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Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 12 (2004) 2451–2458

Synthesis of dihydronaphthofurandiones and dihydrofuroquinolinediones with trypanocidal activity and analysis of their stereoelectronic properties

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Received 23 October 2003; accepted 29 January 2004

Abstract—The synthesis of dihydronaphthofurandione and dihydrofuroquinolinedione derivatives **4**–**11** was performed through Diels–Alder reactions of dihydrobenzofurandione **1** with several carbodienes and acrolein *N*,*N*-dimethylhydrazone. Then, the use of 5-bromobenzofurandione **2** toward 1,3-pentadiene and the 1-azadiene afforded quinones **6** and **11** with a total regioselectivity.

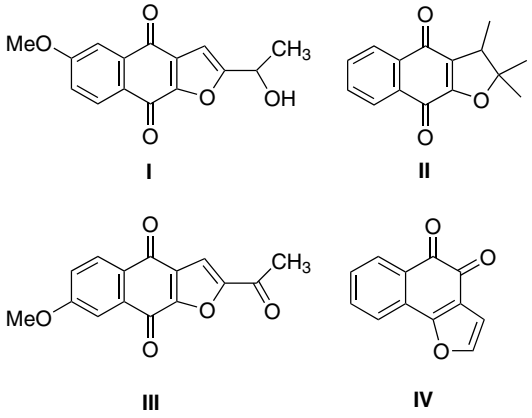
All the prepared quinones were tested for trypanocidal activity in vitro against *Trypanosoma* epimastigotes, Tulahuen strain. Among the tested compounds, the furoquinolinediones **10** and **11** have shown potent trypanocidal activities but, only the 1,5-regioisomer (**11**) was found active as a redox cycling agent. Calculation of their stereoelectronic properties by the density-functional theory method provided a new insight for the trypanocidal activity of these heterocyclic quinones.

1. Introduction

Chagas disease, or American trypanosomiasis, is a serious health problem in several Latin–American countries, where over 20 million people are already infected with *Trypanosoma cruzi*, the protozoan parasite that cause this disease. Mortality indices range from 8% to 12% depending on patient's age and physiological state.[1]

Two nitro heterocyclic drugs, nifurtimox (tetrahydro-3-methyl-4-[{5-nitrofurfurylidene}amino-2*H*-1,4-thiazine 1,1-dioxide) and benznidazole (*N*-benzyl-2-nitroimidazole-1-acetamide), have been used to treat this disease, but the serious side effects produced might force to stop treatment. Furthermore, important differences in susceptibility to these drugs have been detected among the many different parasites isolated.[2] Nifurtimox is no longer being used in some countries because of its toxicity and ineffectiveness in chronic stages of Chagas disease. Due to the inadequacy of the available chemotherapeutic regimens, hundred of chemical compounds—both natural and synthetic—have been tested as anti-chagasic agents.[3]

For many years, the anti-protozoal activity of naphthoquinones have been known.[4] Various naphthofurandiones such as compounds **I**–**IV** (Scheme 1), as well as some sulfur analogs,[5,6] have been tested against *T. cruzi* parasites. Most of them showed an inhibitory effect on



Scheme 1. Examples of natural or synthetic naphthofurandiones displaying trypanocidal activity.

Keywords: Naphthofurandiones; Furoquinolinediones; Density-functional theory; *Trypanosoma cruzi*.
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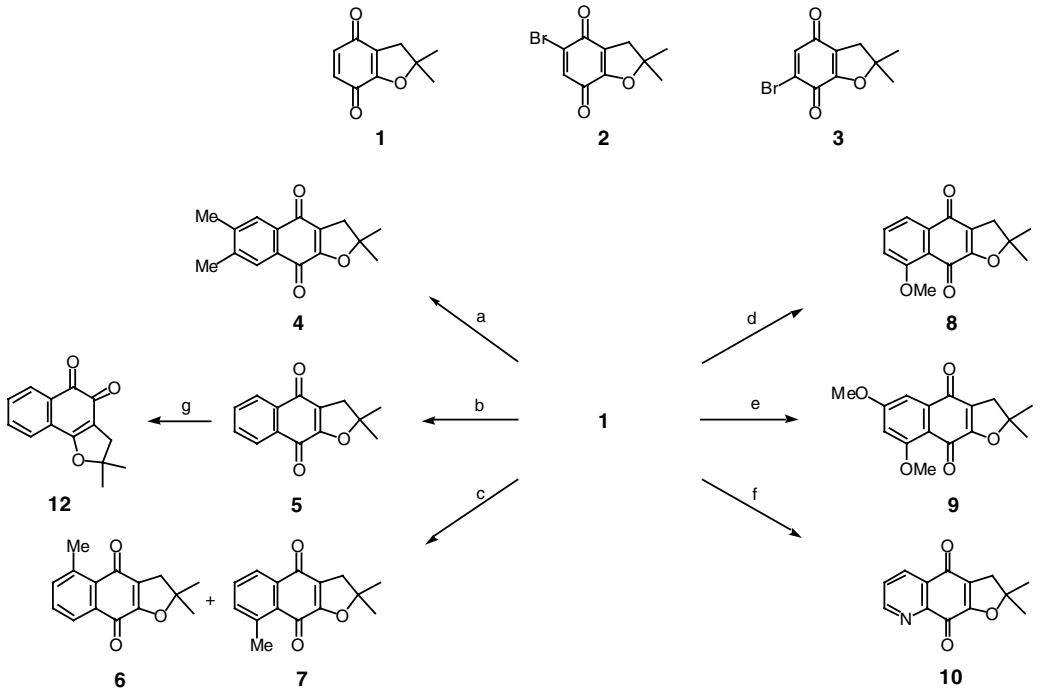
doi:10.1016/j.bmc.2004.01.046



Scheme 2. Reagents and conditions: (a) i. 2,3-dimethyl-1,3-butadiene, C_6H_6, reflux, 15 h; ii. DDQ, reflux, 4 h, 78%; (b) i. 1-trimethylsilyloxy-1,3-butadiene, CH_2Cl_2, rt, 3 days; ii. SiO_2, air, rt, 24 h, 73%; (c) i. 1,3-pentadiene, CH_2Cl_2, reflux, 18 h; ii. SiO_2, air, rt, 24 h; iii. DDQ, reflux, 4 h, 96%; (d) i. 1-methoxy-1,3-cyclohexadiene, MeOH, rt, 1 h; ii. NaH, THF, 0 °C, 30 min; iii. Ag_2O, THF, rt, 2 h; iv. xylene, reflux, 3 h, 40%; (e) i. 1,3-dimethoxy-1-trimethylsilyloxy-1,3-butadiene, CH_2Cl_2, rt, 1 h; ii. SiO_2, air, rt, 2 h; iii. MeI, Ag_2O, rt, 2 h, 80%; (f) i. acrolein *N*,*N*-dimethylhydrazone, CH_2Cl_2, rt, 2 h; ii. SiO_2, air, rt, 10 min; iii. Ag_2O, rt, 5 h, 65%; (g) H_2SO_4, 0 °C, 20 min, 88%.

culture growth and on the parasite respiration.[7,8] The anti-trypanosomal activity has been attributed to free radical production by redox cycling of the quinone system. A correlation between the trypanocidal activity and the redox potential of naphthofurandiones has been investigated and no linear relationship was found. However, the angular naphthofurandiones (*o*-quinones), which are easier to reduce than linear isomers (*p*-quinones) showed higher trypanocidal activity than the latter, suggesting a contribution of the easiness of reduction on the biological activity.[9]

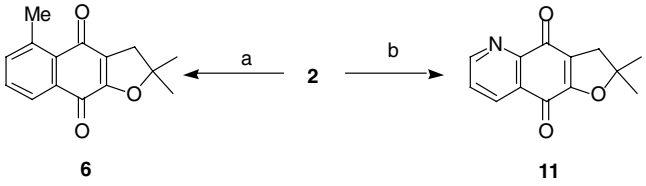
Interestingly calculation of electron affinities for *p*-benzoquinones using Hartree–Fock/density-functional (HF/DF) methods recently reported[10] represent a new challenge to correlate electronic properties of quinones with trypanocidal activity. Thus, we decided to prepare some naphthofurandiones in order to determine whether their trypanocidal activity could be correlated with their electronic properties in an attempt to understand this biological activity and to contribute to the design of more effective trypanocidal drugs.

2. Results and discussion

2.1. Chemistry

In the present study, new dihydronaphthofurandiones and dihydrofuroquinolinediones were synthesized through a Diels-Alder reaction of 2,3-dihydrobenzo[*b*]furan-4,7-dione **1**[11] with 1,3-dienes (Scheme 2). First, reaction of quinone **1** with 2,3-dimethyl-1,3-butadiene in refluxing benzene for 15 h and aromatization of the cycloadduct by treatment with silica gel and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave naphthofurandione **4** in 78% yield. Cycloaddition reaction of **1** with 1-trimethylsilyloxy-1,3-butadiene for 3 days at room temperature and purification of the adduct by column chromatography gave 73% yield of naphthofurandione **5**. Next, use of unsymmetrical dienes was considered. Thus, reaction of quinone **1** with 1,3-pentadiene in refluxing dichloromethane for 18 h and aromatization of the crude adduct by treatment with silica gel and DDQ gave a mixture of the aromatized quinones **6** and **7** in 1:9 ratio (96%). The regioisomers **6** and **7** were separated by column chromatography with dichloromethane–hexane (4:1) as the eluent. Then, treatment of quinone **1** with 1-methoxy-1,3-cyclohexadiene in methanol for 1 h at room temperature followed by enolization, oxidation, and thermal aromatization gave naphthofurandione **8** in 40% yield. Reaction of quinone **1** with 1,3-dimethoxy-1-trimethylsilyloxy-1,3-butadiene in dichloromethane for 1 h at room temperature, aromatization and methylation of the reaction mixture with methyl iodide and silver(I) oxide provided naphthofurandione **9** in 80% yield. Finally, addition of acrolein *N*,*N*-dimethylhydrazone[12] to quinone **1** gave 65% yield of the aromatized cycloadduct **10**.

In order to obtain better yields of the minor regioisomer **6** and the opposite regioisomer of **10** (**11**), we used the



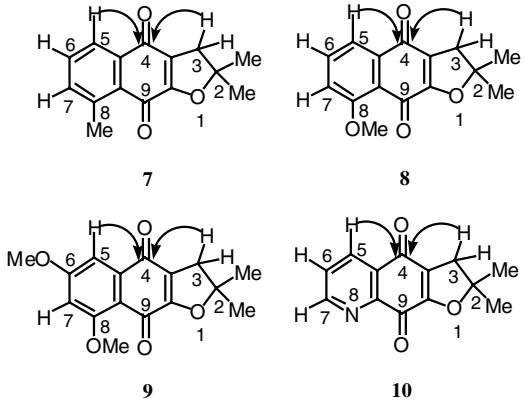
Scheme 3. Reagents and conditions: (a) i. 1,3-pentadiene, $NaHCO_3$, CH_2Cl_2, reflux, 18 h; ii. DBU, rt, 1 h, 77%; (b) i. acrolein *N*,*N*-dimethylhydrazone, $NaHCO_3$, CH_2Cl_2, rt, 2 h; ii. DBU, rt, 1 h, 95%.

blocking effect of the halogen atom. Indeed, it is well known that the presence of a chlorine or bromine atom on the dienophilic double bond of a quinone could orient the nucleophilic end of the diene to attack the unhalogenated carbon of such dienophiles.[13–15] Thus, bromoquinone **2**[11] was reacted separately with 1,3-pentadiene and acrolein *N*,*N*-dimethylhydrazone. The Diels–Alder reactions afford compounds **6** and **11** in 77% and 95% yield, respectively, and with a total regioselectivity (Scheme 3).

The regiochemical assignment for quinones **6**–**11** was established by 1D and 2D NMR studies, including heteronuclear multiple-bond correlations (HMBC). Compounds **7**–**10** showed long range ^3J couplings between C-4 and H-5 and H-3 (Scheme 4). Finally *p*-quinone **5** was converted to the *o*-isomer **12** (88%) by treatment with sulfuric acid (Scheme 2). All the above compounds and 6-bromobenzoquinone **3**[11] were subjected to preliminary screening for trypanocidal activity.

2.2. Biological evaluation

The in vitro trypanocidal activity of benzofurandione **1**, 5-bromobenzofurandione **2**,[11] 6-bromobenzofurandione **3**,[11] and tricyclic quinones **4**–**12** was evaluated against Tulahuén strain at 10 and 5 μM concentrations, as described earlier (Table 1).[16] The IC_{k_c50} for compounds with inhibition activity higher than 80% at 5 μM concentrations, was then determined in order to establish their relative efficacy in vitro compared to the standard drugs nifurtimox and benznidazole (Table 2). The quinones **2**, **3**, **10**, **11**, and **12**, with IC_{k_c50} between 1 and 4 μM, had more potent trypanocidal activity than nifurtimox and benznidazole. The introduction of a bromine atom in quinone **1** contributes substantially to the trypanocidal activity of bromobenzofurandiones **2** and **3**, indicating that the presence of the halogen atom in the molecule is valuable. In the group of tricyclic quinones, the most active compounds are **10** and **11**, showing that the presence of a pyridine instead of a benzene ring in the structure increases the trypanocidal activity. Also the relative position of N and O atoms in the molecule is important.



Scheme 4. Selected 2D ^1H–^{13}C HMBC correlations for **7**–**10**.

Table 1. Culture growth inhibition, *I* (%) of compounds **1**–**12** upon *T. cruzi* epimastigotes

Compound	*I* (%)	
	10 μM	5 μM
1	56.5	38.9
2	96.2	83.5
3	98.7	97.1
4	100.0	44.5
5	93.0	49.8
6	61.2	32.1
7	95.0	65.1
8	49.8	22.6
9	28.3	21.7
10	100.0	98.6
11	95.5	91.7
12	98.5	91.1

The anti-trypanosomal activity of quinones has been attributed to oxygen radicals formation and consequently strong oxidative stress. To evaluate this possibility, oxygen uptake experiments with or without cyanide, were undertaken. Respiration is a composite value where oxygen uptake is performed by mitochondrial reactions as well as by other extra mitochondrial reactions such as redox cycling. Thus the cyanide addition inhibits the oxygen consumption in the respiratory chain and allows seeing more clearly a possible redox cycling induced by the drug. Table 2 shows that compounds **11** and **12**, like nifurtimox, produce an increase of oxygen uptake indicating a significant redox cycling, while bromoquinones **2** and **3** and benznidazole inhibit overall oxygen uptake. On the other hand compound **10**, which is highly active in culture growth, produces neither overall respiration inhibition nor redox cycling.

Table 2. Effect of compounds **2**, **3**, **10**–**12** upon culture growth and oxygen uptake in *T. cruzi* epimastigotes

Compound	IC_{k_c50} (μM)	Respiration[a]	Oxygen redox cycling[a]
Control	—	22.6 ± 1.5	2.1 ± 0.5
Nifurtimox	9.9 ± 0.2	40.0 ± 0.8	4.0 ± 1.5
Benznidazole	11.4 ± 1.5	19.2 ± 0.7	1.8 ± 0.3
2	2.8 ± 1.5	14.2 ± 0.8	0.8 ± 0.1
3	2.4 ± 1.5	10.0 ± 0.5	1.2 ± 0.3
10	2.3 ± 1.5	22.6 ± 0.8	2.1 ± 0.4
11	1.1 ± 1.5	35.1 ± 1.1	3.3 ± 0.5
12	4.0 ± 1.5	53.7 ± 3.1	5.0 ± 0.5

[a] Respiration and oxygen uptake values are expressed as nanoatoms-gram of oxygen/min/mg protein.

2.3. Computational models

Density-functional theory (DFT) is now a useful tool to understand molecular structure, properties, bonding, dynamics, and reactivity. Recently chemical concepts like hardness (η), chemical potential (μ), and electrophilicity index (ω) have been successful to rationalize molecular reactivity and chemical bonding.[17–19] Maynard et al.[20] have already shown the usefulness of these DFT-based reactivity descriptors in the rationalization of the reactivity of complex systems such as the HIV-1 nucleocapsid protein p7. This result prompt us to attempt a characterization of the trypanocidal activity of heterocyclic quinones through the use of the above mentioned global reactivity descriptors.

Physically, μ represent the escaping tendency of electrons from the equilibrium state, formally it is the Lagrange multiplier associated to the normalization constraint of DFT that the electron density integrates to N, the total number of electrons. The link with classical structural chemistry is achieved by making $\mu = -\chi$, where χ is the well known electronegativity. On the other hand, η can be seen as a resistance to charge transfer. Chemical potential and hardness are derivatives of the energy with respect to N, a discrete variable, and in most numerical applications they are calculated using the finite difference approximation,[18]

$$\chi = -\mu = -(\partial E/\partial N)_{v(r)} \cong (\mathrm{IP} + \mathrm{EA})/2, \quad (1)$$

$$\eta = 1/2(\partial\mu/\partial N)_{v(r)} = 1/2(\partial^2 E/\partial N^2)_{v(r)} \cong (\mathrm{IP} - \mathrm{EA})/2, \quad (2)$$

where E is the electronic energy, N the number of electrons, and v the external potential. IP and EA are the ionization energy and the electron affinity, respectively.[17,21] Further approximations involving the use of the Koopman's theorem give access to χ and η in terms of the energies of frontier molecular orbitals HOMO and LUMO. The following approximate versions of χ and η have been widely used:

$$\chi \cong -1/2(\varepsilon_{\mathrm{HOMO}} + \varepsilon_{\mathrm{LUMO}}), \quad (3)$$

$$\eta \cong 1/2(\varepsilon_{\mathrm{HOMO}} - \varepsilon_{\mathrm{LUMO}}), \quad (4)$$

where $\varepsilon_{\mathrm{HOMO}}$ and $\varepsilon_{\mathrm{LUMO}}$ are the energy for the frontier orbitals. It has been widely shown that Koopman's theorem IPs are remarkably good, often between 0.3 eV of experimental data.[22] On the other hand, Koopman's theorem can formally be applied to obtain electron affinities, the result show a systematic underestimation of experimental EAs. Of course, the Koopman's theorem is not exactly correct and will fail when strong electronic relaxation and correlation effects occur. In the present case, since we are dealing with systems that are structurally related, the relaxation and correlation effects are expected to be about the same for all systems allowing the comparison of electronic properties obtained from the Koopman's theorem to give insights on the relative behavior of the systems.

Recently the electrophilicity power ω of an atom or molecule has been introduced by Parr et al.[19] through the simple expression (Eq. 5),

$$\omega = \mu^2/2\eta, \quad (5)$$

where μ and η are the already defined chemical potential and chemical hardness. The electrophilicity power ω measures the energy stabilization upon electronic saturation of the system when electrons flow from the surroundings with a higher chemical potential than that of the system.[23]

Considering that quinones are good electron acceptors we explored a possible relationship between the electron affinity (EA), electronegativity (χ), and electrophilicity (ω) of the structurally related quinones **4**–**11** with their trypanocidal activity. Hartree–Fock/6-31G (HF) and B3LYP/6-31G calculations have been performed using the GAUSSIAN 98 package of programs.[24] All molecular geometries have been optimized using the HF/6-31G level of theory, this gives a quite good average reproduction of bond distances when compared to the solid state X-ray diffraction structure of related substances.[25] It is well known that 6-31G basis set gives quite reliable structures and electronic properties of organic molecules.[26] Of course the use of polarization or diffuse functions should decreases the variational total energy, however since the systems under study are structurally related we expect the energy change due to enlargement of the basis set be almost completely systematic so it changes the relative energies and properties very little. An exhaustive study of adiabatic electron affinities of neutral hydroquinone radicals confirms the above statement.[27]

The electron affinity, absolute electronegativity, and electrophilicity index of neutral as well as ionic species for quinones of similar structures such as compounds **4**–**11** were calculated using HF and DFT methods. Table 3 shows the calculated values of electron affinity, absolute electronegativity (Eq. 3), and electrophilicity index (Eq. 5) for neutral species using B3LYP/6-31G method, which gave more consistent data.

Figures 1–3 show the plots of calculated electron affinity (EA), electronegativity (χ), and electrophilicity (ω) values versus in vitro trypanocidal activity of quinones **4**–**11**. Even though the observed correlations may not be

Table 3. Calculated electron affinity (EA), electronegativity (χ), and electrophilicity (ω) for quinones **4**–**11** (B3LYP/6-31G, values given in eV)

Compound	I (%)[a]	EA	χ	ω
4	44.5	2.807	4.600	5.902
5	49.8	2.957	4.754	6.290
6	32.1	2.884	4.486	6.094
7	65.1	2.861	4.675	6.025
8	22.6	2.889	4.700	6.099
9	21.7	2.705	4.495	5.643
10	98.6	3.133	4.920	6.774
11	91.7	3.159	4.919	6.878

[a] At 5 μM.

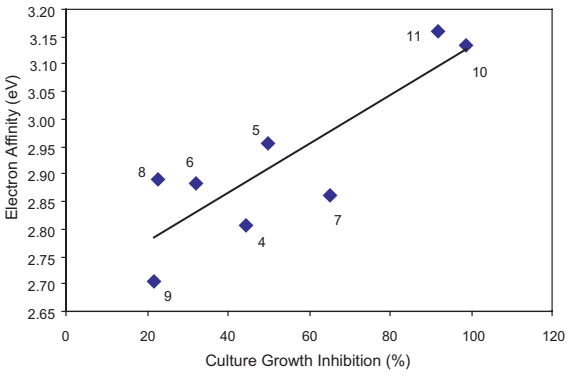


Figure 1.

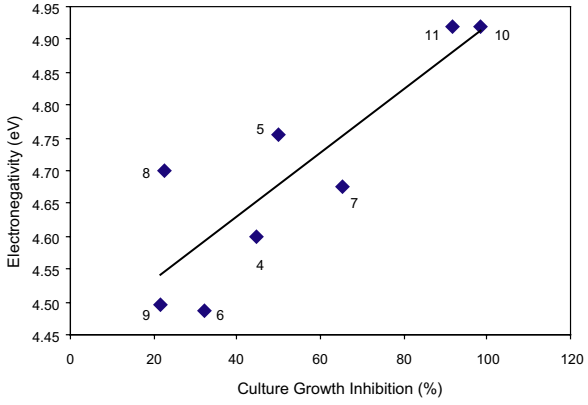


Figure 2.

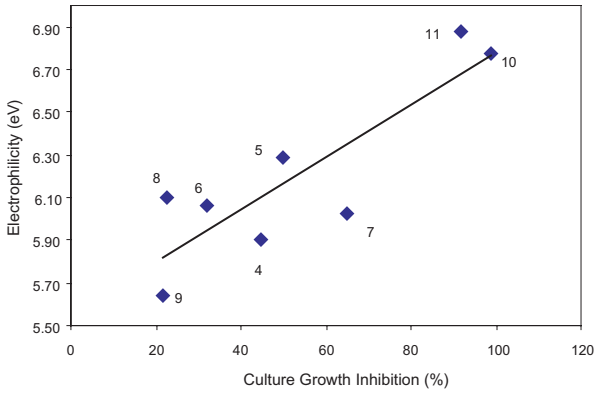


Figure 3.

conclusive (correlation coefficients are in the range 0.85–0.87), trends are quite consistent indicating that the greatest values of these parameters were obtained for the more active quinones **10** and **11**.

In conclusion, we have described new 2,3-dihydro-2,2-dimethylnaphtho[2,3-*b*]furan-4,9-diones and aza-analogs that were evaluated in vitro against *T. cruzi* epimastigotes, Tulahuen strains. Among the tested compounds, quinones **10** and **11** showed potent trypanocidal activities, and the latter is essentially active as a redox cycling agent, leading to oxidative stress. Although more examples and more sophisticated calculations are required for quantitative estimation of optimal values, the calculated electron affinity, electronegativity, and electrophilicity provides a new insight for the trypanocidal activity of some heterocyclic quinones. Our results show that trypanocidal activity is related to the capacity of the system to acquire electronic charge from the surroundings, thus indicating the validity of the use of EA, χ, and ω as reactivity descriptors.

3. Experimental

Melting points were measured with a Stuart Scientific SMP3 apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 22 spectrophotometer using KBr discs. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-200 instrument, using tetramethylsilane as internal reference. Column chromatography was performed on silica gel Merck 60 (70–230 mesh). Thin layer chromatography separations were performed on Merck Kieselgel 60 (70–230 mesh). Elemental analyses were carried out on a FISONS EA 1108 CHNS–O analyzer.

3.1. 2,3-Dihydro-2,2,6,7-tetramethylnaphtho[2,3-*b*]furan-4,9-dione (4)

To a solution of quinone **1**[11] (238 mg, 1.34 mmol) in benzene (10 mL) was added freshly distilled 2,3-dimethyl-1,3-butadiene (220 mg, 2.69 mmol) and the mixture was heated at reflux for 15 h. Silica gel (2.0 g) was added and the mixture was stirred for 24 h at room temperature. The suspension was filtered and to the filtrate was added DDQ (640 mg, 2.82 mmol) and the mixture was heated at reflux for 4 h. The reaction mixture was poured into a saturated sodium bicarbonate solution (50 mL) and extracted with dichloromethane (2×30 mL). The extracts were washed with water (2×15 mL) and dried (magnesium sulfate). After evaporation of the solvent the residue was purified by column chromatography on silica gel using dichloromethane as eluent to afford naphthofurandione **4** (257 mg, 78%), mp 170–171 °C (dec). IR (KBr) ν cm^{-1} 1675, 1645 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.58 (6H, s, 2×Me), 2.36 (3H, s, Me), 2.37 (3H, s, Me), 2.98 (2H, s, CH_2), 7.78 (1H, s, H-5), 7.81 (1H, s, H-8); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 20.0, 20.2, 28.4 (2C), 40.1, 91.8, 122.9, 127.2, 127.4, 129.5, 131.6, 142.3, 143.9, 158.9, 178.7, 183.0. Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.80; H, 6.25.

3.2. 2,3-Dihydro-2,2,-dimethylnaphtho[2,3-*b*]furan-4,9-dione (5)

To a solution of quinone **1**[11] (75 mg, 0.32 mmol) in dichloromethane (7.5 mL) was added 1-trimethylsilyloxy-1,3-butadiene (54 mg, 0.38 mmol) and the mixture was stirred for 3 days at room temperature. Silica gel (2.0 g) was added and the mixture was further stirred for 24 h at room temperature in the presence of air. The suspension was filtered and the solvent was removed at reduced

pressure to give naphthofurandione **5** (70 mg, 73%) after purification by column chromatography (dichloromethane), mp 186–188 °C (lit.[28] mp 188–190 °C). IR (KBr) ν cm^{-1} 1680, 1640 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.55 (6H, s, 2×Me), 2.96 (2H, s, CH_2), 7.60–7.70 (2H, m, H-6 and H-7), 7.90–8.00 (2H, m, H-5 and H-8); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 28.3 (2C), 40.0, 91.8, 123.4, 125.9, 126.2, 131.5, 132.8, 133.0, 134.1, 158.7, 178.2, 182.4.

3.3. 2,3-Dihydro-2,2,5-trimethylnaphtho[2,3-*b*]furan-4,9-dione (6) and 2,3-dihydro-2,2,8-trimethylnaphtho[2,3-*b*]furan-4,9-dione (7)

Method A. To a solution of quinone **1**[11] (200 mg, 1.12 mmol) in dichloromethane (10 mL) was added 1,3-pentadiene (116 mg, 1.70 mmol) and the mixture was heated at reflux for 18 h. Silica gel (2.0 g) was added and the mixture was stirred for 24 h at room temperature. The suspension was filtered and to the filtrate was added DDQ (640 mg, 2.82 mmol) and the mixture was heated at reflux for 4 h. The reaction mixture was poured into a saturated sodium bicarbonate solution (50 mL) and extracted with dichloromethane (2×30 mL). The extracts were washed with water (2×15 mL) and dried (magnesium sulfate). Removal of the solvent at reduced pressure afforded a mixture of the isomers **6** and **7** (260 mg, 96%). Separation of the regioisomers was performed by column chromatography on silica gel using dichloromethane–hexane as eluent. Compound **6** R_f 0.63 (dichloromethane–hexane, 4:1) (24.5 mg, 9%), mp 206–207 °C. IR (KBr) ν cm^{-1} 1675 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.63 (6H, s, 2×Me), 2.80 (3H, s, Me), 3.03 (2H, s, CH_2), 7.51–7.60 (2H, m, H-6 and H-7), 8.07 (1H, dd, $J = 2.9$ and 6.1 Hz, H-8); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 22.8, 28.4 (2C), 40.5, 91.6, 124.8, 125.4, 130.2, 131.9, 133.2, 138.9, 140.8, 157.4, 178.5, 185.3. Compound **7** R_f 0.72 (dichloromethane–hexane, 4:1) (220.5 mg, 81%), mp 152–153 °C. IR (KBr) ν cm^{-1} 1665, 1645 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.57 (6H, s, 2×Me), 2.73 (3H, s, Me), 2.98 (2H, s, CH_2), 7.40–7.58 (2H, m, H-6 and H-7), 7.99 (1H, dd, $J = 2.9$ and 6.1 Hz, H-8); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 22.8, 29.7 (2C), 39.9, 91.7, 121.5, 124.8, 128.9, 130.5, 133.2, 137.0, 141.7, 159.7, 180.1, 182.9. Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.05; H, 5.75.

Method B. To a mixture of quinone **2**[11] (110 mg, 0.39 mmol) in dichloromethane (15 mL) and $NaHCO_3$ (200 mg, 2.38 mmol) was added 1,3-pentadiene (40 mg, 0.59 mmol) and the mixture was heated at reflux for 18 h. DBU (0.5 mL) was added and the mixture was stirred for 1 h at room temperature. After removal of the solvent at reduced pressure the mixture was purified by column chromatography on silica gel using dichloromethane to give compound **6** (80 mg, 77%).

3.4. 2,3-Dihydro-8-methoxy-2,2-dimethylnaphtho[2,3-*b*]furan-4,9-dione (8)

To a solution of quinone **1**[11] (200 mg, 1.12 mmol) in methanol (10 mL), 1-methoxy-1,3-cyclohexadiene (130 mg, 1.18 mmol) was added and the mixture was stirred for 1 h at room temperature. After evaporation of the solvent the residue was passed through a short column chromatography on silica gel using dichloromethane as eluent. After evaporation of the solvent, the residue was dissolved in tetrahydrofuran (10 mL) and the solution was added to a suspension of 80% sodium hydride (80 mg, 2.7 mmol) in tetrahydrofuran (10 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C, and then quenched by the addition of a saturated ammonium chloride solution. The resulting mixture was diluted with ethyl acetate and washed with water followed by drying over magnesium sulfate. After evaporation of the solvent, the residue was dissolved in THF (10 mL) and then silver(I) oxide (200 mg, 0.86 mmol) and magnesium sulfate (200 mg, 1.66 mmol) were added. The suspension was stirred for 2 h, filtered through Celite and after removal of the solvent the residue was dissolved in xylene (20 mL) and the solution was heated under reflux for 3 h. After removal of the solvent, the residue was purified by column chromatography on silica gel using dichloromethane as eluent to afford naphthofurandione **8** (115 mg, 40%), mp 173–174 °C. IR (KBr) ν cm^{-1} 1660, 1630 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.56 (6H, s, 2×Me), 2.95 (2H, s, CH_2), 3.98 (3H, s, OMe), 7.22 (1H, dd, $J = 1.3$ and 8.2 Hz, H-7), 7.62 (1H, d, $J = 2.4$ Hz, H-6), 7.72 (1H, dd, $J = 1.3$ and 7.6 Hz, H-5); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 28.3 (2C), 39.3, 56.5, 91.5, 117.4, 118.8, 119.2, 120.8, 135.2, 135.7, 159.9, 160.2, 177.3, 182.1. Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.08; H, 5.50. Found: C, 69.26; H, 5.46.

3.5. 2,3-Dihydro-6,8-dimethoxy-2,2-dimethylnaphtho[2,3-*b*]furan-4,9-dione (9)

To a solution of quinone **1**[11] (100 mg, 0.56 mmol) in dichloromethane (15 mL) was added 1,3-dimethoxy-1-trimethylsilyloxy-1,3-butadiene (113 mg, 0.56 mmol) and the mixture was stirred for 1 h at room temperature. Silica gel (2.0 g) was added and the mixture was stirred for 2 h at room temperature. The suspension was filtered and to the filtrate was added methyl iodide (0.5 mL, 3.3 mmol) and Ag_2O (20 mg, 0.09 mmol) and the mixture was stirred for further 2 h. The suspension was filtered through Celite, and the solvent was removed at reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane as eluent to afford quinone **9** (130 mg, 80%), mp 222 °C (dec). IR (KBr) ν cm^{-1} 1660, 1630 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.62 (6H, s, 2×Me), 3.0 (2H, s, CH_2), 4.0 (6H, s, 2×OMe), 6.69 (1H, d, $J = 2.4$ Hz, H-7), 7.32 (1H, d, $J = 2.4$ Hz, H-5); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 28.3 (2C), 39.3, 56.0, 56.4, 91.8, 102.7, 104.1, 113.5, 120.0, 137.8, 160.5, 162.5, 165.1, 176.1, 181.8. Anal. Calcd for $C_{16}H_{16}O_4$: C, 66.66; H, 5.59. Found: C, 66.34; H, 5.54.

3.6. 2,3-Dihydro-2,2-dimethylfuro[3,2-*g*]quinoline-4,9-dione (10)

To a solution of quinone **1**[11] (100 mg, 0.56 mmol) in dichloromethane (15 mL) was added a solution of

acrolein *N*,*N*-dimethylhydrazone[10] (130 mg, 1.32 mmol) in dichloromethane (2.0 mL) and the mixture was stirred for 2 h at room temperature. Silica gel (1.5 g) was added and the mixture was stirred for 10 min at room temperature. After the addition of Ag_2O (500 mg, 2.16 mmol) the mixture was stirred for further 5 h. The suspension was filtered through Celite, and the solvent was removed at reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane–ethyl acetate (19:1) as eluent to afford quinone **10** (83.5 mg, 65%), mp 196–197 °C. IR (KBr) ν cm^{-1} 1695, 1640 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.61 (6H, s, 2×Me), 3.05 (2H, s, CH_2), 7.65 (1H, dd, $J = 4.8$ and 7.8 Hz, H-6), 8.41 (1H, dd, $J = 1.7$ and 7.8 Hz, H-5), 8.97 (1H, dd, $J = 1.7$ and 4.8 Hz, H-7); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 28.4 (2C), 39.9, 92.8, 123.4, 127.6, 130.1, 134.0, 147.6, 153.5, 159.5, 176.4, 181.0. Anal. Calcd for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 67.87; H, 4.63; N, 6.15.

3.7. 2,3-Dihydro-2,2-dimethylfuro[2,3-*g*]quinoline-4,9-dione (11)

To a mixture of bromoquinone **2**[11] (110 mg, 0.39 mmol) in dichloromethane (10 mL) and $NaHCO_3$ (200 mg, 2.38 mmol) was added acrolein *N*,*N*-dimethylhydrazone[12] (55 mg, 0.54 mmol) and the mixture was stirred at room temperature for 2 h. DBU (0.5 mL) was added and the mixture was stirred for 1 h at room temperature. After removal of the solvent at reduced pressure the mixture was purified by column chromatography on silica gel using dichloromethane–ethyl acetate (19:1) as eluent to give quinone **11** (93.5 mg, 95%), mp 229 °C (dec). IR (KBr) ν cm^{-1} 1680, 1650 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.59 (6H, s, 2×Me), 3.06 (2H, s, CH_2), 7.60 (1H, dd, $J = 4.8$ and 7.8 Hz, H-7), 8.38 (1H, dd, $J = 1.7$ and 7.8 Hz, H-8), 8.97 (1H, dd, $J = 1.7$ and 4.8 Hz, H-6); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 28.3 (2C), 40.1, 92.8, 124.6, 126.6, 128.3, 134.2, 148.9, 154.2, 158.6, 177.3, 180.5. Anal. Calcd for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 67.80; H, 5.09; N, 5.79.

3.8. 2,3-Dihydro-2,2-dimethylnaphtho[1,2-*b*]furan-4,5-dione (12)

A mixture of quinone **5** (50 mg, 0.22 mmol) in cold concentrated sulfuric acid (5 mL) was stirred for 20 min at 0 °C. The solution was poured into 60 mL of cold water and extracted with ethyl acetate. After removal of the solvent the product was purified by column chromatography on silica gel using dichloromethane–ethyl acetate (19:1) as eluent to give *o*-quinone **12** (44 mg, 88%), mp 188–189 °C (lit.[29] 187–188 °C). IR (KBr) ν cm^{-1} 1695, 1645 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.58 (6H, s, 2×Me), 2.92 (2H, s, CH_2), 7.50–7.60 (3H, m, H-7, H-8, and H-9), 8.00 (1H, m, H-6); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 28.4 (2C), 39.3, 93.7, 108.7, 115.0, 124.6, 127.9, 129.3, 130.8, 131.9, 134.5, 175.6, 181.3.

3.9. Parasites and culture growth inhibition

A suspension of 3×10^6 *T. cruzi* epimastigotes per milliliter, Tulahuen strain, were grown at 28 °C in monophasic diamond's culture medium supplemented with 4 μM of hemin. Inactivated bovine fetal calf serum at a final concentration of 4% was added.[30] Compounds **1**–**12** dissolved in dimethylsulfoxide (DMSO, 1% final concentration) were added to the culture media to give 100 to 0.5 μM final concentrations. Parasite growth was followed by nephelometry for 7–10 days.[31,32] No toxic effect of DMSO alone was observed. Values are expressed as mean ± SD for three independent experiments.

The growth culture constant (k_c) for each drug concentration employed was calculated using an exponential growth curve (regression coefficient >0.97, $p < 0.05$). This constant correspond to the slope resulting from plotting the natural logarithm (Ln) of nephelometry lecture versus time. IC_{k_c50} is the drug concentration needed to reduce the k_c in 50% and it is calculated by linear regression analysis from the k_c values and the concentrations used. Pearson's correlation and linear regression analysis were performed using Prism GraphPad software (GraphPad Software Inc.).

3.10. Oxygen uptake and redox cycling

T. cruzi epimastigotes, Tulahuen strain, were harvested by 500g centrifugation, followed by washing and re-suspension in 0.05 M sodium phosphate buffer, pH 7.4, containing 0.107 M sodium chloride. Respiration measurements were carried out polarographically with a Clark 5331 electrode (Yellow Springs Instruments) in a 53 YSI model (Simpson Electric Co.). The chamber volume was 2.0 mL and the temperature 28 °C. The amount of parasite used was equivalent to 2.0 mg of protein. In order to evaluate redox cycling, mitochondrial respiration was inhibited with 20 μM sodium cyanide. To maintain the parasite mass–drug ratio constant in these experiments, the original IC_{k_c50} was corrected by the parasite mass increase in the oxygen uptake experiments.[32]

Acknowledgements

We are grateful to FONDECYT (Research Grants 8980003, 2990098, 2010080, 1020095, 1020534, and 1020874) for financial support.

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