Synthesis and *in Vitro* Antiprotozoal Activity of Thiophene Ring-Containing Quinones

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A series of quinones (3a—i, 4—9, 11) and aromatic compounds (2a, 2d, 2g) containing the thiophene ring were tested *in vitro* against the trypomastigote form of *Trypanosoma cruzi* and the promastigote forms of *Leishmania*. The quinones 3a—i, 4, 5a, b, 6 and 9 having the thiophene ring fused to a quinone nucleus were the most active members of the series. The electron affinities of the benzo[*b*]thiophene-4,7-quinones 3, evaluated by their LUMO energies and halfwave potentials, are reported.

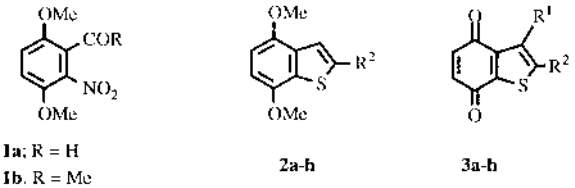
Key words benzo[*b*]thiophene-4,7-quinones; synthesis; biological activity; *Leishmania*; *Trypanosoma cruzi*; electron affinity

Leishmaniasis and Chagas' disease are common protozoal parasitic diseases in South America which cause considerable morbidity and mortality. Leishmaniasis is initiated by inoculation of *Leishmania* species into the skin *via* sand fly bites. Drugs currently available for treatment of Leishmaniasis are potentially toxic, inconvenient to administer and frequently give rise to clinical resistance.[1,2] The infection is classically treated with pentavalent antimony in the form of sodium stibogluconate (Pentostam®) or *N*-methylglucamine antimonate (Glucantime®) and with pentamidine or amphotericin B. Chagas' disease is a widespread infection in Latin America which currently infects 16 to 20 millions people leading to over 45000 deaths each year.[3] It is caused by *Trypanosoma cruzi* and is naturally transmitted by Reduviidae bugs. The chemotherapy of Chagas' disease is limited to the drugs benznidazole and nifurtimox. Both drugs are not very active and have severe side effects. The absence of new drugs to control Chagas' disease makes the search for active chemotherapeutic agents an urgent priority in parasitic research.[4]

The quinonoid compounds occupy a special place among the broad variety of natural and synthetic agents with antibacterial, antifungal, antiprotozoal, and antitumor activity.[5—8] Some of these pharmacological effects have been attributed to the formation of DNA-damaging anion–radical intermediates by bioreduction of the quinone system.[9] Among the diversity of quinones with cytotoxic activity, those having a thiophene nucleus fused to a quinone system have received relatively little attention[10,11] despite the antitumoral activity of thiophene analogues of daunomycin and mitoxantrone.[12,13]

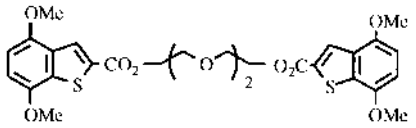
As part of a program involving the design and synthesis of polycyclic quinones with cytotoxic activity, we wish to describe here our results on the *in vitro* activity of a series of thiophene ring-containing compounds against the bloodstream forms of *T. cruzi* (Y strain) and the promastigote forms of three strains of *Leishmania*, *L. amazonensis* (LV-79), *L. braziliensis* (2903) and *L. donovani* (PP-75).

The benzo[*b*]thiophenes **2a**—**g** (Chat 1) were prepared from *o*-acylnitroarenes **1a**, **b** and methyl thioglycolate according to a recently reported method.[14] Compound **2h** was prepared in 75% yield by reaction of **2e** with excess triethylene glycol and *N*,*N*′-dicyclohexylcarbodiimide (DCC) as shown in Chart 2. Dimer **2i** was obtained in 72% yield by condensation of **2e** and **2h** with DCC. Benzo[*b*]thiophene-4,7-quinones **3a**—**i** were prepared by oxidative demethylation of the corresponding benzo[*b*]thiophenes **2a**—**i** with ceric ammonium nitrate (CAN) in acetonitrile–water solution following the procedure reported recently.[14]

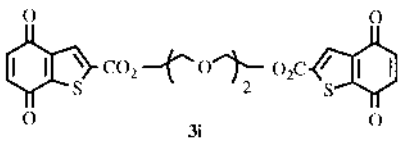


1a; R = H
1b, R = Me

2a-h **3a-h**



2i



3i

2,3	R¹	R²
a	H	H
b	H	CO_2Me
c	Me	CO_2Me
d	H	CH_2OH
e	H	CO_2H
f	H	COCl
g	H	COMorph
h	H	$CO_2(CH_2CH_2O)_3H$

Chart 1

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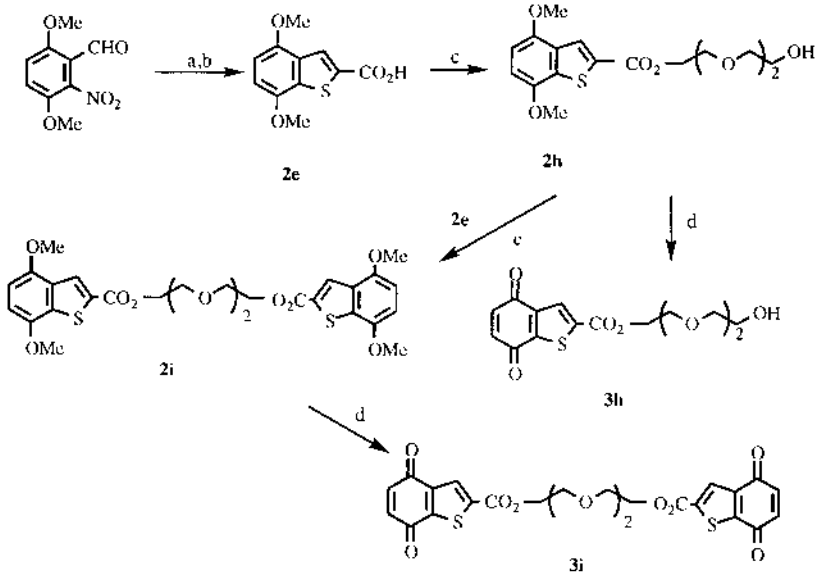


a: HS–CH_2–CO_2Me, K_2CO_3, DMF; b: KOH, MeOH; c: triethylene glycol, DCC, CH_2Cl_2; d: CAN, MeCN–H_2O

Chart 2

Table 1. *In Vitro* Activity of Benzo[*b*]thiophene-4,7-quinones against Three Strains of Promastigote Forms of *Leishmania* spp. (IC_{100} in μg/ml), Bloodstream Forms of *Trypanosoma cruzi* Percentage Reduction in Parasite Number at 250 μg/ml and Electron Affinities

Quinone	*T. cruzi*[a] (%)	*L. braziliensis*[b] 2903	*L. amazonensis*[b] LV-79	*L. donovani*[b] PP-75	E_{LUMO}	$E_{1/2}$ (V)
3a	85	25	25	25	−1.669793	−0.47
3b	91	10	10	10	−2.000877	−0.33
3c	82	5	5	5	−2.007004	−0.47
3d	41	100	100	100	−1.661554	−0.48
3e	62	>100	>100	>100	—	—
3g	74	10	10	10	−1.916452	−0.32
3h	14	10	10	10	−2.021383	—
3i	18	10	5	5	−2.089511	—

a) Gentian violet as the reference substance for *T. cruzi* (100% inhibition at 250 μg/ml). *b*) Pentamidine as the reference substance for *Leishmania* spp. (5 μg/ml for 100% inhibition).

Heterocyclic quinones **3** were evaluated *in vitro* against the trypomastigote forms of *T. cruzi* and the promastigote forms of *Leishmania*, and the results are given in Table 1. Considering that the antiprotozoal activity of quinones has been attributed to an oxidant stress mechanism,[15] we decided to investigate the effect of the oxidant capability of quinones **3** on the trypanocidal and leishmanicidal activity. To this end a variety of aromatic (**2a**, **2d**, **2g**) and quinone analogues (**4**—**9**, **11**) of compounds **3** (Chart 3) were submitted to screening and the results are summarized in Table 2.

Quinone **4** was prepared in 37% yield by reaction of dimethylamine and benzothiophenequinone **3b** at room temperature in dichloromethane solution, in air. The mixture of regioisomers **5a**, **b** was obtained by cycloaddition of quinone **3a** with 2-methylbuta-1,3-diene followed by air oxidation of the 50 : 50 mixture of the cycloadducts. Our attempts to separate regioisomers **5a** and **5b** by means of recrystallization and chromatography were unsuccessful.

Thienoquinolinquinones **6** and **9** were obtained by reaction of **3a** and **3c** with 1-dimethylamino-4-methyl-1-azabuta-1,3-diene and 1-dimethylamino-3-methyl-1-azabuta-1,3-diene, respectively, followed by air oxidation. Purification of the reaction mixtures by chromatography on silica gel afforded products **6** and **9** in 48 and 49% yield and no further attempts were made to increase the yields. The cycloaddition involved in the formation of compounds **6** and **9** occurs regiospecifically since no isomers of **6** and **9** were detected in the reaction mixture (TLC, ^{1}H-NMR).

Compounds **7** and **8** were obtained from **3b** and **3c** and buta-1,3-diene as reported.[16] Quinone **11** was prepared by cycloaddition of quinone **3c** with (*E*)-1-trimethylsilyloxybuta-1,3-diene followed by hydrolysis of the Diels–Alder adduct and oxidation of **10** with pyridinium chlorochromate (PCC). The ^{1}H-NMR spectrum of crude compound **11** displayed a weak singlet signal at δ 12.51 ppm attributed to the chelated protons of the regiosimer of quinone **11**, indicating high regiocontrol of the cycloaddition between the polarized diene and quinone **3c**.

The regiochemistry of the cycloaddition of quinones **3a** and **3c** with the 1-azadienes and (*E*)-1-trimethylsilyloxybuta-1,3-diene was established on the basis of HOMO–LUMO interactions. Figure 1 shows the LUMO coefficients of quinones **3a** and **3c**[17] and the reported HOMO coefficients of (*E*)-1-trimethylsilyloxybuta-1,3-diene[18] and the 1azadienes.[19]

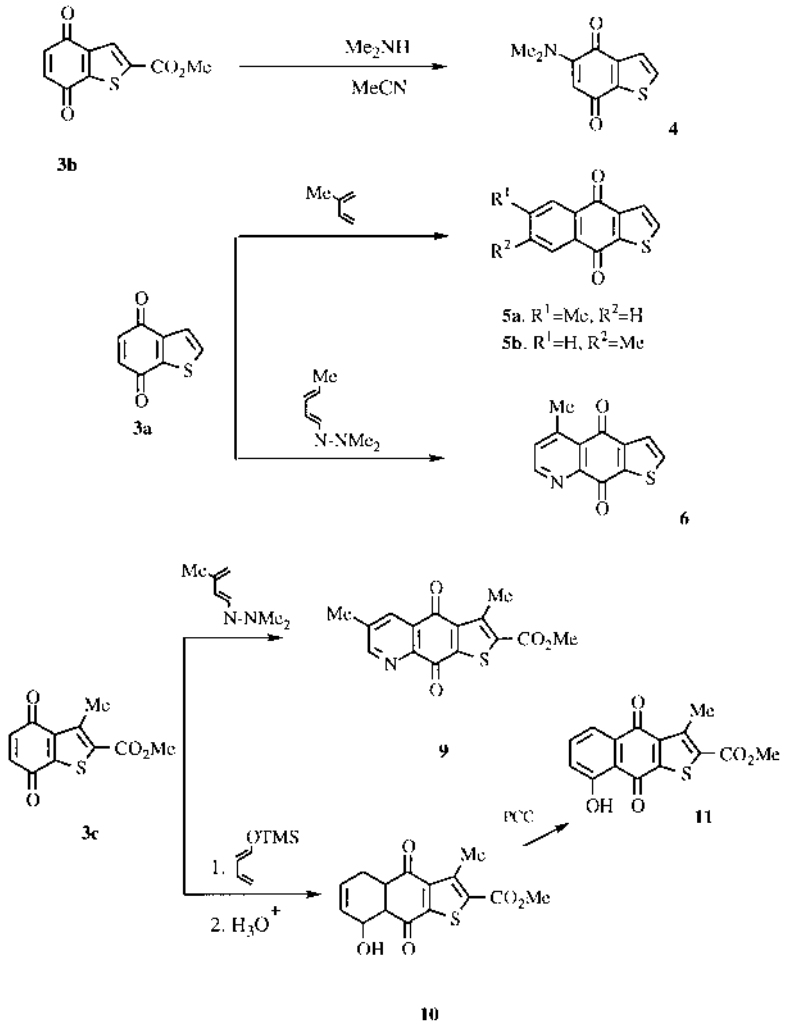
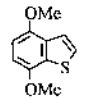
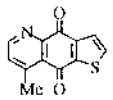
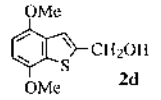
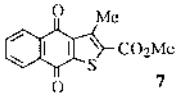
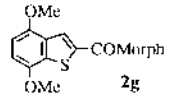
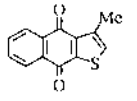
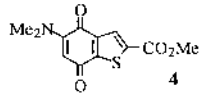
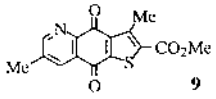
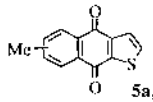
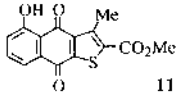


Chart 3

Table 2. Activity of Thiophene Fused Ring Compounds against the Bloodstream Forms of *Trypanosoma cruzi* (Percentage Reduction in Parasite Number at 250 μg/ml

Thiophenes	*T. cruzi* (%)	Thiophenes	*T. cruzi* (%)
 **2a**	32	 **6**	41
 **2d**	59	 **7**	0
 **2g**	0	 **8**	9
 **4**	50	 **9**	74
 **5a,b**	52	 **11**	0

The HOMO and LUMO orbital coefficients indicate that the larger coefficients were located at C-4 for the 1,3-dienes and at C-5 for quinones **3a**, **b**. This allows in to predict that compounds **6**, **9** and **11** are the favoured regioisomers generated *via* the corresponding Diels–Alder adducts.

These results suggest that the antiprotozoal activities of compounds **3** depend on the presence of the quinone nucleus because the aromatic analogues **2a** and **2g** exhibited only low or no activity. On the other hand, the activity of quinone **3b** was significantly altered by introduction of the electron-donating NMe_2 substituent at C-6, as in benzo[*b*]thiophenequinone **4**. It is noteworthy that compounds **3h** and **3i** having the benzo[*b*]thiophene-4,7-quinone cytotoxic moiety, display low activity against *T. cruzi*. However, they were active against strains of *Leishmania* sp. These properties are probably related to the polarity of the polyether chain of these compounds.

The benzene nucleus fused on the quinone ring of the benzo[*b*]thiophene-4,7-quinone **3c** (compounds **7**, **11**) induces a dramatic reduction in antiparasital activity. Nevertheless, the pyridine-fused nucleus in quinones **6** and **9** has only a minor effect on bioactivity, relative to the benzo[*b*]thiophene-4,7-quinones **3a** and **3c**.

The above weaker effect of the pyridine nucleus compared with the benzene ring is probably due to the electron-with-

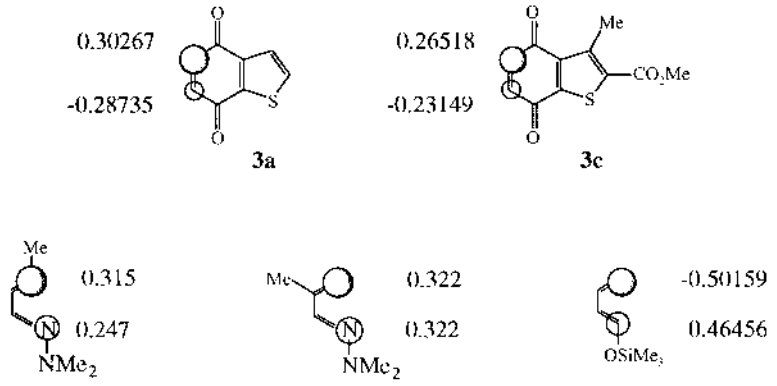


Fig. 1. Orbital Coefficients of Quinones **3a**, **c** and the 1,3-Dienes

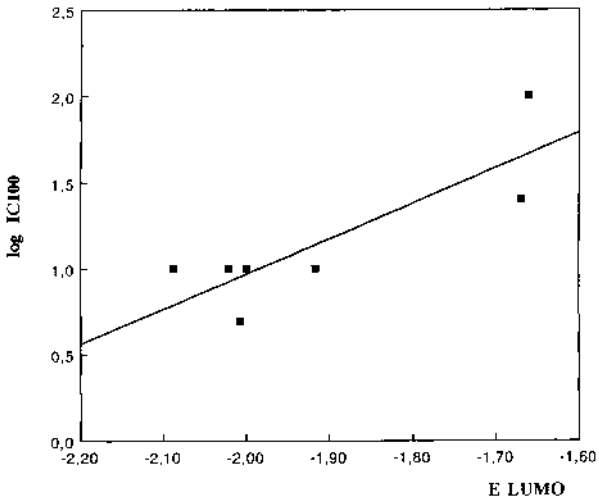


Fig. 2. Plot of the Linear Regression in Eq. 1

Equation 1 indicates the correlation between the Leishmanicidal activity of *L. braziliensis* and the LUMO energy.

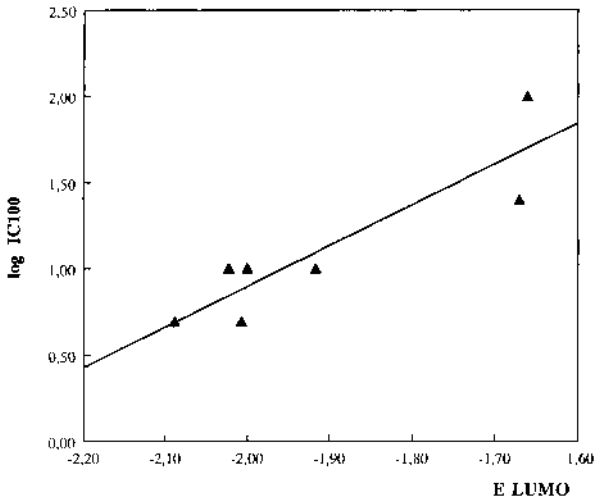


Fig. 3. Plot of the Linear Regression in Eq. 2

Equation 2 indicates the correlation between the Leishmanicidal activity of *L. amazonensis* and *L. donovani* and the LUMO energy.

drawing effect of the *N*-heterocyclic ring that results in a less marked effect on the oxidant ability of the quinone system. It is noteworthy that doxorubicin, a well known cytotoxic drug, was tested together with quinones **3** against *T. cruzi* and exhibited an inhibition activity of 48%.

The screening of the thiophene derivatives in Tables 1 and 2 indicates that the highest inhibition of *T. cruzi* was displayed by those members having a quinone nucleus. The aromatic members were less active than the corresponding quinones (except for **2d**) suggesting that the thiophene nucleus also plays a role in the antiparasitic activity. It is noteworthy that the quinones in Table 1 were equally active on *Leishmania*, independent of the species.

In order to investigate a possible relationship between the antiparasitic activity and electronic affinities of benzo[*b*]-thiophene-4,7-quinones (oxidative stress mechanism) the E_{LUMO}[19] and halfwave potential parameters were determined. Statistical analysis of the data in Table 1 indicates that no significative correlations were obtained for the affinity parameters ($E_{1/2}$ and E_{LUMO}) and trypanocidal activity. However, the regression Eqs. 1 and 2, obtained between Leishmanicidal activity and E_{LUMO} do indicate a significant correlation (see also Figs. 2 and 3). Compound **3e** was excluded from this analysis because its inhibition value was unknown.

Relationship between leishmanicidal activity (*L. braziliensis*) and E_{LUMO}:

$$\log(IC_{100})=(5.1\pm1.1)+(2.05\pm0.59)E_{LUMO} \quad n=7,\ R=0.8432,\ s=0.2495 \tag{1}$$

Relationship between leishmanicidal activity (*L. amazonensis* and *L. donovani*) and E_{LUMO}:

$$\log(IC_{100})=(5.6\pm1.0)+(2.35\pm0.52)E_{LUMO} \quad n=7,\ R=0.8965,\ s=0.2214 \tag{2}$$

Eqs. 1 and 2 indicate that the Leishmanicidal activity depends on the electron-withdrawing capability of the drugs. The coefficient of the E_{LUMO} parameter is positive implying that the high LUMO energy of the molecules leads to potent antileishmania activity. Therefore, it can be concluded that the quinone nucleus of compounds **3** is probably involved in the inhibition mechanism.

In conclusion, the results reported here demonstrate that compounds having the benzo[*b*]thiophene-4,7-quinones and thieno[3,2-*g*]quinoline-4,9-quinone system are potential antiprotozoal agents. In the series of benzo[*b*]thiophene-4,7-quinones **3** the activity against *Leishmania* strains is in relative good agreement with the E_{LUMO} suggesting that an electron-transfer interaction between the drug and some electron donor in the receptor may be involved.

Experimental

Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. FT-IR spectra were recorded on a Bruker vector 22-FT spectrophotometer using KBr discs and the wave numbers are given in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker AC-200P spectrometer in deuterated chloroform. Chemical shifts are reported in δ ppm downfield to tetramethylsilane (TMS), and *J*-values are given in Hertz. Mass spectra were obtained on a VG-12-250 spectrometer at 70 eV. Silica gel Merck 60 (70—230 mesh) and TLC aluminium foil $60F_{254}$ were normally used for preparative column chromatography and analytical TLC, respectively. 1-Azadienes were prepared from methacrolein, crotonaldehyde and hydrazine as reported previously.[20)]

The halfwave potential ($E_{1/2}$V) measurements were carried out with a Potentiostat Bank (Model Wenking ST-72) coupled to a Voltage Scan Generator (Model USG-72) and a Graphtec Recorder (Model WX-2300). The working electrode used in the cyclic voltametry was a platinum inlay electrode (Beckman). The auxiliary electrode was a platinum-coil electrode, which was isolated from the bulk solution by a glass tube with a porosity glass frit at the end. All the experiments were performed under an argon atmosphere, at room temperature in acetonitrile solution. Tetraethylammonium perchlorate (TEAP) was used as the supporting electrolyte.

1-(4,7-Dimethoxybenzo[*b*]thiophene-2-carbonyloxy)-8-hydroxy-3,6-dioxaoctane (2h) A solution of acid **2e** (500 mg, 2.1 mmol), triethylene glycol (2.8 ml, 21 mmol), DCC (520 mg, 2.52 mmol), a catalytic amount of dimethylamino pyridine and dichloromethane (25 ml) was allowed to stand with stirring at room temperature for 18 h. The reaction mixture was filtered *in vacuo* to afford a liquid residue which following flash chromatography (chloroform) yielded compound **2h** as a yellow-brown oil (590 mg, 75%); FT-IR: 3600—3200, 1710, 1250, 1230, 1090; ^{1}H-NMR δ: 3.60 (dt, 2H, *J*=5, 0.7 Hz, 8-H), 3.65—3.73 (m, 6H, 4-, 5-, 7-H), 3.82 (t, 2H, *J*=5 Hz, 2-H), 3.89 (s, 3H, OMe), 3.92 (s, 3H, OMe), 4,48 (t, 2H, *J*=5 Hz, 1-H), 6.69 (dd, 2H, *J*=8.4 Hz, 5-, 6-H), 8.20 (s, 1H, 3-H); ^{13}C-NMR δ: 55.8; 56.0; 61.8; 64.5, 69.1; 70.4; 70.8; 72.6; 104.7; 106.9; 128.2; 131.1; 132.1; 133.0; 148.4; 150.5; 162.7; *Anal*. Calcd for $C_{17}H_{22}O_7S$: C, 55.12, H, 5.99; S, 8.66. Found: C, 55.23; H, 6.12; S, 8.65.

1,8-Bis(4,7-dimethoxybenzo[*b*]thiophene-2-carbonyloxy)-3,6-dioxaoctane (2i) A mixture of acid **2e** (232 mg, 0.975 mmol), compound **2h** (300 mg, 0.810 mmol), DCC (242 mg, 1.17 mmol) and a catalytic amount of 4-dimethylaminopyridine in dichloromethane (35 ml) was allowed to stand with stirring at room temperature for 18 h. The reaction mixture was filtered and the filtrate was evaporated *in vacuo* to give a liquid residue. Flash chromatogtaphy of the crude (chloroform) afforded **2i** (346 mg, 72%) as a yellow green oil; FT-IR: 1710, 1250, 1230, 1090; ^{1}H-NMR δ: 3.81 (s, 4H, 4-, 5-H), 3.92 (t, 4H, *J*=5 Hz, 2-, 7-H), 3.95 (s, 6H, 2×OMe), 3.98 (s, 6H, 2×OMe), 4.55 (t, 4H, *J*=5 Hz, 1-, 8-H), 6.61 (d, 1H, *J*=8.5 Hz, 5′- or 6′-H), 6.72 (d, 1H, *J*=8.5 Hz, 6′- or 5′-H), 8.24 (s, 2H, 3′-H); ^{13}C-NMR δ: 55.8; 56.0; 61.8; 64.5; 69.1; 70.4; 70.8; 72.6; 104.7; 106.9; 128.2; 131.1; 132.1; 133.0; 148.4; 150.5; 162.7; *Anal*. Calcd for $C_{28}H_{30}O_{10}S_2$: C, 56.94; H, 5.12; S, 10.86. Found: C, 56.67; H, 5.22; S, 10.04.

1-(4,7-Dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carbonyloxy)-8-hydroxy-3,6-dioxaoctane (3h) A magnetically stirred solution of **2h** (100 mg, 0.27 mmol) in acetonitrile (10 ml) was added dropwise to a solution of CAN (280 mg, 0.52 mmol) in water (10 ml) at room temperature, and the stirring was continued for 30 min. The resulting orange solution was diluted with water and extracted with chloroform (3×25 ml). The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by flash chromatography to afford quinone **3h** as a red-brown oil (80 mg, 87%); FT-IR: 3600—3200, 1715, 1670, 1270, 1240, 1085: ^{1}H-NMR δ: 3.61 (t, 2H, *J*=5 Hz, 8-H), 3.69 (m, 6H, 4-, 5-, 7-H), 3.82 (t, 2H, *J*=4.5 Hz, 2-H), 4.50 (t, 2H, *J*=4.5 Hz, 1-H), 6.84 (d, 2H, *J*=10 Hz, 5′- or 6′-H), 6.90 (d, 2H, *J*=10 Hz, 6′- or 5′-H), 8.13 (s, 1H, 3′-H); ^{13}C-NMR δ: 61.7; 65.2; 68.8; 70.4; 70.8; 72.5; 130.8; 138.1; 138.2; 140.3; 140.4; 146.4; 160.9; 179.8; 180.6; *Anal*. Calcd for $C_{15}H_{16}O_7S$: C, 52.94 ; H, 4.74; S, 9.42. Found: C, 53.17 ; H, 4.55; S, 9.72.

1,8-Bis(4,7-dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carbonyloxy)-3,6-dioxaoctane (3i) Compound **2i** (100 mg, 0.27 mmol) was reacted with CAN (280 mg, 0.52 mmol) under the same conditions used for the preparation of **3h**. After work-up, the residue was purified by flash chromatography to give quinone **3i** as a red oil (72 mg, 80%); FT-IR: 1715, 1670, 1270, 1240, 1090: ^{1}H-NMR δ: 3.71 (s, 4H, 4-, 5-H), 3.85 (t, 4H, *J*=5 Hz, 2-, 7-H), 4.49 (t, 4H, *J*=xxx Hz 1-, 8-H), 6.85 (d, *J*=10.5 Hz, 5′- or 6′-H), 6.91 (d, 2H, *J*=10.5 Hz, 6′- or 5′-H), 8.11 (s, 1H, 3′-H); ^{13}C-NMR δ: 61.6; 65.2; 68.7; 70.2; 70.7; 72.4; 130.7; 138.1; 140.2; 140.4; 146.4; 160.8; 179.8; 180.5; *Anal*. Calcd for $C_{24}H_{18}O_{10}S_2$: C, 54.34; H, 3.42; S, 12.09. Found: C, 54.64; H, 3.18; S, 11.85.

2-Methoxycarbonyl-5-dimethylaminobenzo[*b*]thiophene-4,7-quinone (4) To a solution of quinone **3b** (100 mg, 0.45 mmol) in acetonitrile (10 ml) was added dropwise 48% aqueous dimethylamine (0.05 ml, 21 mg, 0.45 mmol) and the mixture was left for 20 min at room temperature The mixture was diluted with water (20 ml) and then extracted with dichloromethane (2×15 ml). The organic layer was washed with water and dried over magnesium sulfate. Evaporation of the solvent followed by column chromatography of the residue (chloroform) afforded quinone **4** as a violet solid (44 mg, 37%), mp 167—168 °C; FT-IR 1712, 1674, 1617, ^{1}H-NMR δ 3.24 (s, 6H, NMe_2), 3.93 (s, 3H, OCOMe), 5.70 (s, 1H, 6-H), 8.06 (s, 1H, 3-H), ^{13}C-NMR δ: 43.00; 43.82; 53.82; 105.50; 131.04; 136.96; 138.53; 150.29; 152.27; 161.68; 177.44; 178.30; *Anal*. Calcd for $C_{12}H_{11}NO_4S$: C, 54.33; H, 4.18; N, 5.28; S, 12.09. Found: C, 54.21; H, 4.30; N, 5.17; S, 11.89.

6-Methyl- and 7-methylnaphtho[*b*]thiophene-4,9-quinone (5) A solution of quinone **3a** (53 mg, 0.32 mmol) and 2-methylbuta-1,3-diene (0.5 ml) in benzene (5 ml) was left for 5 d at room temperature. The mixture was evaporated, the residue was dissolved in ethanol and the solution was stirred in an open flask for 3 d at room temperature. The mixture was then evaporated *in vacuo* and the residue was chromatographed (chloroform) to afford a 50 : 50 mixture (evaluated by ^{13}C-NMR) of regioisomers **5a**, **b** (42 mg, 57%) as a yellow solid, mp 160—164 °C; FT-IR 1660; ^{1}H-NMR δ: 2.53 (s, 3H, Me), 7.53 (d with fine coupling, 1H, *J*=7.8 Hz, C-7), 7.70 (s, 2H, 2-, 3-H), 8.04 (s with fine coupling, 5-, 8-H for **5a** and **5b**, respectively), 8.13 (d, 1H, *J*=7.8 Hz, 8- and 5-H for **5a** and **5b**, respectively); ^{13}C-NMR δ 21.84; 126.85; 127.19; 127.44; 127.58; 127.84; 131.20; 131.46; 133.41; 133.80; 133.99; 134.35; 134.57; 142.88; 143.06; 144.88; 145.12; 145.41; 145.72; 178.20; 178.55; 179.37; 179.71; *Anal*. Calcd for $C_{13}H_8O_2S$: C, 68.40; H, 3.53; S, 14.04. Found: C, 68.63; H, 3.42; S, 14.72.

5-Methylthieno[3,2-*g*]quinoline-4,9-quinone (6) A solution of quinone **3a** (53 mg, 0.32 mmol) and 4-methyl-1-dimethylamino-1-azabuta-1,3-diene (50 mg, 0.45 mmol) in dichloromethane (5 ml) was allowed to stand for 1 d at room temperature. The mixture was evaporated, the residue was poured into ethanol and the solution was stirred in an open flask for 3 d at room temperature. The resulting mixture was evaporated *in vacuo* and chromatographed (chloroform) to give quinone **6** (36 mg, 48%) as a yellow-brown solid, mp 156—158 °C; FT-IR 1680; ^{1}H-NMR δ: 2.90 (s, 3H, Me), 7.46 (d, 1H, *J*=4.8 Hz, 6-H), 7.70 (d, 1H, *J*=5 Hz, 3-H), 7.79 (d, 1H, *J*=5 Hz, 2-H), 8.85 (d, 1H, *J*=5 Hz, 7-H); ^{13}C-NMR δ 22.66; 126.98; 128.54; 131.06; 135.03; 143.62; 150.78; 151.68; 152.69; 176.34; 181.04; *Anal*. Calcd for $C_{12}H_7NO_2S$: C, 62.87; H, 3.08; N, 6.11; S, 13.98. Found: C, 63.12; H, 2.98; N, 6.05; S, 14.20.

3,6-Dimethyl-2-methoxycarbonylthieno[3,2-*g*]quinoline-4,9-quinone (9) To a solution of quinone **3c** (100 mg, 0.424 mmol) in dichloromethane (10 ml) was added dropwise with stirring a solution of 3-methyl-1-dimethylamino-1-azabuta-1,3-diene (116 mg, 1.03 mmol). The mixture was allowed to stand overnight at room temperature and then the solvent was evaporated. The residue was chromatographed on preparative TLC (dichloromethane) to give heterocyclic quinone **9** (65.25 mg; 49%) as a yellow solid, mp >250 °C; FT-IR: 1720, 1660; ^{1}H-NMR δ: 8.86 (d, 1H, *J*=2 Hz, 5-H), 8.34 (d, 1H, *J*=2 Hz, 7-H), 3.95 (s, 3H, CO_2Me), 2.96 (s, 3H, C_3-Me), 2.56 (s, 3H, C_6-Me); ^{13}C-NMR δ 179.53; 176.82; 161.93; 154.97; 148.41; 147.54; 146.33; 139.10; 139.08; 135.31; 135.06; 130.78; 52.69; 18.96; 14.77; LS-MS *m*/*z* (rel. int.%): 301 (M^+, 47), 286 (20), 271 (15), 270 (71), 269 (31), 39 (15); *Anal*. Calcd for $C_{15}H_{11}NO_4S$: C, 59.79; H, 3.68; N, 4.65; S, 10.64. Found: C, 59.58; H, 3.90; N, 4.73; S, 10.28.

8-Hydroxy-2-methoxycarbonyl-3-methylnaphtho[2,3-*b*]thiophene-4,9-quinone (11) A solution of **3c** (200 mg, 0.85 mmol) and (*E*)-1-trimethylsilyloxybuta-1,3-diene (260 mg, 1.83 mmol) in dichloromethane (20 ml) was stirred for 3 d at room temperature. The solvent was removed, the residue was poured into a solution of 9 : 1 tetrahydrofuran (THF)–water (30 ml) and then 5% hydrochloric acid (1 ml) was added. The resulting solution was left at room temperature for 1 h. The mixture was diluted with water and extracted with chloroform. The organic layer was washed with water and dried over sodium sulfate. Evaporation of the solvent afforded crude 4a,5,8,8a-tetrahydro-5-hydroxy-2-methoxycarbonyl-3-methylnaphtho-[2,3-*b*]thiophene-4,9-quinone **10** as a red-brown solid (245 mg, 95%), mp 55—57 °C; FT-IR: 3240, 1720, 1665; ^{1}H-NMR δ: 2.20 (dd, 1H, *J*=16 Hz, 3, 5-H), 2.76 (s, 3H, 3-Me), 2.96 (m, 1H, 5′-H), 3.20 (m, 2H, 4a-, 8a-H), 3.89 (s, 3H, CO_2Me), 4.44 (m, 1H, 8-H), 5.90 (m, 2H, 6-, 7-H). To a stirred solution of **10** (100 mg; 0.328 mmol) in dichloromethane (4.5 ml) at room temperature was added dropwise a solution of PCC (615 mg, 2.85 mmol) and dry sodium acetate (205 mg, 2.5 mmol) in dichloromethane (20.5 ml). The mixture was stirred for 2 h and then filtered through silica gel. Evaporation of the solvent

afforded quinone **11** (92 mg, 93%) as an orange solid, mp >250 °C; FT-IR: 3400, 2950, 1720, 1600; ^{1}H-NMR δ 2.95 (s, 3H, 3-Me), 3.95 (s, 3H, CO_2Me), 7.26 (dd, 1H, J=7.8, 1.3 Hz, 7-H), 7.63 (t, 1H, J=7.8 Hz, 6-H), 7.74 (dd, 1H, J=7.8, 1.3 Hz, 5-H), 11,98 (s, 1H, OH); LR-MS m/z (%)=302 (M^+, 22), 287 (18), 271 (56), 270 (26), 242 (43); *Anal*. Calcd for $C_{15}H_{10}O_5S$: C, 59.60; H, 3.33; S, 10.61. Found: C, 59.50; H, 3.16; S, 10.74.

Bioassays. *In Vitro* Activity against *Leishmania* Cultures of *Leishmania* species were obtained from IICS (Instituto de Investigaciones en Ciencias de la Salud, Asunción, Paraguay) and identified by isoenzyme analysis. Three strains of *Leishmania* were used during these investigations: *L. braziliensis* (MHOM/BR/75/M 2903), *L. amazonensis* (IFLA/BR/67/PH8) and *L. donovani* (MHOM/IN/83/HS-70)) grown at 22 °C in Schneider's drosophila medium containing 20% fetal bovine serum. Compounds were dissolved in 5 μl dimethyl sulfoxide (DMSO), then in medium and placed in microtitre plates in triplicate. The minimum amount (μg) of compound to inhibit growth of *Leishmania* sp. was evaluated after 48 h by optical microscopy using a drop of each cell culture and comparing this with control cells and reference drug (pentamidine). The maintenance, cultivation, and isolation of promastigote-stage parasites have been described in detail elsewhere.[21)]

In Vitro* Activity against *Trypanosoma cruzi Albino mice infected with *T. cruzi* were used 7 d after infection. Blood was obtained by cardiac puncture using 3.8% sodium citrate as anticoagulant in a 7 : 3 blood/anticoagulant ratio. The parasitemia in infected mice ranged from 1×10^5 to 5×10^5 parasites per millilitre. The compounds were dissolved in cold DMSO to give a final concentration of 250 μg/ml. Aliquots (10 μl) of each extract at different concentrations (4, 20, 40, 100, 250 μg/ml) were mixed in microtitre plates with 100 μl infected blood containing different parasite concentrations (1×10^5 and 10^6 parasites per ml). Infected blood and infected blood containing gentian violet, 250 μg/ml were used as controls. The plates were shaken for 10 min at room temperature and kept at 4 °C for 24 h. Each solution was examined microscopically at 400×, placing a 5 μl-sample on a slide and covering it with a 22×22 mm coverglass for parasite counting.[22,23)]

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