Studies on Quinones. Part 37.[1] Synthesis and Biological Activity of *o*-Aminoester Functionalised Benzo- and Naphtho[2,3-*b*]-thiophenequinones

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The synthesis of 3-amino-2-methoxycarbonyl-4,7-dimethoxybenzo[*b*]thiophene (5) and benzothieno[3,2-*d*][1,3]oxazin 15 from 3,6-dimethoxy-2-nitrobenzaldehyde (1) is reported. Benzo[*b*]thiophene-4,7-quinones 9 and 10 were prepared in good yields by oxidative deprotection of the corresponding dimethoxybenzothiophenes 8 and 7. Cycloaddition reaction of quinone 8 with 1-(*E*)-trimethylsilyloxy-1,3-butadiene followed by acid-induced aromatization provides access to naphtho[2,3-*b*]thiophene-4,9-quinone 13 and 14. The *in vitro* activity of the new quinones against *Leishmania amazonensis* and human-T-cell leukemia virus type 1 (HTLV-1) is reported.

Key words benzo[*b*]thiophene-4,7-quinone; oxidative demethylation; Diels–Alder reaction; benzothieno[3,2-*d*][1,3]oxazin-4-one; *Leishmania amazonensis*; HTLV-1

Natural and synthetic quinonoid compounds are well-known substances which possess a variety of biological properties such as antibacterial, antifungal, antiprotozoal, inhibition of the human immunodeficiency virus (HIV)-1 reverse transcriptase and antitumor activity.[2—7] Some of these pharmacological effects have been attributed to the formation of DNA-damaging anion-radical intermediates formed by bioreduction of the quinone nucleus.[8] Among heterocyclic quinones with cytotoxic activity, those containing a thiophene nucleus fused to a quinone system have received little attention[9,10] despite the antitumoral activity of thiophene analogues of daunomycin and mitoxantrone.[11,12]

As part of a program directed towards the design and synthesis of carbo- and heterocyclic quinones as potential antiprotozoal agents, we have reported the synthesis and antiprotozoal activity of benzopyrano- and benzo[*b*]thiophenequinones.[1,13,14] Since the thiophene ring-containing quinones showed promising antiprotozoal activity against *trypanosome cruzi* and strains of *Leishmania* spp.[1,14] we decided to continue the studies on synthesis and biological evaluation of new members of this series containing functional groups on C-2 and C-3 positions of the thiophene ring.

This paper describes the preparation of functionalised benzo[*b*]thiophenes and their application to the synthesis of benzo- and naphthothiophenequinones with *ortho*-aminoester functionality on the thiophene ring. Moreover we report the cytotoxic activity of the new quinones against *Leishmania amazonensis* and human-T-cell leukemia virus type 1 (HTLV-I), the causative agent of two well-defined diseases: adult T-cell leukemia/lymphoma (ATLL) and tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM).[15]

In a previous work we have described the synthesis of a variety of 2-substituted benzo[*b*]thiophene-4,7-quinones using a two step sequence which involves the reaction of dimethoxy-*o*-acylnitroarenes with methyl thioglycolate followed by oxidative deprotection of the corresponding dimethoxybenzo[*b*]thiophene with ceric ammonium nitrate (CAN).[16] On the basis of these results we investigated the synthesis of *ortho*-aminoesters **5** *via* cyclisation of 3,6-dimethoxy-2-nitrobenzonitrile (**3**) with methyl thioglycolate. Compound **5** could be a valuable intermediate in the synthesis of angular tetracyclic thiophene-containing quinones due to the possibility to extend the bicyclic system through either the thiophene and/or the quinone rings.

3,6-Dimethoxy-2-nitrobenzaldehyde (**1**), prepared as reported,[17] was converted into 2,5-dimethoxy-6-nitrobenzonitrile (**3**) in 54% total yield, by reaction with hydroxylamine followed by dehydration of 3,6-dimethoxy-2-nitrobenzaldehyde oxime (**2**) with acetic anhydride. The thiophene ring formation on **3** was attempted by reaction with methyl thioglycolate in *N*,*N*-dimethylformamide (DMF) using potassium carbonate however, methyl (2-cyano-3,6-dimethoxyphenylsulfanyl)acetate (**4**) was isolated as the main product along with small amounts of the methyl 3-amino-4,7-dimethoxybenzo[*b*]thiophene-2-carboxylate (**5**) (^{1}H-NMR). Better results were obtained when the reaction of **3** with methyl thioglycolate was performed using potassium hydroxide in DMF which afforded **5** in 80% yield (Chart 1). Isolation of compound **4** provides evidence for the pathway in which the formation of the thiophene ring is initiated by displacement of the nitro group followed by base-induced aldol condensation.

In order to prepare methyl 3-amino-4,7-dioxo-4,7-dihydrobenzo[*b*]thiophen-2-carboxylate (**6**) from **5**, oxidative demethylation with CAN[16] was attempted; nevertheless, the treatment gave a complex reaction mixture. This result was attributed to intermolecular amination reactions between substrate **5** and the nascent quinone **6**.

In view of this result we decided to protect the amino group in **5** before attempting the release of the corresponding quinone with CAN. Protection of the amino group in **5**, as the corresponding amide, was tried by acetylation of **5** with acetic anhydride at room temperature. This treatment gave a mixture of methyl 3-acetylamino-4,7-dimethoxybenzo[*b*]-

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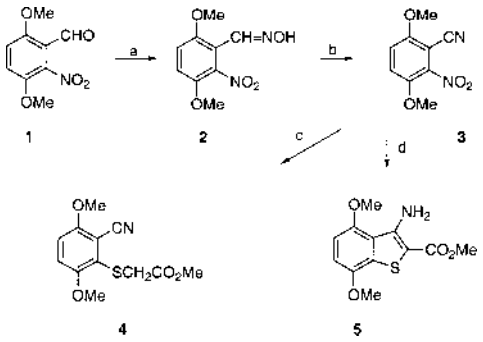
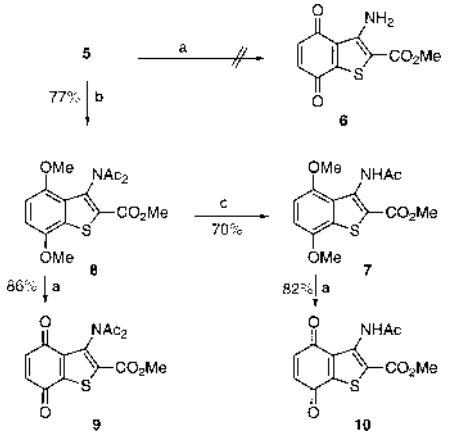


Reagents: (a) $NH_2OH.HCl$, NaOH, EtOH, 67%; (b) Ac_2O, 80%; (c) $HSCH_2CO_2Me$, K_2CO_3, DMF, 43%; (d) $HSCH_2CO_2Me$, KOH, DMF, 80%

Chart 1



Reagents: (a) CAN, MeCN, H_2O; (b) Ac_2O; (c) HCl, MeOH, H_2O

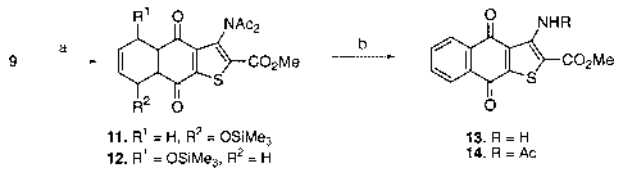
Chart 2

thiophene-2-carboxylate (**7**) together with methyl 3-diacetylamino-4,7-dimethoxybenzo[*b*]thiophene-2-carboxylate (**8**), and all attempts to obtain **7** as the main product were unsuccessful. Preparation of **7** was achieved in 58% total yield by reaction of **5** with acetic anhydride at reflux followed by selective deacylation of **8** (Chart 2).

Compounds **7** and **8** were then submitted to oxidative deprotection with CAN, providing the corresponding stable quinones, methyl 3-acetylamino-4,7-dioxo-4,7-dihydrobenzo-[*b*]thiophene-2-carboxylate (**10**) and methyl 3-diacetylamino-4,7-dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carboxylate (**9**) in 82 and 86% yield, respectively (Chart 2).

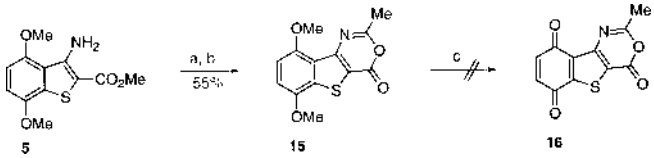
The synthesis of naphtho[2,3-*b*]thiophenquinones from quinone **9** and 1-(*E*)-trimethylsilyloxybuta-1,3-diene was attempted. The reaction of **9** and the diene at room temperature gave a mixture of regioisomers, methyl 3-diacetylamino-5-trimethylsilyloxy-4,9-dioxo-4,4a,5,8,8a,9-hexahydronaphtho[2,3-*b*]thiophene-2-carboxylate (**11**) and methyl 3-diacetylamino-8-trimethylsilyloxy-4,9-dioxo-4,4a,5,8,8a,9-hexahydronaphtho[2,3-*b*]thiophene-2-carboxylate (**12**) which, by reaction with 5% hydrochloric acid in THF gave a mixture of methyl 3-amino-4,9-dioxo-4,4a,5,8,8a,9-hexahydronaphtho[2,3-*b*]thiophene-2-carboxylate (**13**) and methyl 3-acetylamino-4,9-dioxo-4,4a,5,8,8a,9-hexahydronaphtho[2,3-*b*]thiophene-2-carboxylate (**14**) in 74 and 26% yields, respectively (Chart 3).

Heterocyclisation of compound **5** to 6,9-dimethoxy-4*H*-



Reagents: (a) 1-TMSO-1,3-butadiene, CH_2Cl_2 ; (b) THF-HCl aq

Chart 3



Reagents: (a) NaOH aq; (b) Ac_2O; (c) CAN, MaCN, H_2O

Chart 4

Table 1. IC_{50} and TC_{50} Values of Quinones **9**, **10**, **13** and **14**

Quinone	IC_{50} (μM)	TC_{50} (μM)
9	15	15
10	>75	8
13	>80	>80
14	1.56	4
Amphotericin B	0.1	5

[1]benzothieno[3,2-*d*][1,3]oxazin-4-one (**15**) was attempted by reaction with sodium hydroxide, followed by reaction of the resulting sodium carboxylate with acetic anhydride using a reported methodology.[18] The treatment provided heterocycle (**15**) and all attempts to obtain quinone **16** by oxidative demethylation of **15** with CAN were unsuccessful and the starting material was recovered in these experiments (Chart 4).

Compounds **9**, **10**, **13** and **14** were evaluated *in vitro* against intracellular *Leishmania amazonensis* amastigotes stage in mouse peritoneal macrophages. The IC_{50} and TC_{50} were determined using amphotericin B as the reference drug, and the results are summarized in Table 1. The results indicate that the thiophene-containing quinones **9**, **10** and **14** have cytotoxic activity which is probably due in part to the quinone nucleus. Comparison of the activity between quinones **13** and **14** suggest that the electron-donating effect of the amino group decreases the biological effect probably by lowering the oxidant capacity of the quinone system.

Compounds **9**, **10**, **13** and **14** were evaluated on HTLV-1 transformed HUT-102 cell lines infected with 250000 cells/ml. These compounds displayed a reduction in cellular proliferation by 75% (see Table 2) but induced a weak cellular viability after the quinone treatment.

In conclusion, we have reported the synthesis and the *in vitro* leishmanicidal and anti-HTLV-1 activity of benzo- and naphtho[2,3-*b*]thiophene-4,7-quinones containing the *ortho*-aminoester functionality on the thiophen ring. The biological activity of these highly functionalised heterocyclic quinones and the possibility to construct nitrogen heterocycles fused to the thiophene ring represents an advance in the search for novel antiprotozoal and antiviral agents.

Table 2. Inhibition of HTLV-1 Transformed Cell Lines (HUT-102) by Quinones **9**, **10**, **13** and **14**

Quinone	% Inhibition
9	10 μM : 75
	1 μM : 5
10	10 μM : 84
	1 μM : 0
13	10 μM : 41
	1 μM : 0
14	10 μM : 86
	1 μM : 0

Experimental

All reagents were of commercial quality, reagent grade, and were used without further purification. Mps were determined on a Köfler hot-stage apparatus and are uncorrected. The IR spectra were recorded on a FT Bruker spectrophotometer using KBr discs and the wave numbers are given in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were acquired at 200 and 50 MHz, respectively, on a Bruker AC-200P in deuteriochloroform. Chemical shifts are reported in δ ppm downfield to tetramethylsilane (TMS), and *J*- values are given in Hertz. Silica gel Merck 60 (70—230 mesh), and TLC aluminiun foil 60 F_{254} were used for preparative column and analytical TLC, respectively. Compound **1** was prepared from commercially available 2,5-dimethoxybenzaldehyde as previously reported.[19]

2,5-Dimethoxy-6-nitrobenzaldehyde Oxime (2) A suspension of 2,5-dimethoxy-6-nitrobenzaldehyde (**1**) (300 mg, 1.42 mmol), hydroxylamine hydrochloride (117.5 mg, 1.69 mmol), aqueous sodium hydroxide (2 ml, 10%) and ethanol (10 ml) was stirred for 30 min at 60 °C. The mixture was poured into acetic acid (15 ml, 5%) and the precipitate was recrystallized from ethanol to afford **2** as a pale yellow solid (214 mg, 67%). mp 115—117 °C (ethanol); ^{1}H-NMR δ: 3.86 (s, 6H, OMe), 6.96 (d, 1H, *J*=9.2), 7.04 (d, 1H, *J*=9.2), 7.59 (br s, 1H), 8.34 (s, 1H); ^{13}C-NMR δ: 186.1, 155.3, 144.5, 120.1, 116.2, 114.2, 57.3, 56.8; *Anal.* Calcd for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.38. Found: C, 47.66; H, 4.51; N, 12.32.

3,6-Dimethoxy-2-nitrobenzonitrile (3) A solution of **2** (200 mg, 0.88 mmol) in acetic anhydride (20 ml) was heated at reflux for 3 h. The mixture was poured into ice-water and the precipitate was filtered and washed with water. The solid was purified by column chromatography over silica gel with chloroform to give compound **3** as yellow solid (147 mg, 80%). mp 150—152 °C (hexane); IR: 2231 and 1534; ^{1}H-NMR δ: 3.91 (s, 3H), 3.96 (s, 3H), 7.11 (d, 1H, *J*=9.4), 7.29 (d, 1H, *J*=9.4); ^{13}C-NMR δ: 155.3, 143.2, 119.4, 114.7 111.0, 97.0, 57.5, 57.1; *Anal.* Calcd for $C_9H_8N_2O_4$: C, 51.93; H, 3.87; N, 13.46. Found: C, 51.73; H, 3.89; N, 13.29.

(2-Cyano-3,6-dimethoxyphenylsulfanyl)acetate (4) A suspension of **3** (200 mg, 0.96 mmol), methyl thioglycolate (110 mg, 0.95 mmol), and potassium carbonate (158 mg) in DMF (5 ml) was stirred for 2 h at 60 °C. The mixture was poured into cooled diluted hydrochloric acid (50 ml, 10%) The precipitate was filtered, washed with water and the solid was recrystallized from ethanol to afford pure **4** as a pale pink solid (110 mg, 43%). mp 139—140 °C (hexane); IR: 2224, 1740; ^{1}H-NMR δ: 3.65 (s, 3H), 3.70 (s, 2H), 3.88 (s, 6H), 6.92 (d, 1H, *J*=9.2), 7.06 (d, 1H, *J*=9.2); ^{13}C-NMR δ: 169.6, 156.0, 154.1 125.9, 116.2, 114.5, 112.6, 108.5, 56.6, 56.5, 52.5, 35.7; *Anal.* Calcd for $C_{12}H_{13}NO_4S$: C, 53.92; H, 4.90; N, 5.24; S, 11.99. Found: C, 53.78; H, 4.95; N, 5.39: S, 11.86.

Methyl 3-Amino-4,7-dimethoxybenzo[*b*]thiophene-2-carboxylate (5) A solution of **3** (200 mg, 0.96 mmol), methyl thioglycolate (110 mg, 0.95 mmol), and potassium hydroxide (65 mg) in DMF (5.0 ml) was stirred for 2 h at 80 °C. The solution was diluted with water and the precipitate was filtered and washed with water. The solid was purified by flash chromatography with chloroform to afford **5** as a white solid (205 mg, 80%). mp 146—147 °C (ethanol); IR: 3479, 3372, 1674; ^{1}H-NMR δ: 3.86 (s, 3H), 3.89 (s, 3H), 3.90 (s, 3H), 6.56 (d, 1H, *J*=8.5), 6.71 (d, 1H, *J*=8.5), 6.71 (br s, 2H, exchangeable with D_2O); ^{13}C-NMR δ: 165.9, 151.8, 151.1, 148.6, 131.3; 122.1, 107.6, 104.3, 95.7, 56.0, 55.8, 51.2; *Anal.* Calcd for $C_{12}H_{13}NO_4S$: C, 53.92; H, 4.90; N, 5.24; S, 11.99. Found: C, 53.81: H, 4.92; N, 5.21; S, 12.02.

Methyl 3-Diacetylamino-4,7-dimethoxybenzo[*b*]thiophene-2-carboxylate (8) A solution of **5** (200 mg, 0.75 mmol) in acetic anhydride (20 ml) was refluxed for 3 h. The mixture was poured into ice-water and the precipitate was filtered and washed with water. Purification of the solid by column chromatography on silica gel (chloroform) gave pure **8** as a pale yellow solid (202 mg, 77%). mp 157—159 °C (ethanol); IR: 3372, 1719, 1699; ^{1}H-NMR δ: 2.30 (s, 6H), 3.82 (s, 3H), 3.89 (s, 3H), 3.95 (s, 3H), 6.69 (d, 1H, *J*=8.5), 6.81 (d, 1H, *J*=8.5); ^{13}C-NMR δ: 172.4, 161.5, 150.4, 148.4, 136.6, 130.1, 127.4, 126.5, 107.4, 106.1, 56.1, 56.0, 52.6, 26.0; *Anal.* Calcd for $C_{16}H_{17}NO_6S$: C, 54.69; H, 4.88; N, 3.99; S, 9.12. Found: C. 54.68; H, 4.86; N, 4.03; S, 9.26.

Methyl 3-Acetylamino-4,7-dimethoxybenzo[*b*]thiophene-2-carboxylate (7) A solution of **8** (110 mg, 0.31 mmol), hydrochloric acid (1 ml, 18%) and methanol (5 ml) was refluxed for 1 h. The mixture was diluted with water and the solid was column chromatographed on silica gel (dichloromethane) to yield pure **7** as a white solid (67 mg, 70%). mp 185—187 °C (ethanol); IR: 3254, 1700 and 1667; ^{1}H-NMR δ: 2.20 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 6.65 (d, 1H, *J*=8.5), 6.72 (d, 1H, *J*=8.5), 8.62 (s, 1H); ^{13}C-NMR δ: 168.4, 163.2, 150.6, 148.3, 134.4, 129.7, 125.1, 119.1, 106.7, 105.8, 56.1, 23.8; *Anal.* Calcd for $C_{14}H_{15}NO_5S$: C, 54.36; H, 4.89; N, 4.53; S, 10.36. Found: C, 54.24; H, 4.91; N, 4.50; S, 10.35.

Methyl 3-Diacetylamino-4,7-dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carboxylate (9) To a solution of compound **8** (200 mg, 0.57 mmol) in acetonitrile (6 ml) was added with stirring a solution of CAN (1.25 g, 2.28 mmol) in water (6 ml). The mixture was stirred for 30 min at room temperature, diluted with water, and extracted with chloroform (3×10 ml). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was chromatographed on silica gel (chloroform) to yield pure **9** as yellow crystals (157 mg, 86%). mp 128—130 °C (hexane); IR: 1720, 1598, 1587; ^{1}H-NMR δ: 2.32 (s, 6H), 3.94 (s, 3H), 6.83 (d, 1H, *J*=10.5), 6.97, (d, 1H, *J*=10.5); ^{13}C-NMR δ: 179.9, 179.3, 171.7, 159.8, 144.8, 139.7, 139.0, 137.5, 135.0, 134.6, 53.3, 26.0; *Anal.* Calcd for $C_{14}H_{11}NO_6S$: C, 52.33; H, 3.45; N, 4.36; S, 9.98. Found: C, 52.21; H, 3.48; N, 4.41; S, 9.94.

Methyl 3-Acetylamino-4,7-dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carboxylate (10) A solution of compound **7** (100 mg, 0.32 mmol) in acetonitrile (6 ml) was added with stirring to a solution of CAN (702 mg, 1.28 mmol) in water (6 ml) and the mixture was stirred for 30 min at room temperature. Work-up of the mixture followed by purification of the crude by column chromatography over silica gel (chloroform) afforded **10** (73 mg, 82%). mp 122—124 °C (hexane); IR; 3325, 1727, 1583; ^{1}H-NMR δ: 2.27 (s, 3H), 3.94 (s, 3H), 6.83 (d, 1H, *J*=10), 6.91 (d, 1H, *J*=10), 9.11 (s, 1H); ^{13}C-NMR δ: 181.2, 179.5, 168.0, 162.1, 143.5, 139.0, 138.1, 137.5, 131.7, 125.1, 52.9, 23.9; *Anal.* Calcd for $C_{12}H_9NO_5S$: C, 51.61; H, 3.25; N, 5.02; S, 11.48. Found: C, 51.23; H, 3.19; N, 5.01; S, 11.05.

3-Acetylamino- and 3-Amino-2-methoxycarbonynaphtho[*b*]thiophene-4,9-quinone (13) and (14) A solution of **9** (100 mg, 0.31 mmol) and (*E*)-1-trimethylsilyloxy-1,3-butadiene (0.811 g, 5.7 mmol) in dichloromethane (3 ml) was stirred for 2 d at room temperature. After the solvent was removed under reduced pressure, the residue was dissolved in THF-hydrochloric acid (20 ml, 5%), and the solution was left at room temperature for 1 h. The mixture was diluted with water, extracted with chloroform (3×10 ml) and the organic extract was dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography (chloroform) of the residue afforded **13** (less polar compound) as a deep red solid (66 mg, 74%). mp 179—181 °C (petroleum ether); IR: 3463, 3346, 1657; ^{1}H-NMR δ: 3.91 (s, 3H), 7.07 (br s, 2H), 7.77 (m, 2H), 8.22 (m, 2H); ^{13}C-NMR δ: 180.2, 178.4, 164.3, 153.5, 146.9, 134.3, 133.7, 133.6, 133.3, 127.7, 127.1, 127.0, 107.1, 51.9; *Anal.* Calcd for $C_{14}H_9NO_4S$: C, 58.53; H, 3.16; N, 4.88; S, 11.16. Found: C, 58.32; H, 3.24; N, 4.76; S, 10.86.

Further elution with chloroform afforded pure **14** (25 mg, 26%) as yellow solid. mp 163—165 °C (1 : 1 hexane–benzene); IR: 3380, 1753, 1595; ^{1}H-NMR δ: 2.31 (s, 3H), 3.95 (s, 3H), 7.81 (m, 2H), 8.23 (m, 2H), 9.45 (br s, 1H); ^{13}C-NMR δ: 179.2, 176.3, 165.2, 160.6, 156.9, 147.7, 136.9, 133.0, 132.6, 130.5, 129.1, 127.9, 127.5, 118.9, 50.9, 24.1; *Anal.* Calcd for $C_{16}H_{11}N_5O_5S$: C, 58.35; H, 3.37; N, 4.25; S. 9.74. Found: C, 58.30; H, 3.33; N, 4.21; S, 9.80.

6,9-Dimethoxy-4*H*-[1]benzothieno[3,2-*d*][1,3]oxazin-4-one (15) A solution of **5** (300 mg, 1.12 mmol), sodium hydroxide (70 mg, 1.75 mmol) and ethanol (30 ml) was refluxed for 2 h. The mixture was cooled and the precipitate was filtered, washed with ethanol and dried. A suspension of the sodium salt (200 mg, 0.6 mmol) in acetic anhydride (10 ml) was refluxed for 3 h and the mixture was cooled overnight. The precipitate was filtered, washed with ethanol, and purified by column chromatography over silica gel (chloroform) to give pure **15** as a pale green solid (170 mg, 55%), mp 293—294 °C (methanol); IR: 1740; ^{1}H-NMR δ: 2.53 (s, 3H), 3.91 and 3.94 (2s, 6H), 6.76 (d, 1H, *J*=8.5), 6.86 (d, 1H, *J*=8.5); ^{13}C-NMR δ: 194.1, 163.9, 151.9, 150.8, 148.6, 118.2, 109.2, 107.0, 100.5, 56.6, 56.2, 21.7. *Anal.* Calcd for $C_{13}H_{11}NO_4S$: C, 56.31; H, 4.00; N, 5.05; S, 11.56. Found: C, 56.21; H,

3.98; N, 4.98: S, 11.44.

Bioassays. ***In Vitro* Activity against *Leishmania amazonensis*** The isolation of macrophages and parasites (*L. amazonensis*, strains LV79) was described previously in full details.[19] For all drugs, stock solutions were prepared in DMSO at a concentration of 100 mg/ml. Two fold serial dilutions were made from 500 μg/ml in culture medium supplemented with 0.5% DMSO final. Twenty-four hours after infection, freshly prepared drugs were added to the infected cultures decreasing both the first final drug concentration to 100 μg/ml and the final DMSO concentration to 0.1%. This DMSO concentration was proven to have no effect on control cultures. Thirty hours after drug addition, infected cultures were examined using an inverted phase contrast Zeiss microscope (magnification of 400). Note was made of toxic effects in the host cells as evidenced by the change in morphological features, *i.e.*, loss of refringency, vacuolation of cytoplasm or loss of cytoplasmic material. Leishmanicidal effects of drugs are easily detectable by observing the regression of parasitophorous vacuoles (PV) and the overall decrease in parasite number. Under the best conditions, complete clearance of amastigotes can be achieved.

Cell Lines and Cell Proliferation Assay[20] HUT-102 are HLTV-1-transformed cell lines and were grown in RPMI-1640 medium supplemented with 10% fetal calf serum, L-Gln, and penicillin–streptomycin. To measure cellular proliferation or viability, a cell proloferation-viability kit (XTT; Roche Molecular Biochemicals) was used. In this assay, tetrazolium salt XTT is cleaved to form an orange formazan dye by metabolic active cells. This dye is directly quantified using an enzyme-linked immunosorbent reader at 492 nm.

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References and Notes

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