction of

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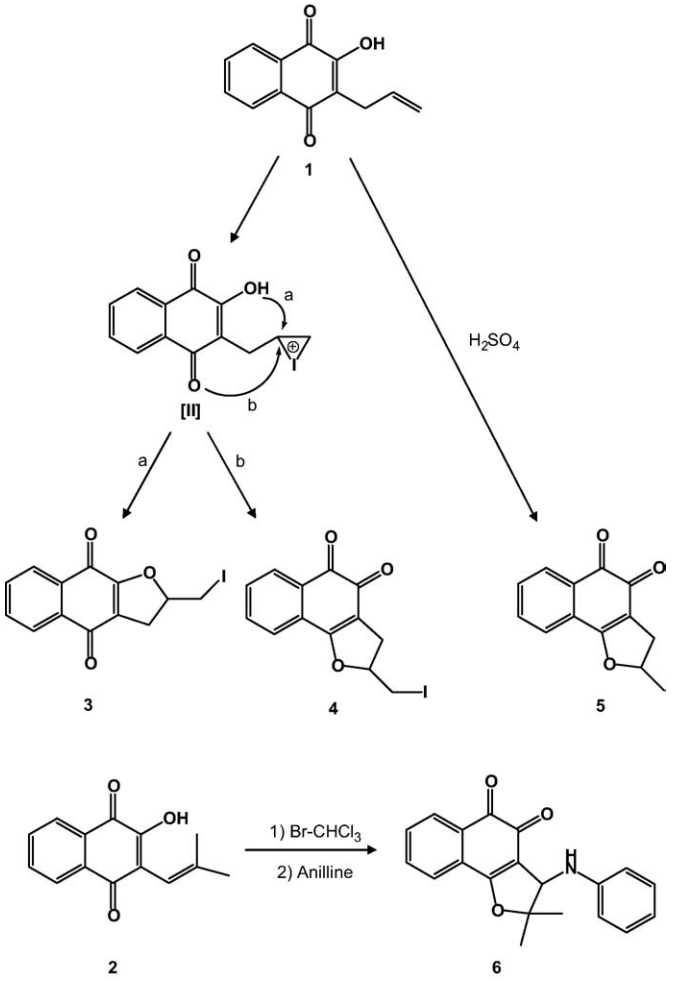

Scheme 1. Structure of the naphtoquinones obtained from 2-hydroxy-3-allyl-1,4-naphthoquinone (**1**) and 2-hydroxy-3-(2′-methyl-1-propenyl)-1,4-naphthoquinone (**2**).

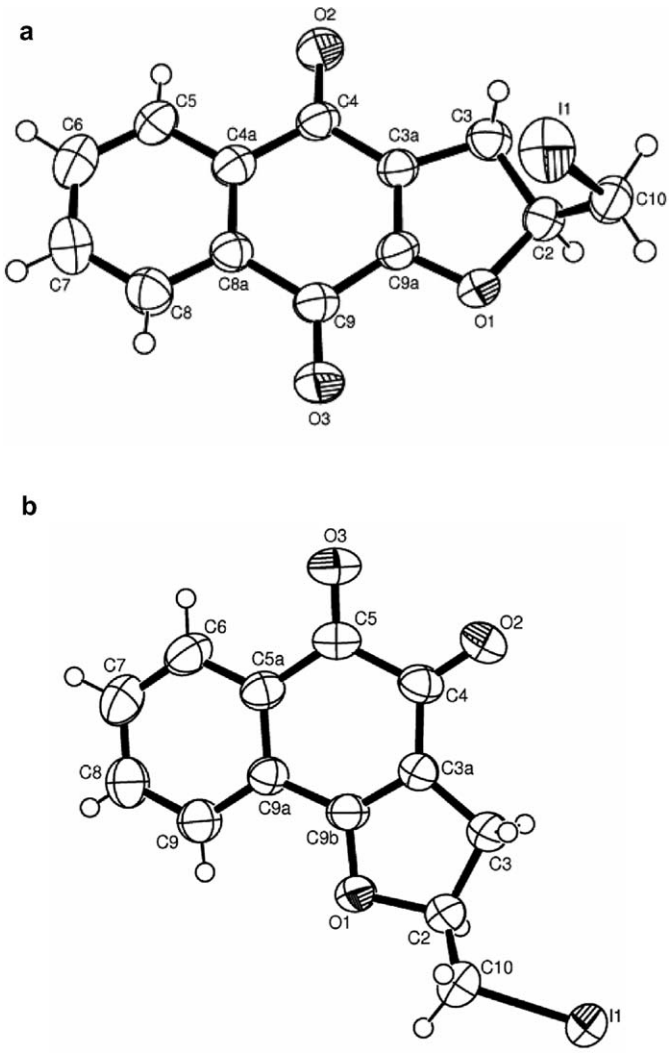

Fig. 1. ORTEP projections of the iodinated naphthoquinones: (a) **3**; (b) **4**.

1 with metallic iodine dissolved in a mixture of dichloromethane and pyridine, and **5** by dissolution of **1** in sulfuric acid. Compound **6** was prepared from **2** by addition of bromine followed by aniline. The physical and spectroscopic data for the synthesized compounds are in agreement with the structures depicted in Scheme 1.

Crystal data, data collection procedures, structure determination methods and refinement results for the iodinated compounds **3** and **4** are summarized in Table 1. The programs used were: graphic presentation: ORTEP3 for Windows [9]; material to publication: WinGX Routine [10].

The structures of the iodinated compounds **3** and **4** were solved by direct methods and refined by full-matrix least-squares calculations. Compound **3** crystallizes in the monoclinic system, space group $P2_1/c$, with one independent molecule in asymmetric unit ($Z = 4$), while compound **4** crystallizes in the orthorhombic system, space group *Cmca*, with one independent molecule in asymmetric unit ($Z = 8$). All H atoms were located by geometric considerations. In the final difference Fourier map there are no peaks greater than 0.555 Å^3 for **3** and 0.721 Å^3 for **4**. Bond lengths and angles are in good agreement, within experimental accuracy, with the values found in the literature [11]. The ORTEP representations of the compounds are shown in Fig. 1. Complete crystallographic data have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 278381 for compound **3** and no. CCDC 278380 for compound **4**. Copies of available material can be obtained, free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CH21EZ, UK (fax: +44 1223 336 033 or e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

3. Results and discussion

In folk medicine, plants containing naphthoquinones have been employed for the treatment of different diseases [12]. The molecular structure of naphthoquinones endow them with redox properties that could interfere in different biological oxidative processes [13]. Although there are several reports on the biological activity of naphthopyranquinones, such as β-lapachone, data in the literature about naphthofuranic analogues are scarce. The present work describes three unpublished compounds of our screening program. Compounds **3**, a naphtho

Table 1
Crystal data and structure refinement of the iodinated naphthoquinones

	Compound **3**	Compound **4**
Empirical formula	$C_{13}H_9O_3I$	$C_{13}H_9O_3I$
Formula weight (g mol^{-1})	340.10	340.10
Temperature (K)	293(2)	210(2)
Wavelength (Å)	0.71070	0.71070
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1/c$	*Cmca*
Unit cell dimensions	a = 7.7600(4) Å	a = 6.7500(2) Å
	b = 8.4330(2), β = 112.456(2)°	b = 19.0970(7) Å
	c = 19.2930(9) Å	c = 18.3250(7) Å
Volume (Å^3)	1166.80(9)	2362.18(14)
Z	4	8
Density (calculated) (Mg m^{-3})	1.936	1.963
Absorption coefficient (mm^{-1})	2.737	2.707
F(000)	656	1336
Crystal size (mm)	0.083 × 0.212 × 0.174	0.1200 × 0.1225 × 0.0345
Theta range for data collection (°)	2.3–27.5	2.22–27.5
Index ranges	$10 \le h \le 10$, $10 \le k \le 10$, $24 \le l \le 24$	$8 \le h \le 8$, $24 \le k \le 24$, $23 \le l \le 23$
Reflections collected	4902	2592
Independent reflections	2660 [R(int) = 0.0201]	1457 [R(int) = 0.0120]
Absorption correction	Multiscan	Multiscan
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	1.096	1.099
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.036, wR_2 = 0.0825	R_1 = 0.0481, wR_2 = 0.1325

[2,3-*b*]furan quinone and **4**, a naphtho[1,2-*b*]furan quinone were obtained by electrophylic addition of iodine to the lateral double bond of **1** followed by cyclization generating a furan ring [14]. Formation of the naphthopyrane ring in **3** and **4** could follow two routes, oxygen attack either on the less substituted carbon, CH_2, giving rise to a six-membered ring or on the more substituted carbon, CH, forming a five-membered ring. The exclusive formation of the latter indicates that on the transition state entropic factors prevail over esteric ones [15]. The quinone **5** was obtained through the acid-catalyzed reaction of ring formation by the dissolution of **1** in sulfuric acid. The quinone **6** was synthesized by addition of bromine to **2** in chloroform, followed by aniline, aiming the evaluation of the presence of a nitrogen group linked to a furane in the trypanocidal activity. Naphthoquinones **3**, **4** and **6** are described here for the first time in the literature and, although the synthesis of **5** has been described by Fieser [14], we found no report about its biological activity. In vitro assays with bloodstream forms of *T. cruzi* resulted in to IC_{50}/24 h values for **3**–**6** in the range of 157–640 μM, while the corresponding value for crystal violet, the standard compound, was 536 ± 3 μM (Table 2). The quinone **3** showed the highest activity among the assayed naphthoquinones and comparison between **4** and **5** revealed that the presence of an iodine atom led to a 1.6-fold increase in the trypanocidal activity. Similar increase in the activity against *T. cruzi* has been reported in studies with culture epimastigote forms treated with bromine furanquinones [16]. The quinones **3**–**5** also inhibited epimastigote proliferation (Table 3).

A point related to the derivatives of **1** is the analysis of the iodinated compounds, which shows that the compound with an α-ring (**3**) is more active than the counterpart with a β-ring (**4**). This is an unexpected result since *ortho*-quinones, such as β-lapachone, are known to exhibit higher trypanocidal activity than the corresponding *para* isomers, such as α-lapachone [5,17]. The aminated compound **6** was 6.4-times more active against trypomastigote forms than the original quinone **2**. The high trypanocidal activity of **6** and the lack of previous register in the literature on amino naphthofuran quinones stimulate the synthesis of compounds with aminated groups linked to the furane ring.

In a previous report of this series we observed that **1**, lapachol and β-lapachone displayed similar activities against *T. cruzi*, which were higher than that of crystal violet, whereas nor-β-lapachone, α-lapachone, and lawsone were inactive [5]. These results suggest that minor structural features such as an

Table 2
Values of IC_{50} expressed in μM, for the activity of the naphthoquinones on bloodstream trypomastigote forms of *T. cruzi*

#	IC_{50}/1 day
1	352 ± 29[a]
2	1281 ± 167[b]
3	158 ± 9
4	398 ± 56
5	641 ± 38
6	199 ± 19
CV[c]	536 ± 3

[a] Mean ± standard deviation for four independent experiments.
[b] Ref. [5].
[c] CV, crystal violet.

Table 3
Values of IC_{50}, expressed in μM, for the activity of naphthoquinones on culture epimastigote forms of *T. cruzi*

#	IC_{50}/1 day	IC_{50}/2 days	IC_{50}/3 days	IC_{50}/4 days
3	7.9 ± 1.3[a]	3.7 ± 0.3	3.0 ± 0.7	2.6 ± 0.3
4	24.9 ± 1.8	21.8 ± 2.4	19.5 ± 2.4	18.3 ± 4.9
5	13.2 ± 2.2	12.4 ± 1.4	11.7 ± 1.5	12.7 ± 2.0

[a] Mean ± standard deviation for three independent experiments.

increase in lipophilicity and the presence of an aliphatic side chain lead to higher activity, possibly associated with a better penetration of the compound through the plasma membrane of the parasite [7]. Similar relationships were observed when these compounds were studied aiming the prophylaxis of schistosomiasis [18].

The biological activity of new naphthoquinones points to the synthesis of new analogues endowed with redox properties, reinforcing a rational approach in the development of drugs active against Chagas' disease. Due to the broad spectrum of activity of these compounds and their easy obtention, assays involving other pathogenic trypanosomatids could be also performed.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in a capillary Thomas Hoover apparatus (Thomas Co., Philadelphia, PA, USA) and are uncorrected. ^{1}H- and ^{13}C-NMR were recorded at room temperature using a Varian Gemini 200 (Varian, Palo Alto, CA, USA) and Bruker 200 MHz spectrophotometer (Bruker-Franzen Analytic, Bremen, Germany), in the solvents indicated, with TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. The mass spectra were obtained at 70 eV in a VG Autospec apparatus (Micromass, Manchester, UK). The 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. Elemental analysis was performed in a Perkin–Elmer CHN 2400 analyzer (Perkin–Elmer Inc., Wellesley, MA, USA).

4.2. Synthesis of 2-iodomethyl-2,3-dihydro-naphtho[2,3-b] furan-4,9-dione (3) and 2-iodomethyl-2,3-dihydro-naphtho [1,2-b]furan-4,9-dione (4)

2-Hydroxy-3-allyl-1,4-naphthoquinone (**1**) (250 mg, 1.18 mmol) in dichloromethane (20 ml) was treated at room temperature with a solution of metallic iodine (1.21 g, 3.34 mmol) dissolved in a mixture of 30 ml dichloromethane plus 4 ml pyridine. The reactional system was stirred for 1 h at room temperature, followed by addition of 100 ml cold water. The lower organic phase was washed with 10% sodium carbonate (3 × 50 ml), followed by cold water (3 × 50 ml). After drying on sodium sulfate, the solvent was evaporated under vacuum. The residue was submitted to column chromatography over silica gel and the quinones **3** and **4** were eluted with a mixture of hexane/ethyl acetate at the ratios of 90:10 and 75:25, respectively. Compound **3** was obtained as yellow crystals (48.14 mg, 12% yield, m.p. 136–138 °C), and **4** as orange red crystals (102.3 mg 25.5% yield, m.p. 145–147 °C).

4.2.1. Compound 3

^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.1 (m, 2H), 7.7 (m, 2H), 5.1 (m, 1H), 3.4 (m, 3H), 3.1 (dd, 1H); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 184.71 (C=O), 179.85 (C=O), 159.28 (C), 134.1 (CH), 133.16 (CH), 132.17 (C), 131.77 (C), 130.94 (C), 126.51 (CH), 125.45 (CH), 83.76 (CH), 33.77 (CH_2), 7.21 (CH_2I); MS (70 eV, m/z) (%) 340 (24), 312 (50), 213 (32), 185 (100), 157 (56), 143 (18), 129 (73), 127 (65), 104 (22), 76 (84). Anal. calcd. for $C_{13}H_9IO_3$: C 45.91, H 2.67, I 37.31, O 14.11. Found: C 45.2, H 3.1.

4.2.2. Compound 4

^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.1 (d, 1H), 7.6 (m, 3H), 5.1 (m, 1H), 3.5 (d, 2H), 3.3 (dd, 1H), 3.0 (dd, 1H); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 181.61 (C=O), 175.12 (C=O), 150.05 (C), 134.52 (CH), 131.99 (CH), 130.4 (C), 129.43 (CH), 126.99 (C), 124.47 (CH), 114.71 (C), 85.7 (CH), 32.8 (CH_2), 7.28 (CH_2I); MS (70 eV, m/z) (%) 340 (24), 312 (50), 213 (32), 185 (100), 157 (56), 143 (18), 129 (73), 127 (65), 104 (22), 76 (84). Anal. calcd. for $C_{13}H_9IO_3$: C 45.91, H 2.67, I 37.31, O 14.11. Found: C 45.2, H 3.1.

4.3. Synthesis of 2-methyl-2,3-dihydro-naphtho[1,2-b]furan-4,9-dione (5)

2-Hydroxy-3-allyl-1,4-naphthoquinone (**1**) (214 mg, 1 mmol) was dissolved in 2 ml of a concentrated acid sulfuric solution, and after 10 min, 80 g ice was added. After melting of the ice, the reactional mixture was extracted with ethyl acetate (3 × 50 ml). The organic fraction was collected, dried over sodium sulfate, filtered and evaporated under vacuum. The residue consisted of compound **5** obtained as red micro crystals (203.5 mg 95% yield, m.p. 133 °C).

^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.1 (t, 1H), 7.6 (m, 3H), 5.25 (m, 1H), 3.3 (dd, 1H), 2.75 (dd, 1H), 1.6 (d, 3H); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 181.01 (C=O), 175.25 (C=O), 169.06 (C), 134.7 (CH), 131.74 (CH), 130.4 (C), 129.11 (CH), 127.23 (C), 124.34 (CH), 115.5 (C), 85.5 (CH), 33.2 (CH_2), 21.8 (CH_3). Anal. calcd. for $C_{13}H_{10}O_3$: C 72.89, H 4.71, O 22.41. Found: C 71.9, H 4.8.

4.4. Synthesis of 2,2-dimethyl-3-phenylamino-2,3-dihydro-naphtho[1,2-b]furan-4,5-dione (6)

2-Hydroxy-3-(2′-methyl-1-propenyl)-1,4-naphthoquinone (**2**) (242 mg, 1 mmol) was dissolved in 25 ml of chloroform, followed by addition of 2 ml bromine (26.22 mg, 38 mmol), and after about 10 s, an orange precipitate was formed. Addition of 5 ml aniline (2.1 g, 58 mmol) to the reactional mixture was followed by 50 ml water. The organic fraction was washed with 10% HCl (3 × 50 ml) and dried over sodium sulfate, filtered and evaporated under vacuum. The residue consisted of compound **6** obtained as an amorphous solid, which purity was checked by thin layer chromatography (300.1 mg, 94% yield, m.p. 126 °C).

^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.1 (d, 1H), 7.7 (m, 3H), 7.2 (m, 3H), 6.9 (t, 1H), 6.8 (t, 1H), 4.9 (s, 1H), 4.0 (bs, 1H change with D_2O), 1.55 (CH_3), 1.45 (CH_3); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 180.78 (C=O), 175.21 (C=O), 169.48 (C),

147.13 (C), 134.48 (CH), 132.37 (CH), 130.95 (C), 129.34 (2CH), 129.16 (2CH), 127.23 (C), 124.97 (CH), 117.92 (CH), 115.03 (C), 112.91 (CH), 96.74 (C), 61.41 (CH), 27.2 (CH_3), 21.61 (CH_3). Anal. calcd. for $C_{20}H_{17}NO_3$. C 79.47, H 6.03, N 4.41, O 10.08. Found: C 78.8, H 6.1.

4.5. Trypanocidal assay

Stock solutions of the napthoquinones were prepared in dimethyl sulfoxide (DMSO), with the final concentration of the solvent in the experiments never exceeding 0.5%. The experiments were performed with the Y strain of *T. cruzi* [19]. Bloodstream trypomastigotes were obtained at the peak of parasitaemia from infected albino mice, isolated by differential centrifugation and resuspended in Dulbecco's modified Eagle medium (DMEM) to a parasite concentration of 10^7 cells per ml in the presence of 10% of blood. Untreated and crystal violet-treated parasites were used as controls [5]. Epimastigote forms maintained axenically at 28 °C with weekly transfers in LIT medium were harvested during the exponential phase of growth and resuspended in LIT (10^7 cells per ml). This suspension (500 μl) was added to the same volume of a solution of each quinone, previously prepared at twice the desired concentration in LIT in 24-well plates and then incubated at 28 °C for 4 days. The activities of the compounds were expressed as IC_{50} values, corresponding to the concentration that causes lysis of 50% of the parasites.

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