STUDIES ON QUINONES . PART 36.[1] SYNTHESIS AND TRYPANOCIDAL ACTIVITY OF 2-ALKOXYCARBONYLBENZO[*b*]THIOPHENE-4,7-QUINONES

Carlos D. Pessoa-Mahana,*[a] Jaime. A. Valderrama,[a] María G. Olmos,[a] Omar A. Espinoza,[a] Hernán Pessoa-Mahana,[b] Antonieta Rojas de Arias,[c] Héctor Nakayama,[c] Susana Torres,[c] Jorge Miret[c]

[a]Facultad de Química. Pontificia Universidad Católica de Chile. Casilla 306, Santiago 22. Chile. [b]Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. Casilla 233. Santiago 1 Chile. [c]IICS-UNA (Instituto de Investigaciones en Ciencias de la Salud-Universidad Nacional de Asunción), Río de la Plata y Lagerenza, Casilla 2511, Asunción, Paraguay

Abstract: A series of 2-alkoxycarbonylbenzo[*b*]thiophene-4,7-quinones (**11-15**), prepared from the corresponding acid **5**, were tested *in vitro* against trypomastigote form of *Trypanosome cruzi*. The influence of the lypophilia and oxidant capacity upon the trypanocidal activity of these members is discussed.

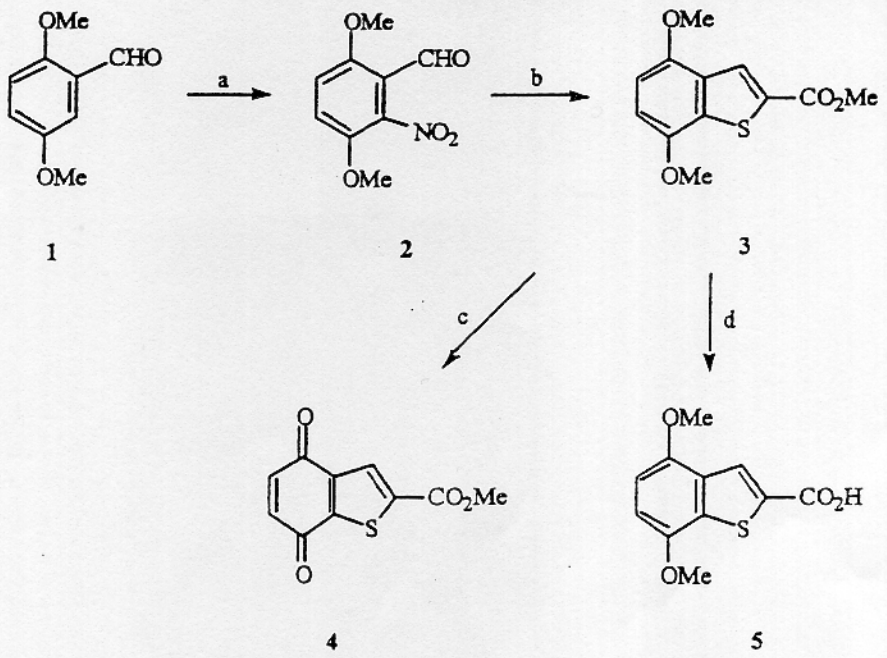
Introduction

Chagas' disease, caused by the flagellate protozoal *Trypanosome cruzi*, is responsible for much pain in Central and South America, where about 24 millions people are infected with this parasite.[2,3] The current chemotherapy against the chronic Chagas' disease is inadequate because the two principal drugs (nifurtimox, benznidazol) have low efficacy and severe side effects.[4] In a previous paper we have reported the synthesis and antiprotozoan activity of benzo[*b*]thiophene-4,7-quinone derivatives.[5] The activity of these compounds was attributed to an electron transfer process between the drug and the receptor. Among the members of the reported series, quinone **4**, easily obtained from 2,5-dimethoxybenzaldehyde (**1**) (Scheme 1), stands out for its significant activity which correlates with the LUMO energy. This suggests an electron transfer inhibition mechanism. Here we wish to report the *in vitro* activity of 2-alkoxycarbonylbenzo[*b*]thiophene-4,7-quinones against *T. cruzi*. The results indicate that lipophilicity also have an important influence upon the protozoal activity of the members of this series.

Results and Discussion

The synthesis of acid **5** required for the preparation of the 2-alkoxycarbonylbenzo[*b*]thiophene-4,7-quinones was carried out according to a procedure developed in our laboratory (Scheme 1).[6] The preparation of precursor **2** was improved with respect to our previously published method.[7] In fact, compound **2** was obtained in 66% by nitration of 2,5-dimethoxybenzaldehyde (**1**) in dichloromethane at –10°C and was easily isolated by filtration from the reaction mixture.

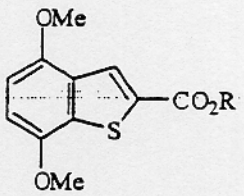
* Corresponding author. Tel.: +56-2-6864391; fax: + 56-2-6864744; e-mail: cpessoa@puc.cl



Scheme 1. Reagents: a) HNO_3, CH_2Cl_2, -10°C; b) $HSCH_2CO_2Me$, KOH, DMF; c) CAN, MeCN, H_2O; d) MeOH, KOH, H_2O

The synthesis of 2-alkoxycarbonyl-4,7-dimethoxybenzo[*b*]thiophenes **6**, **7** and **9** was achieved by acid-induced condensation of **5** with ethyl, *n*-propyl, and *n*-butyl alcohol respectively. Compounds **8** and **10** were prepared by reaction of **5** with the respective alcohols and dicyclohexylcarbodiimide (DCC). The results are summarized in Table 1.

Table 1. Esters 6-10 prepared from compound 5



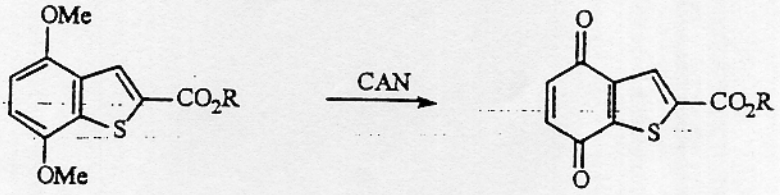
6-10

Product	R	Yield (%)
6	ethyl	59
7	*n*-propyl	52
8	*iso*-propyl	35
9	*n*-butyl	69
10	(-)menthyl	25

The access to the selected benzothiophenequinones **11-15** was performed by oxidative deprotection of the corresponding dimethyethers **6-10** with ceric ammonium nitrate (CAN) according to a procedure previously reported [6] and the results are summarized in Table 2.

The *in vitro* antiprotozoan efficacy of the heterocyclic quinones **11-15** against *Trypanosome cruzi* was tested using the trypomastigotes (blood) forms of the parasite obtained from mice inoculated intraperitoneally, as was described in detail in a previous work.[5] Quinone **4** was also tested in order to compare its activity with those of the new members of the series. Table 3 summarizes the bioassays.

Table 2. Benzothiophenequinones 11-15 obtained by oxidative demethylation



6-10 **11-15**

Quinone	R	Yield (%)
11	ethyl	50
12	*n*-propyl	44
13	*iso*-propyl	48
14	*n*-butyl	48
15	(-)menthyl	60

The lipophilicity and LUMO energies of 2-alkoxycarbonylbenzothiophene-4,7-quinones were calculated in order to gain information on the influence of the oxidant capacity upon the inhibition activity.

The results summarized in Table 3, suggest that, in spite of the fact quinones **4**, **11-15** have similar LUMO energies, the inhibition depends on the size, shape and lipophilicity of the alkoxycarbonyl group.

The least lipophilic substituent methyl (entry 1), displays the highest inhibition activity and the most lipophilic menthyl group lacks antiprotozoan activity (entry 7).

Recently we have prepared 2-formylbenzo[*b*]thiophene-4,6-quinone (**16**), which showed high inhibition activity against *leishmania amazonensis*. Since **16** is structurally related to the 2-alkoxycarbonylbenzothiophene-4,7-quinones **4**, **11-15**, it was also evaluated against *Trypanosome cruzi* and the LUMO energy and lipophilia were also calculated (Table 3).

According to these data compounds **4** and **16** showed the highest activity against *T. cruzi*, and the rest of the compounds displayed weak or no activity at the test concentrations. The results demonstrate that the lower lipophilicity and LUMO energy of the members of this series the higher is the inhibition activity.

Table 3. ***In vitro*** **activity, LUMO energy and log P of quinones 4, 11-16**

Entry	Quinone	LUMO (eV)[a]	Log P[b]	Inhibition (%)[c,d]
1	**4**	-2.152	1.99	91
2	**11**	-2.128	3.25	0
3	**12**	-2.125	3.73	50
4	**13**	-2.095	3.76	0
6	**14**	-2.059	4.23	78
7	**15**	-2.075	7.18	0
8	**16**	-2.161	2.07	100

a) [a]Performed using the semiempirical PM3 method. [b]Calculated using the Dixon method in the Spartan package. [c]Percentage reduction at 250 μg/mL. [d]Gentian violet as the reference drug; 100% inhibition at 250μg/mL

Conclusions

In conclusion we have prepared a variety of 2-alkoxycarbonylbenzo[*b*]thiophene-4,7-quinones by oxidative deprotection of the corresponding dimethylethers. The lipophilicity together with the oxidant capacity of the members of this series exert an influence upon the tripanocidal activity.

Experimental

Melting points are uncorrected. The IR spectra were recorded on a FT Bruker spectrophotometer for KBr discs and the wave numbers are given in cm^{-1}. The ^1H and ^{13}C NMR spectra were acquired at 200 and 50 MHz respectively on a Bruker AM-200 in $CDCl_3$. Chemical shifts are reported in δ ppm downfield to TMS, and *J* values are given in Hertz. Silica gel Merck 60 (70-230 mesh), and DC-Alufolien $60F_{254}$ were used for preparative column and analytical TLC, respectively.

3,6-Dimethoxy-2-nitrobenzaldehyde (2) A solution of 2,5-dimethoxybenzaldehyde 1 (2.4 g, 14.4 mmol) was added dropwise to a stirred solution of concentrated nitric acid (2 mL) in dichloromethane (10 mL) at –10 °C and the mixture was stirred at 0 °C for 4 h. The precipitate was filtered and washed with water to afford pure compound 2 (2.0 g, 66%) as white crystals, mp. 162-164 °C (lit.[8] mp 164-165 °C).; IR ν 1680 (CO) and 1540 (NO_2).

4,7-Dimethoxy-2-ethoxycarbonylbenzo[*b*]thiophene (6) A solution of compound 5 (150 mg, 0.63 mmol), ethanol (5 mL), and *p*-toluensulfonic acid (10 mg) in benzene (35 mL) was refluxed in a Dean Stark apparatus for 6 h. The reaction mixture was washed with water, aqueuos sodium hydrogencarbonate and water. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed over silica gel (dichloromethane) to afford ester 6 (126 mg, 69%) as white crystals (ethanol), mp 105-107 °C; IR ν 1712 (C=O); 1293 (C-O); ^1H NMR δ 8.19 (s, 1H, 3-H); 6.77 (d, 1H, J = 9 Hz, 5- H); 6.66 (d, 1H, J = 9 Hz, 6-H); 4.40 (quart, 2H, J = 14.3 Hz, OCH_2); 3.96 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 1.41 (t, 3H, J = 7.1Hz, CH_3); ^{13}C NMR δ 162.9, 150.5, 148.5, 133.0, 132.9, 131.2, 127.7, 106.7, 104.6, 61.46, 56.0, 55.8, 14.3; *Anal.* Calcd for $C_{13}H_{14}O_4S$: C, 58.63; H, 5.30; S, 12.04. Found: C, 58.67; H, 5.40; S, 12.09.

4,7-Dimethoxy-2-*n*-propoxycarbonylbenzo[*b*]thiophene (7) According to the procedure described for 6, compound 5 (155 mg, 0.65 mmol), and *n*-propyl alcohol (10 mL) were reacted for 6 h to give 7 (80 mg, 52%) as white crystals, mp 93-94 °C; IR ν 1712, 1290; ^1H NMR δ 8.19 (s, 1H, 3-H); 6.77 (d, 1H, J = 9 Hz, 5- H); 6.66 (d, 1H, J = 9 Hz, 6-H); 4.30 (t, 2H,

J = 6.6 Hz, OCH_2); 3.95 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 1.79 (qt, 2H, J = 6.8 Hz, J = 7.3 Hz CH_2); 1.04 (t, 3H, J = 7.3, CH_3); ^{13}C NMR δ 162.9, 150.5, 148.5, 133.0, 132.9, 131.2, 127.7, 106.7, 104.6, 67.0, 56.0, 55.8, 22.1, 10.5; *Anal.* Calc. for $C_{14}H_{16}O_4S$: C, 59.58; H, 5.75; S, 11.44. Found: C, 60.03; H, 5.89; S, 11.43.

***n*-Butyl 4,7-dimethoxybenzo[b]thiophene-2-carboxylate (9)** According to the procedure described for **6**, compound **5** (150 mg, 0.63 mmol) and *n*-butyl alcohol (10 mL) were reacted for 12 h to give **9** as white crystals (88 mg, 59%) (ethanol); mp 78-79 °C; IR ν 1671, 1260; ^{1}H NMR δ 8.05 (s, 1H, 3-H), 6.82 (d, 1H, J = 8 Hz, 5- H); 6.64 (d, 1H, J = 8 Hz, 6-H); 4.34 (t, 2H, J = 6.5 Hz, OCH_2); 3.96 (s, 3H, OCH_3); 3.93 (s, 3H, OCH_3); 1.74 (q, 2H, J=14 Hz, CH_2); 1.48 (q, 2H, J=7 Hz, J=14 Hz, CH_2); 0.98 (t, 3H, J=7.3 Hz, CH_3); ^{13}C NMR (50 MHz) δ 163.0, 150.5, 148.5, 133.0, 132.9, 131.2, 127.6, 106.7, 104.6, 65.3, 55.8, 55.1, 30.7, 19.2, 13.7; *Anal.* Calcd for $C_{15}H_{18}O_4S$ (294,37): C, 61.20; H, 6.16; S, 10.89. Found: C, 61.63; H, 6.17; S, 10.54.

4,7-Dimethoxy-2-isoproxycarbonylbenzo[*b*]thiophen (8) A solution of acid **5** (99 mg, 0.42 mmol), *N,N*-dicyclohexylcarbodiimide (110 mg, 0,53), DMAP (10 mg), *iso*-propyl alcohol (26 mg, 0.43 mmol) and dichloromethane (50 mL) was stirred at room temperature for 18 h. The solution was filtered, and washed successively with water (3x15 mL), acetic acid 15% (3 x 15) and water (3 x 15 mL). The organic extract was dried over magnesium sulfate and the solvent was removed under vacuum. The residue was purified by column chromatography on silica gel, eluting with dichloromethane to give ester **8** (41 mg, 35%) as white solid, mp 91-93 °C; IR ν 1717, 1280; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.18 (s, 1H, 3-H); 6.77 (d, 1H, J = 10 Hz, 5- H); 6.66 (d, 1H, J = 10 Hz, 6-H); 5.25 (q, 1H, 1-H); 3.96 (s, 3H, OCH_3); 3.93 (s, 3H, OCH_3); 1.39 (d, 6H, J = 6 Hz, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.4, 150.4, 148.5, 133.6, 132.8, 131.2 127.4, 106.6, 104.5, 69.1, 56.0 55.76; 21.94; *Anal.* Calcd. for $C_{14}H_{16}O_4S$ (280,34); C. 59,98; H. 5,75; S. 11,44. Found: C. 59.77; H. 5.71; S. 11.93.

4,7-Dimethoxy-2-menthyloxybenzo[*b*]thiophen (10) According to the procedure described for **8**, acid **5** (115 mg, 0.30 mmol) and (-)-menthol (85 mg, 0.54 mmol) were reacted for 12 h to give 10 (45 mg, 25%) as white crystals mp 82-83 °C; IR ν 1714 (CO, ester); 1275 (C-O); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.18 (s, 1H, 3-H); 6.76 (d, 1H, J = 10 Hz, 5- H); 6.66 (d, 1H, J = 10 Hz, 6-H); 4.91 (dt, 1H, J = 4.4 Hz, J = 10.8 Hz H-1); 3.95 (s, 3H, OCH_3); 3.93 (s, 3H, OCH_3); 2.19-0,79 (m, 18H, menthyl); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.4, 150.45, 148.5, 133.5, 132.9 131.2, 127.4, 106.6, 104.6, 75.6, 56.0 55.8, 47.2, 40.9, 34.3 31.5, 26.5, 23,7 22.0, 20.8, 16.6; *Anal.* Calcd for $C_{21}H_{28}O_4S$ (376,51); C, 67.02; H, 7.45; S, 8.51. Found: C, 67.17; H, 7.76; S, 8.54.

2-Etoxycarbonylbenzo[*b*]thiophen-4,7-quinone (11) A solution of compound **6** (219 mg, 0.82 mmol) in acetonitrile (10 ml) was added dropwise to a stirred solution of CAN (880 mg, 1.61mmol) in 5 mL of water and the stirring was mantained for 15 min. The resulting orange solution was extracted with ethyl acetate (3x15mL) and the dry extract was evaporated under vacuum. The residue was purified by column chromatography (dichloromethane) to afford pure **11** (110 mg, 50%), mp 99-100 °C; IR ν 1704 (CO, ester); 1656 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.08 (s, 1H, 3-H); 6.76 (d, 1H, J = 10 Hz, 5- H); 6.67 (d, 1H, J = 10 Hz, 6-H); 4.40 (c, 2H, J = 14.3 Hz, OCH_2); 1.41 (t, 3H, J = 7.1Hz, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.6, 179.9, 160.9, 146.2, 140.9, 140.9, 138.2, 138,1, 130.4, 62.4, 14.3; *Anal.* Calcd for $C_{11}H_8O_4S$ (236,24); C, 55.93; H, 3.41; S, 13.57. Found: C, 55.96; H, 3.42; S, 13.49.

2-*n*-Propoxycarbonylbenzo[*b*]thiophen-4,7-quinone (12) According to the procedure described for **11**, compound **7** (236 mg, 0.84 mmol) and CAN (950 mg, 1,73 mmol) were reacted to give **12** (104 mg, 44%) mp 103-104 °C; IR ν 1718 (CO, ester); 1660 (CO quinone); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.11 (s, 1H, 3-H); 6.76 (d, 1H, J = 10 Hz, 5- H); 6.66 (d, 1H, J =

10 Hz, 6-H); 4.31 (t, 2H, *J*=6.6 Hz, OCH_2); 1.78 (qt, 2H, *J* = 14 Hz, *J* = 7 Hz, CH_2); 1.00 (t, 3H, *J* = 7,3, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.6, 179.9, 161.0, 146.2, 140.5, 138.2, 130.3, 67.9, 21.9, 10.4; *Anal.* Calcd for $C_{12}H_{10}O_4S$ (250,27); C, 57.59; H, 4.03; S, 12.81. Found: C, 57.32; H, 4.00; S, 12.60.

2-*iso*-Proxycarbonylbenzo[b]thiophene-4,7-quinone (13) The reaction of compound **8** (58 mg, 0.21mmol) and CAN (242mg, 0.42 mmol) gave **13** (25 mg, 48%), mp 76-77°C; IR ν 1705 (CO ester); 1654 (CO quinone); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.09 (s, 1H, 3-H); 6.87 (d, 1H, *J* = 10 Hz, 5- H); 6.81 (d, 1H, *J* = 10 Hz, 6-H); 5.26 (m, 1H, 1'-H); 1.38 (d, 6H, *J*=6 Hz, $C(CH_3)_2$; ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.7, 179.9, 160.5, 141.6, 140.5, 138.2, 138.1, 130.2, 70.5, 21.8 ; *Anal.* Calcd for $C_{12}H_{10}O_4S$ (250.27); C, 57,59; H, 4.04; S, 12.81. Found: C, 57.23; H, 3.61; S, 12.93.

2-*n*-Butoxycarbonylbenzo[*b*]thiophen-4,7-quinone (14) The reaction of **9** (49 mg, 0.16 mmol) and CAN (220 mg, 0.40 mmol) afforded quinone **14** (21mg, 48%) mp 82-83 °C; IR ν 1718 (CO, ester); 1659 (CO quinone); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.11 (s, 1H, 3-H); 6.77 (d, 1H, *J* = 10 Hz, 5- H); 6.66 (d, 1H, *J* = 10 Hz, 6-H); 4.36 (t, 2H, *J* = 6.5 Hz, OCH_2); 1.76 (q, 2H, *J* = 14 Hz, J= 7Hz, CH_2); 1.46 (q, 2H, *J* = 7 Hz, J=15 Hz, CH_2); 0.98 (t, 3H, *J*=7.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180,6, 179,88; 161.0, 146.2, 140.9, 140.5, 138.2, 138.12, 130.4, 66.3, 30.6, 19.2, 13.7; *Anal.* Calcd for $C_{13}H_{12}O_4S$ (264,30); C, 59.09; H, 4.54; S, 12.12. Found: C, 59.04; H, 4.63; S, 12.08.

2-Menthyloxybenzo[*b*]thiophene-4,7-quinone (15) The reaction of **10** (45 mg, 0.12 mmol) with CAN (159 mg, 0,29 mmol) afforded **15** (25 mg, 60%), mp 96-97 °C; IR ν 1712 (CO ester); 1660 (CO quinone); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.11 (s, 1H, 3-H); 6.87 (d, 1H, *J* = 10 Hz, 5- H); 6.81 (d, 1H, *J* = 10 Hz, 6-H); 4.91 (dt, 1H, *J* = 4.4 Hz, *J* = 10 Hz, H-1'); 2.19-0,79 (m, 18H, menthyl); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 180.6, 179.9, 160.3, 141.2, 140.9, 138.2, 138.1, 130.4, 75.7, 47.1, 41.0, 34.3, 31.5, 26.6, 23,7, 21.9, 20.8, 16.3. This compound decomposes on standing, therefore no satisfactory microanalysis was obtained.

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