Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 12, pp 2185–2192, 1997

PII: S0968-0896(97)00155-7
0968-0896/97 $17.00 + 0.00

Anti-Plasmodial and Anti-Trypanosomal Activity of Synthetic Naphtho[2,3-*b*]thiophen-4,9-quinones

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Abstract—Naphtho[2,3-*b*]thiophen-4,9-quinone and five derivatives were prepared using the Friedel–Crafts reaction and tandem-lithiation of aromatic diethylamides. These quinones were evaluated for their trypanocidal and anti-plasmodial activities by their effects on: (1) growth of epimastigote forms of *Trypanosoma cruzi* in vitro, (2) lysis of trypomastigote forms of *T.cruzi* in murine blood, (3) growth of *Plasmodium falciparum* in vitro, and (4) inhibition of the recombinant enzyme trypanothione reductase. The parent compound, naphtho[2,3-*b*]thiophen-4,9-quinone (**3a**), was among the most active quinone tested in vitro against *P. falciparum* at 0.2 μM. However, it was inactive against *P. berghei*-infected mice treated with 2.3 mmol/kg daily for 5 days. Most of the quinones prepared were active against *T. cruzi* epimastigotes in culture but exhibited weak activity at 4 °C against trypomastigotes in murine blood as well against the enzyme trypanothione reductase. Further structural modifications will be necessary to improve the in vivo activity of the naphthothiophenquinones © 1997 Elsevier Science Ltd.

Introduction

Chagas' disease is an illness that affects 18 million people in Latin America, while malaria is one of the most important parasitic infections on the planet, causing the death of more than a million patients per year.[1] Quinonoid compounds display a wide spectrum of biological and pharmacological activities, including antiprotozoan activity against parasites such as *Trypanosoma cruzi* and *Plasmodium falciparum*,[2,3] the causative agents of Chagas' disease and human malaria, respectively.

The life cycle of *T. cruzi* involves a nondividing, trypomastigote form of the parasite, that circulates in the blood of the patients and is transferred to hematophagous *Triatomine* insects during their blood meal or directly to other people via blood transfusion. Natural transmission occurs by contamination with the infective form of the parasite present in the insect feces at the site of the insect bite or via neighboring intact mucosa. At present, the Triatomine vectors are under control in most affected areas such that blood transfusion now causes the majority of new cases of Chagas' disease.[4] In highly endemic areas, it is strongly recommended to use chemoprophylatic measures such as the addition of gentian violet (crystal violet) to clear trypomastigotes from blood banked for transfusion.[5,6] However, despite being effective, gentian violet is not completely accepted by clinicians or patients because of undesirable effects such as coloring the skin.[7]

The treatment of Chagas' disease relies on two available drugs, nifurtimox and benznidazole, introduced in the 1970s. Although efficient in most cases of the acute phase of the disease, these drugs are almost ineffective in the chronic phase.[8,9] Thus, the development of new compounds to replace the currently available prophylactic and therapeutic drugs remains a highly desirable goal. Recently, the discovery of trypanothione reductase (TR-EC 1.6.4.8)[10] and its role in the maintenance of the redox equilibrium within the parasite has opened new horizons for the development of trypanocidal drugs. Although this enzyme has 41% homology in its primary structure with the analogous human glutathione reductase (GR),[13] their active sites are sufficiently different to allow the development of selective TR inhibitors that could kill the parasites through reduction of their defenses to oxidant stress.

Key words: Naphthothiophenquinones, *Plasmodium falciparum*, *Plasmodium berghei*, *Trypanosoma cruzi*, trypanothione reductase.
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Malaria, caused by protozoan parasites of the genus *Plasmodium*, continues to be one of most important parasitic disease in the world.[1] Since the World War II, an impressive effort has been devoted to the development of new antimalarial drugs, resulting in the introduction of potent chemotherapeutic agents for clinical use.[14] However, the emergence of resistance to many of these drugs has seriously impaired their therapeutic value. Associated with the resistance of the insect vector to organochlorines, this has resulted in the resurgence of malaria in areas where it was thought to be under control.[15,16] Thus, new drugs are urgently required. During the 1940s, Fieser and colleagues synthesized more than 300 substituted naphthoquinones as potential antimalarials,[3] a work that paved the way to the development of the recently introduced antiprotozoal drug Atovaquone (**1**), a 2-hydroxynaphthoquinone derivative.[2,17]

Within the quinone group, it has been known for over 100 years that naphthofuranquinones (NFQs; e.g., **2**) can be obtained via cyclization of the natural product lapachol.[18] However, the biological activities of NFQs against a variety of diseases has only been recognized in the last two decades.[19,20] A number of natural and synthetic NFQs have been evaluated in vitro against *P. falciparum*[21] and *T. cruzi*,[21-24] and some were found to be active at the nanomolar level.

These observations prompted us to synthesize quinones related to NFQs such as naphtho[2,3-*b*]thiophen-4,9-quinones (NTQs, **3a**) and investigate their antiprotozoal activity in a variety of biological assays. The synthesis of NTQs has been known since 1914 but, as for NFQs, only recently have their biological activities started to be explored.[25,26] Besides the classical strategies for their synthesis, based on Friedel–Crafts arylation,[25,27-34] new methodologies using organometallics have been successfully employed.[26,35]

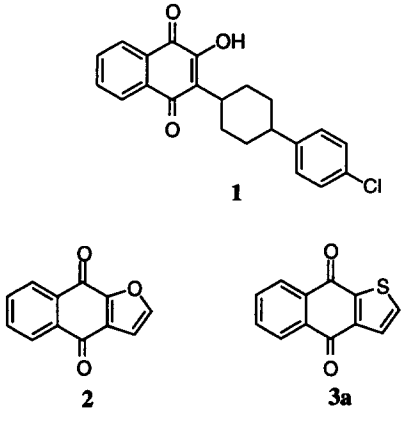


Figure 1. Structures of atovaquone (**1**), naphtho[2,3-*b*]furan-4,9-quinone (NFQ, **2**) and naphtho[2,3-*b*]thiophen-4,9-quinone (NTQ, **3a**).

Results and Discussion

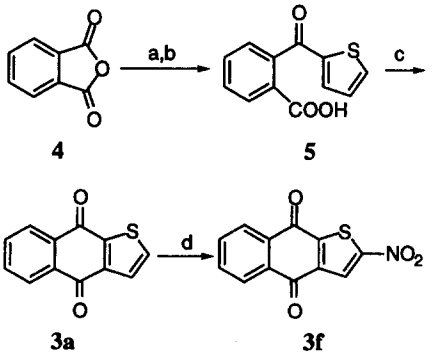
Synthesis

Six naphthothiophenquinones (NTQs) (**3a–f**) were synthesized using classical and modern synthetic methods (Schemes 1 and 2). The synthesis of **3a** and **3f** were carried out using Friedel–Crafts conditions, similar to those reported in the literature[29] (Scheme 1).

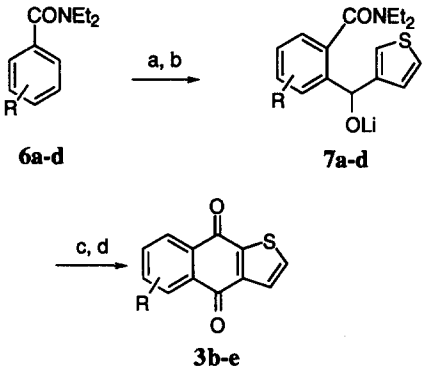
For the synthesis of methoxylated NTQs (**3b–3e**) we used the one-pot tandem lithiation strategy developed by Watanabe and Snieckus[35] (Scheme 2). This latter procedure was chosen because it gives access to the desired quinones from commercially available starting materials in only two steps with control of regioselectivity. After the electrophilic reaction between the lithiated amide and the 3-thiophene carboxaldehyde, cyclization is promoted by metallation at the 2-position of the thiophene by using excess of *sec*-butyl lithium reagent.[35] Following the removal of solvents, the crude reaction mixture was oxidized with Jones' reagent to generate the corresponding quinones that were purified by flash chromatography over silica gel and recrystallization. All compounds appeared as single spots on TLC after eluting with several solvent systems and visualization methods. The global yields of the pure quinones are indicated in Scheme 2. The preparation of quinone **3b** was published recently.[36] However, the conditions used do not ensure control of regioselectivity and could result in the production of isomers. Quinones **3c–3e** are reported here for the first time and their structures are fully compatible with the spectroscopic data (see Experimental).

Biological activities

When evaluated in vitro against *P. falciparum*, all quinones, except **3b**, were significantly active at 0.2 μM (Table 1). NTQs **3c** and **3d** were the most active. Quinone **3a**, produced in a larger amount, was also tested in vivo in mice experimentally infected with *P.*



Scheme 1. (a) $AlCl_3/CH_2Cl_2$/30 °C/30 min. (b) Thiophene/ 30 °C/2 h. (c) Concentrated H_2SO_4/100 °C/2 h. The global yield for steps a–c was 53% of the pure quinone. (d) Fuming HNO_3/80 °C/2 h, 30% yield of the pure nitro derivative.



Scheme 2. (a) *sec*-BuLi (1.1 equiv)/TMEDA/THF/−80 °C/1 h (b) thiophene-3-carboxaldehyde (1 equiv)/−80 °C/30 min. (c) *sec*-BuLi (4 equiv)/−78 °C to rt. (d) Jones' oxidation. The global yields of the isolated, pure quinones **3b–3e** were 5%, 10%, 23%, and 9%, respectively.

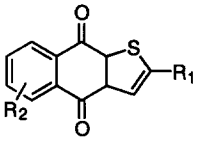
berghei. It was given at five consecutive daily doses of 2.3 mmol/kg, but found to be inactive (data not shown).

In order to understand the lack of correlation between in vitro and in vivo activity of **3a** we performed a preliminary study to evaluate its serum concentration in mice. Using an HPLC method originally developed to quantify Atovaquone, **3a** was found to elute after 14.8 min (Fig. 2b) in a region without interference from serum components (Fig. 2a). Its identity was confirmed by comparing the UV spectrum of the peak registered by a photodiode array detector with that of pure **3a** under the same conditions. The peak areas were measured and the amount of **3a** calculated from a calibration curve [concentration (μM) = $(4.3 \times 10^{-5} \times$ area of the peak) − 0.0588; $r^2 = 0.9988$). The recovery from normal blood spiked with **3a** (see Fig. 2b) was found to be 90 ± 2%.

Two hours after oral treatment with a single 2.3 mmol/kg dose, the serum concentration of **3a** reached its maximum around 40 μM and decreased to about 1 μM after 48 h (Fig. 3). The increase in the serum concentration after 24 h was confirmed by repeating the experiment with another group of mice. This result could be attributed to the accumulation of **3a** in an organ or tissue and posterior release into the blood or to a reversible in vivo transformation of the quinone. In contrast to Atovaquone, which is not extensively metabolized in vivo,[37] some possible metabolites were also noted (e.g., peak at 4.6, 10.6, and 11.7 min in Fig. 2c). However, the chemical identity and the biological activity of these compounds are not known at present. It is possible that after a bioreduction of the quinonoid ring, the hydroquinone formed could be stabilized by conjugation. The identification of the major metabolite will be the subject of future work.

Thus, although the serum concentration of **3a** in treated mice was always higher than that used in vitro against *P. falciparum* (0.2 μM) it was inactive against *P. berghei* in vivo. As the parasites reside inside the erythrocytes most of the time, two possibilities arise: (a) **3a** is able to attain higher concentrations inside the human than in the mice hematias or, (b) the quinone concentration is similar in both but the parasites (*P. falciparum* and *P. berghei* schizonts) have different susceptibility to the

Table 1. Results of the bioassays with the naphtho[2,3-*b*]thiophen-4,9-quinones against *Plasmodium falciparum*, *Trypanosoma cruzi*, and the enzyme trypanothione reductase from *T. cruzi*



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3	Structure R_1	Structure R_2	*Plasmodium falciparum* % red. at 0.2 μM[a]	*Trypanosoma cruzi* epimastigote (GI_{50} μM)[b]	*Trypanosoma cruzi* trypomastigote[c] dose (mM)	*Trypanosoma cruzi* trypomastigote[c] % lysis	Trypanothione reductase inhibition at 100 μM (%)[d]
a	H	H	55	3.7 ± 1	0.23	13 ± 3	72
b	H	8-OMe	7	14.3 ± 3	0.20	26 ± 7	87
c	H	5-OMe	78	9.8 ± 22	0.20	0	80
d	H	6-OMe	78	6.6 ± 2	0.20	9 ± 3	63
e	H	7,8-di-OMe	51	inactive	0.18	19 ± 5	29
f	2-NO_2	H	45	inactive	0.19	54 ± 12	45
	Chloroquine		100	—	—	—	—
	Nifurtimox		—	21 ± 4	—	—	—
	Benznidazole		—	37 ± 6	—	—	—
	Gentian violet		—	—	0.61	100	—

[a]Percentage reduction of in vitro parasitemia of *P. falciparum* at 0.2 μM.
[b]Growth inhibitory activity (GI_{50}) of *T. cruzi* epimastigotes in culture.
[c]Percentage lysis of trypomastigotes of *T. cruzi* in infected murine blood after 24 h contact at 4 °C and at the doses indicated.
[d]Percentage inhibition of trypanothione reductase activity after 30 min of drug incubation with the enzyme.

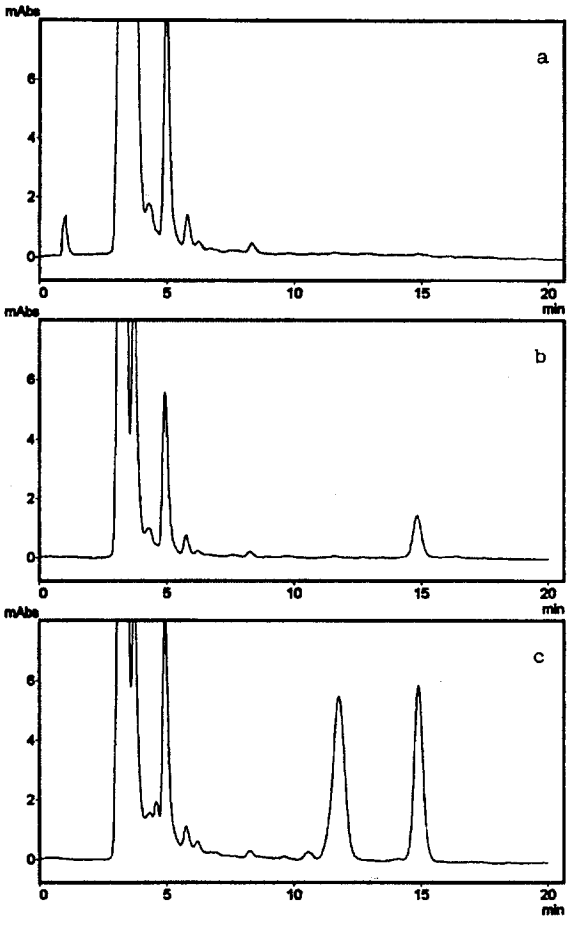

Figure 2. (a) Chromatogram of pooled serum from three untreated mice; (b) Chromatogram of normal serum spiked with standard **3a** solution; (c) Chromatogram of pooled serum from five mice 1 h after oral administration of a single 2.3 mmol/kg dose of **3a**. See Experimental section for conditions.

drug. Experiments to approach these questions are being carried out.

Our results opened new questions about the low in vivo efficacy of **3a**, that is, why the schizonts of *P. berghei* can survive in the mice blood where this quinone attains much higher concentrations than in the in vitro assay. As the parasites live inside the erythrocytes, this could be explained by slow uptake, rapid elimination, intracellular compartmentalization or metabolic inactivation of the drug by *P. berghei* schizonts.

The use of gentian violet to clear banked blood from *T. cruzi* was reported in 1953.[38] The discovery of important side effects[4,39] accelerated the search of substitutes for this triphenylmethane dye.[6,7,40–43] The lysis of trypomastigotes in murine blood incubated at 4 °C is the model of choice to detect active compounds that eliminate *T. cruzi* from banked blood. Of the quinones tested here, **3a–3d** showed significant activity against *T. cruzi* epimastigotes grown at 28 °C but not against trypomastigotes in murine blood (Table 1), indicating a poor correlation between these assays as reported by others.[44] The reason for this difference is not known, but may

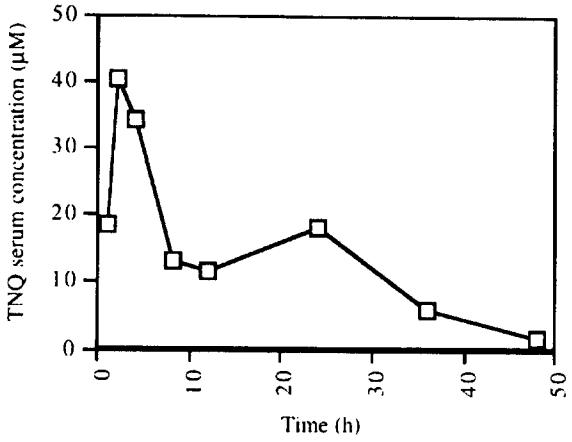

Figure 3. Serum concentration of **3a** in mice after a single oral administration of 2.3 mmol/kg.

result from the lack of metabolic activation of the drug at 4 °C or biochemical differences between these two developmental stages. Thus, despite being safer and simpler than the trypomastigote assay, the epimastigote assay should not be used as a prescreen test for new agents to treat blood infected with *T. cruzi*. Compound **3a** could be produced in larger quantities and was administered at 2.3 mmol/kg in a single oral dose per os to mice experimentally infected with the Y strain of *T. cruzi* (data not shown). This treatment did not affect the parasitaemia when compared to untreated controls, a result we attribute to the metabolism of the drug and/or to differences in the parasites' biochemistry.

In an attempt to understand the mechanism of action of the quinones **3a–3f** against epimastigotes we tested them against the enzyme trypanothione reductase (TR), one of the most promising targets for drug development in Chagas' disease. This enzyme performs a crucial role in the maintenance of the redox equilibrium in these parasites. Following its discovery in 1985,[10] it was shown that certain naphthoquinones (subversive substrates) were potent inhibitors of TR. In the presence of oxygen, these compounds were cyclically reduced and re-oxidized generating deleterious oxygen radicals while at the same time inhibiting TR's ability to reduce its physiological substrate, trypanothione disulfide.[45] Subjected to an assay similar to that used by Henderson[45] the quinones (100 μM) were allowed to interact with the enzyme (1–30 min) and, after addition of substrate, the activity was measured spectrophotometrically by NADPH consumption. The results are summarized in Table 1. In spite of having some activity on epimastigotes and trypomastigote forms of *T. cruzi*, the quinones were almost inactive as inhibitors of TR. Apparently, there is no direct correlation among TR inhibition and the observed trypanocidal effect for the compounds studied here. However, it is worth noting that quinones with one methoxy group, specially at peri-position (**3b** and **3c**), had inhibitory activity against TR which are slightly better than the parent compound (**3a**) or those with two methoxy groups (**3e**).

Conclusion

Our results indicate that despite their significant activity in vitro against *P. falciparum* and against the epimastigote forms of *T. cruzi*, the NTQs described here need to be further modified in order to improve their in vivo activity. Side chains that are positively charged in physiological medium[26,45] or substituents that change the redox potential of the quinonoid ring would be good starting points for development of new derivatives. Furthermore, preliminary results point to a possible difference in the concentration of the drug within human and mice erythrocytes and/or difference in susceptibility between *P. falciparum* and *P. berghei* to the quinone **3a**. Experiments to confirm these hypothesis are in progress.

Experimental

Melting points were determined on a Mettler FP-145 apparatus and are uncorrected. Proton and ^{13}C NMR were run on a Bruker AC-200 spectrometer using TMS as internal standard. Mass spectra were recorded in a Finnigan-MAT 95 spectrometer. IR spectra were obtained on a Mattson–Galaxy series FTIR-3000. Precoated TLC plates of silica gel (Merck) were used to monitor reaction development. Column liquid chromatography (silica gel 200–400 mesh, Merck) was used for product isolation from reaction mixtures.

Synthetic procedures

Naphtho[2,3-*b*]thiophen-4,9-quinone (3a). A procedure based on that published by Gonçalves and Brown[29] (Scheme 1) was adopted: phthalic anhydride (**4**) (20 mmol) was dissolved in methylenedichloride (25 mL) and the solution added dropwise over a suspension of aluminum chloride (44 mmol) in the same solvent (25 mL). The mixture was stirred during 30 min at temperatures below 30 °C after which a solution of thiophene (20 mmol) was slowly added with vigorous stirring. After 2 h, the reaction was stopped by adding 4 N HCl (100 mL) and the aqueous phase extracted with methylenedichloride. The organic phases were combined and extracted with 1 N NaOH. The thenoylbenzoic acid (**5**) was then precipitated by acidification with 2 N HCl. This crude compound was then cyclized and oxidized by heating in concentrated sulfuric acid (100 °C, 2 h). After cooling, crushed ice was added to the dark-red reaction mixture and crude **3a** (4968-81-4) precipitated. Recrystallization of the precipitate from methanol–acetic acid afforded the pure quinone in 53% overall yield. Bright-yellow needles from MeOH–AcOEt, mp 228–231 °C (lit.[29] 227–228 °C, from AcOH); IR ν_{max} (KBr cm^{-1}) $\nu_{C=O}$ 1670, ν_{CS} 1310, δ_{Ar-H} 1180; ^1H NMR (200 MHz, $CDCl_3$) δ 7.75 (m, 4 H, H_1, H_2, H_6, H_7), 8.25 (m, 2 H, H_5 and H_8); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.2 and 179.4 (C_4 and C_9), 145.4 and 142.9 ($C_{3'}$ and $C_{9'}$), 133.6 and 133.4 ($C_{4'}$ and $C_{8'}$), 127.4 (C_5), 126.9 (C_8), 133.7 (C_6), 133.9 (C_7), 134.2 (C_2) and 126.9 (C_3); LREIMS *m/z* 214 (M^+), 186 $(M-CO)^+$, 158 $(M-2CO)^+$. HREIMS *m/z* 214.0093 $(M)^+$, calcd for $C_{12}H_6O_2S$ = 214.0088

2-Nitro-naphtho[2,3-*b*]thiophen-4,9-quinone (3f). Quinone **3a** (10 mmol) was slowly added to ice-cooled fuming nitric acid (50 mL) and after the addition was completed, the temperature was raised to 80 °C. The reaction mixture was stirred for 2 h and the crude product precipitated by the addition of ice.[30] The precipitate was extracted with diethyl-ether and recrystallized from acetic acid affording pure **3f** in 30% yield as bright-yellow needles, mp 238–240 °C (lit.[30] 239–240 °C, from AcOH); IR ν_{max} (KBr, cm^{-1}) ν_{Ar-H} 3080, $\nu_{C=O}$ 1670, $\nu_{Ar\ C=C}$ 1540, ν_{NO_2} 1520, 1340, ν_{NO_2} 820; ^1H NMR (200 MHz, $CDCl_3$) δ 8.40 (s, 2H, H_3), 8.28 (m, 2H, H_5 and H_8), 7.85 (m, 2H, H_6 and H_7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177,8 (C_4 and C_9), 140.9 ($C_{3'}$ and $C_{9'}$), 133.1 ($C_{4'}$) and ($C_{8'}$), 127.5 and 127.9 (C_5 and C_8), 134.9 and 134.4 (C_6 and C_7); 77.2 (C_2); LREIMS *m/z* 259 $(M)^+$, 213 $(M-NO_2)^+$, 185 $(M-NO_2-CO)^+$, 157 $(M-NO_2-2CO)^+$, 229 $(M-NO)^+$, 201 $(M-NO-CO)^+$, 173 $(M-NO-2CO)^+$; HREIMS *m/z* 258.9956 $(M)^+$, calcd for $C_{12}H_5O_4NS$ = 258.9939

General procedure for preparation of methoxylated naphtho[2,3-*b*]thiophen-4,9-quinones. The protocol developed by Watanabe and Snieckus was used (Scheme 2).[35] To a solution of *sec*-butyl lithium (2.1 mmol) and tetramethyl ethylenediamine (2.1 mmol) in dry tetrahydrofuran (THF) (50 mL), cooled to −78 °C and kept under inert atmosphere, a solution of the benzamide (2 mmol) in THF was slowly added and left to react for 1 h. Thiophen-3-carboxaldehyde (2.1 mmol) was then added dropwise and allowed to react for 15 min before a second batch of *sec*-butyl lithium (8 mmol) was added to the reaction mixture. After 1 h at −78 °C, the cooling bath was removed and the reaction mixture allowed to reach room temperature. The reaction was stopped by addition of a saturated solution of NH_4Cl and the THF removed by rotary evaporation under reduced pressure. The reaction products were extracted from the aqueous phase with methylenedichloride or diethyl ether to yield the crude product. This material was oxidized in acetone with Jones' reagent to afford the quinones with the yields indicated in Scheme 2.

8-Methoxy-naphtho[2,3-*b*]thiophen-4,9-quinone (3b) (163459-39-0). Using the tandem procedure described above and 2-methoxy-*N*,*N*-diethylbenzamide (**6a**) as starting material this quinone was produced in 5% overall yield as bright yellow needles from MeOH–AcOEt, mp 164–166 °C (lit.[36] oil); IR ν_{max} (KBr, cm^{-1}) ν_{Ar-H} 3100, ν_{OCH_3} 2920, 2850, $\nu_{C=O}$ 1660, $\delta_{as\ Ar-O-C}$ 1280; ^1H NMR (200 MHz, $CDCl_3$) δ 7.90 (dd, 1H, J = 1 and 8 Hz, H_5), 7.69 (dd, 1H, J = 8.5 and 8 Hz, H_6), 7.65 (d, 1H, J = 5.1 Hz, H_2), 7.62 (d, 1H, J = 5.1 Hz, H_3), 7.32 (dd, 1H, J = 1 and 8.5 Hz, H_7), 4.04 (s, 3H, OMe); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178 and 179 (C_4 and C_9), 141 and 148 ($C_{3'}$ and $C_{9'}$), 136 ($C_{4'}$), 120.9 ($C_{8'}$), 120.3 (C_5), 135.1 (C_6), 118.1 (C_7), 160.6 (C_8), 133.2 (C_2),

126.2 (C_3), 56.6 (OMe); LREIMS *m/z* 244 $(M)^+$, 215 $(M\text{-}CHO)^+$, 186 $(M\text{-}2CHO)^+$, 158 $(M\text{-}2CHO\text{-}CO)^+$; HREIMS *m/z* 244.0188 $(M)^+$, calcd for $C_{13}H_8O_3S$ = 244.0194

5-Methoxy-naphtho[2,3-*b*]thiophen-4,9-quinone (3c). Using the general procedure described above with 3-methoxy-*N*,*N*-diethylbenzamide (**6b**) as starting material this quinone was produced in 10% overall yield as bright-yellow needles from MeOH–AcOEt, mp 179–181 °C; IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar\text{-}H}$ 3080, ν_{OCH_3} 2930, 2850, $\nu_{C=O}$ 1660, $\delta_{as\ Ar\text{-}O\text{-}C}$ 1260 ; ^1H NMR (200 MHz, $CDCl_3$) δ 7.90 (dd, 1H, *J* = 1 and 8.5 Hz, H_8), 7.68 (t, 1H, *J* = 8.5 Hz, H_7), 7.34 (d_{br}, 1H, 8.5 Hz, H_6), 7.66 (d, 1H, *J* = 5.1 Hz, H_3), 7.7 (d, 1H, *J* = 5.1 Hz, H_2), 4.04 (s, 3H, OMe); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.8 and 179.9 (C_4 and C_9), 145.3 and 136.8 ($C_{3'}$, $C_{9'}$ and $C_{8'}$); 161.4 (C_5), 119 (C_6), 134.9 (C_7), 120.5 (C_8 and $C_{4'}$), 135.5 (C_2), 128 (C_3), 57.2 (OMe); LREIMS *m/z* = 244 $(M)^+$, 214 $(M\text{-}CH_2O)^+$, 186, 158 $(M\text{-}CH_2O\text{-}2CO)^+$; HREIMS *m/z* = 244.0189 $(M)^+$, calcd for $C_{13}H_8O_3S$ = 244.0194.

6-Methoxy-naphtho[2,3-*b*]thiophen-4,9-quinone (3d). Using the preceding procedure and the 4-methoxy-*N*,*N*-diethylbenzamide (**6c**) as starting material this quinone was produced in 23% overall yield as bright-yellow needles from MeOH–AcOEt, mp 175–180 °C; IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar\text{-}H}$ 3110, ν_{OCH_3} 2950, 2850, $\nu_{C=O}$ 1670, $\delta_{as\ Ar\text{-}O\text{-}C}$ 1230; ^1H NMR (200 MHz, $CDCl_3$) δ 8,15 (d, 1H, *J* = 8.6 Hz, H_8), 7.69 (s_{br}, 2H, H_2 and H_3), 7.21 (dd, 1H, 8.6 and 2.7 Hz, H_7), 6.65 (d, 1H, *J* = 2,7 Hz, H_5), 3.97 (s, 3H, OMe); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.7 and 179.6 (C_4 and C_9), 142,9 and 146.3 ($C_{3'}$ and $C_{9'}$), 135.8 ($C_{4'}$), 127.2 ($C_{8'}$); 111.3 (C_5), 164.4 (C_6), 120 (C_7), 129.6 (C_8), 133.7 (C_2), 127 (C_3), 56.2 (OMe); LREIMS *m/z* 274 $(M)^+$, 259 $(M\text{-}Me)^+$, 214 $(M\text{-}2CHO)^+$; HREIMS *m/z* 244.0200 $(M)^+$, calcd for $C_{13}H_8O_3S$ = 244.0194

7,8-Dimethoxy-naphtho[2,3-*b*]thiophen-4,9-quinone (3e). Using the tandem procedure and the 2,3-dimethoxy-*N*,*N*-diethylbenzamide (**6d**) as starting material this quinone was produced in 9% overall yield as bright-yellow needles from MeOH–AcOEt, mp 215–217 °C; IR ν_{max} (KBr, cm^{-1}) $\nu_{Ar\text{-}H}$ 3100, ν_{OMe} 2930, 2850, $\nu_{C=O}$ 1660, $\delta_{Ar\text{-}O\text{-}C}$ 1280; ^1H NMR (200 MHz, $CDCl_3$) δ 8,08 (d, 1H, *J* = 8.6 Hz, H_5), 7.68 (d, 1H, *J* = 5.1 Hz H_2), 7.62 (d, 1H, *J* = 5.1 Hz H_3), 7.21 (d, 1H, *J* = 8.6 Hz, H_6), 4.00 (s, 3H) and 3.97 (s, 3H) Ar-O*Me*; ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.9 and 178.5 (C_4 and C_9), 141.9 and 147.6 ($C_{3'}$ and $C_{9'}$), 127.3 ($C_{4'}$), 126.6 ($C_{8'}$), 125.5 (C_5), 115.5 (C_6), 150.3 (C_7), 159 (C_8), 133.7 (C_2), 126.4 (C_3), 61.3 (C_8-O*Me*), 56,3 (C_7-O*Me*); LREIMS *m/z* 274 $(M)^+$, 259 $(M\text{-}Me)^+$, 245 $(M\text{-}CHO)^+$, 214 $(M\text{-}2CHO)^+$, 158 $(M\text{-}2CHO\text{-}CO)^+$; HREIMS *m/z* 274.0306 $(M)^+$, calcd for $C_{14}H_{10}O_4S$ = 274.0300.

Biological studies

In vitro assay with trypomastigotes of *Trypanosoma cruzi*. The bioassays with trypomastigote forms of *T. cruzi* were performed according to a published protocol.[40] In brief, test compounds were dissolved (or suspended) in dimethyl sulphoxide (DMSO) plus Krebs–Ringer-glucose (1:25) and mixed with equal volume of parasitized whole blood diluted in calf serum. A parasite density of 2×10^6/mL was calculated for each flat-bottomed test tube (4 mL, 56 × 13 mm); control experiments with the diluent were run in parallel. After incubation at 4 °C by 24 h, the parasites were counted microscopically. Each experiment was repeated twice, with duplicate tubes.

In vitro assay with epimastigotes of *Trypanosoma cruzi*. All parasites were maintained and tested in liver infusion tryptose (LIT) culture medium supplemented with 10% calf serum. Stock solutions of compounds at 40, 4, 0.4, and 0.04 mM were prepared by dissolving sufficient amount of each quinone in 250 μL of DMSO. Fifty microliters of this solution was diluted in 4 mL of parasite culture containing 10^6 parasites/mL in the logarithmic phase of growth. The final drug concentration was 500, 50, 5, and 0.5 μM. Three aliquots of 1.25 mL/well were transferred to a sterile 24-well tissue culture plate. Positive and negative controls containing either 1.2% DMSO, nifurtimox (IC_{50} 21 μM) or benznidazole (IC_{50} 37 μM) were run in parallel. After four days at 28 °C, the number of parasites was determined using an electronic cell counter. The percentage of growth inhibition was calculated by comparison with the controls containing DMSO. Microscopic observation of the parasites was also performed to check for motility and morphological alterations.

In vitro assay with *T. cruzi* trypanothione reductase (TR). Recombinant *T. cruzi* TR was obtained as described by Borges et al.[46] Enzyme activity was determined spectrophotometrically at 340 nm in 20 mM HEPES, 30 mM NaCl, 0.1 mM EDTA, pH 7.4 containing 150 μM NADPH and 18.4 nM TR (assay volume 0.5 mL). Enzyme was pre-incubated at 27 °C with 100 μM test compound for 1 and 30 min prior to addition of the substrate trypanothione disulfide (50 μM). The test compounds were prepared as stock solutions in DMSO such that the final concentration of solvent was 5.0% (v/v). Control assays contained an equivalent amount of solvent. The results were expressed as percentage reduction in the trypanothione reductase activity.

In vitro assay with *P. falciparum*. Erythrocytes infected with asexual stages of the chloroquine-resistant isolate BHz 26/86 was cultured as described.[47] A stock solution of each quinone (100 mM) was diluted in complete culture medium (RPMI 1640 containing 10% human sera, 2% glutamine and 7.5% $NaHCO_3$). When necessary, 0.25% of Tween 80 was used to help drug dissolution. A suspension of

red blood cells with approximately 1% parasitemia was distributed in a 96-well microtiter plate (100 μL/well). The stock solutions were then added to the wells attaining final concentration of 0.2 μM. Controls without drugs and with chloroquine (0.2 μM) were run in parallel. After 24 and 48 h of incubation at 37 °C under a 5% CO_2 atmosphere, the culture medium was replaced with or without drugs. After 72 h in culture, blood smears were prepared, coded, stained with Giemsa, and examined at 1000 × magnification. The percent reduction of parasitemia was calculated by comparison with the controls without drug. All experiments were run in duplicate.

Preliminary studies on the bioavailability of 3a

A single 2.3 mmol/kg dose of the quinone **3a** was administered per os to four normal male Swiss albino mice. After 1, 2, 4, 8, 12, 36, and 48 h of ingestion, 50 μL of blood from each animal was collected by bleeding the retro-orbital plexus. The blood was pooled and allowed to clot at room temperature for 30 min. The sera were separated by centrifugation (5000 *g*, 10 min) and inactivated by heating at 56 °C for 1 h. A protocol developed to quantify Atovaquone (**1**) by HPLC[48] was adapted to quantify **3a** using a RP-18 column. Briefly, to 50 μL of inactivated sera 100 μL ACN–1% aqueous acetic acid (85:15) was added and the precipitated proteins separated by centrifugation (14,000 *g*, 10 min). The supernatant was analyzed by HPLC on a C-18 reversed-phase column (5 μm particle size, 4.6 × 250 mm) using ACN–10 mM triethylamine in 0.2% aqueous trifluoroacetic acid (65:35) with the detector set at 280 nm.

Acknowledgements

We are grateful to NCI-FCRDC for running the mass spectra of the quinones. This work was supported in Brazil by grants from PAPES-FIOCRUZ, PRONEX. AHF was supported by a grant from the Wellcome Trust. We are also thankful to the CNPq fellows H. Marinuzzi and L. S. R. Soares for running some of the in vitro bioassays with *P. falciparum*

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(*Received* 16 *April* 1997; *accepted* 8 *July* 1997)