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Studies on quinones. Part 35: Access to antiprotozoal active euryfurylquinones and hydroquinones☆

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Abstract—(+)-Euryfuran adds regiospecifically to activated monosubstituted 1,4-benzoquinones under mild conditions to give the corresponding Michael adducts which, depending on the quinone substituent, undergo in situ redox reactions to the respective euryfurylbenzoquinones. One of these Michael adducts undergoes a facile stereoselective cyclisation under oxidant conditions to afford a naphthofuro[4,3-*c*]benzopyran derivative. The in vitro activities of the obtained euryfurylquinones and hydroquinones against *Leishmania amazonensis* are described.

1. Introduction

Among the broad structural variety of naturally occurring quinones and hydroquinones those having a sesquiterpene skeleton occupy a special place. These natural products have attracted much interest in recent years due to their inherent biological properties such as antitumour activity,[2] inhibition of the HIV 1 reverse transcriptase,[3] and immunomodulation.[4]

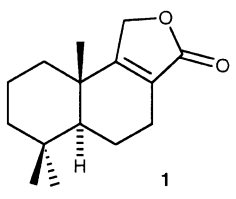
As part of our current research program towards the synthesis of potential bioactive quinones[5–7] we have initiated studies on sesquiterpene quinones and hydroquinones derived from (+)-euryfuran **2**, an antitumoral drimane.[8] It is known that 1,4-benzoquinones bearing an electron-withdrawing substituent (activated quinones) are very reactive with nucleophiles which add regiospecifically on the 3 position to give a variety of products.[9–12]

The behaviour of these 1,4-benzoquinones could be attributed to the quinone substituent which greatly enhances the reactivity of the 3 position, allowing nucleophilic addition to take place at this position, under very mild conditions. Based on these precedents and those reported by Eugster and co-workers[13] and Kraus and Roth[14] on the furylation reaction of some activated quinones we decided to explore the possibility to prepare euryfurylquinones and hydroquinones by linking (+)-euryfuran to activated 1,4-benzoquinones.

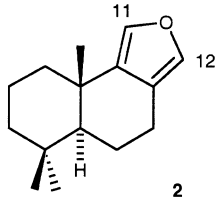
We report here results[15] on the Michael reaction of (+)-euryfuran (**2**) with activated monosubstituted 1,4-benzoquinones which provides a regiospecific access to antiprotozoal active euryfuran derivatives containing a quinone or hydroquinone fragment bonded to the 12 position.

2. Results and discussion

(+)-Euryfuran (**2**) was prepared via a two-step sequence from natural (+)-confertifolin (**1**) according to our



1



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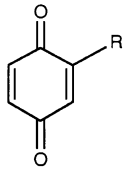

3a. R = CHO
b. R = COMe
c. R = CO_2Me
d. R = NO_2
e. R = CN

☆ For Part 34 of this series see Ref. 1.

Keywords: activated 1,4-benzoquinones; Michael reaction; euryfurylquinones; leishmanicidal activity.

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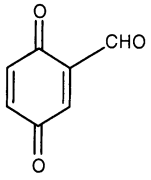
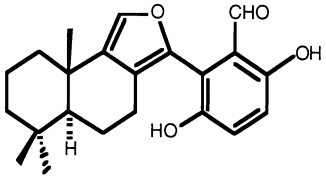
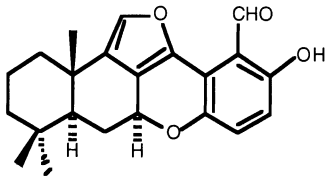
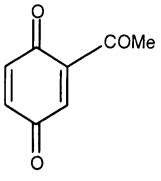
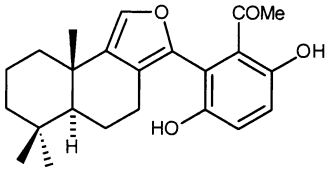
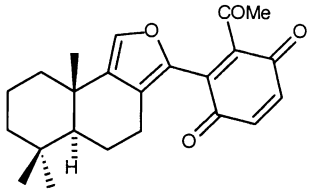
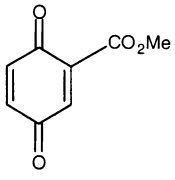
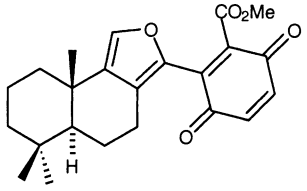
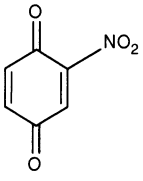
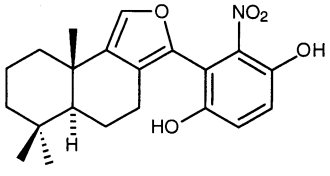
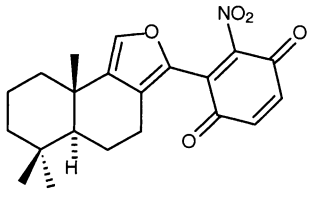
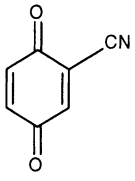
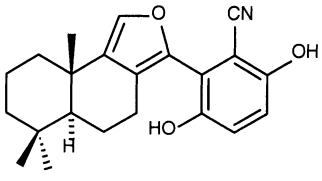
previously published procedure.[16] 1,4-Benzoquinones **3a–e** were selected as Michael acceptors.

The reaction of furan **2** with the unstable quinone **3a** was firstly examined by in situ generating **3a** from 2,5-dihydroxybenzaldehyde and silver(I) oxide in dichloromethane at room temperature. The reaction proceeds rapidly to afford two products, which were isolated by flash chromatography. The major product was characterised as Michael adduct **5a** and the minor showed spectral properties in accord with compound **4** (Table 1). Interestingly, when quinone **3a**, prepared from 2,5-dihydroxybenzaldehyde and silver(I) oxide in a separate procedure, was treated with euryfuran **2** in benzene at room

Table 1. Products generated by reaction of furan **2** with activated quinones **3**

Quinone	Method[a] (yield)	Products
 **3a**	A, **5a** (35) **4** (20) B, **5a** (90)	 **5a**  **4**
 **3b**	B, **5b** (17) **6b** (80)	 **5b**  **6b**
 **3c**	B, **6c** (77)	 **6c**
 **3d**	A, **5d** (27) **6d** (46)	 **5d**  **6d**
 **3e**	A, **5e** (70)	 **5e**

[a] A: Quinone substrates were in situ generated from the corresponding hydroquinones and silver(I) oxide; B: quinone substrates were prepared from the corresponding hydroquinones in separated procedures.

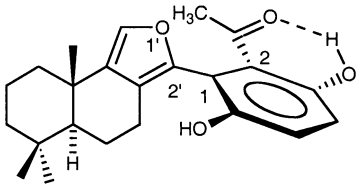


Figure 1. Anisotropy effect of the furan ring on the methyl group in **5b**.

temperature, compound **5a** was obtained as the sole product in high yield (Table 1).

Next, we examined the reaction of furan **2** with 2-acetyl-1,4-benzoquinone (**3b**) in dichloromethane at room temperature. The reaction afforded after 5 h a mixture of Michael adduct **5b**, quinone **6b** and 2,5-dihydroxyacetophenone. The presence of 2,5-dihydroxyacetophenone, detected by ^{1}H NMR and thin layer chromatography (TLC) analysis of the reaction mixture, indicates that **6b** arises from a redox reaction between adduct **5b** and **3b**.

It should be noted that the chemical shifts for the hydrogen and carbon atoms of the acetyl group of **5b**, appear at unusual upfield values (δ_H=1.84 ppm, δ_C=28.3 ppm). An inspection of the optimised conformation of **5b** using CSChem3D software reveals that the aromatic rings are at an angle of 77° (O1′–C2′–C1–C2) and the methyl group is located into the shielding cone of the furan ring as shown in Fig. 1.

We studied the reaction of furan **2** with 2-methoxycarbonyl-1,4-benzoquinone (**3c**) in dichloromethane at room temperature. In this case the reaction occurred slowly affording a mixture of quinone **6c** and methyl 2,5-dihydroxybenzoate. The absence of the corresponding Michael adduct can be ascribed to a slow addition of nucleophile **2** to quinone **3c**, followed by a fast redox reaction between the nascent Michael adduct and **3c**.

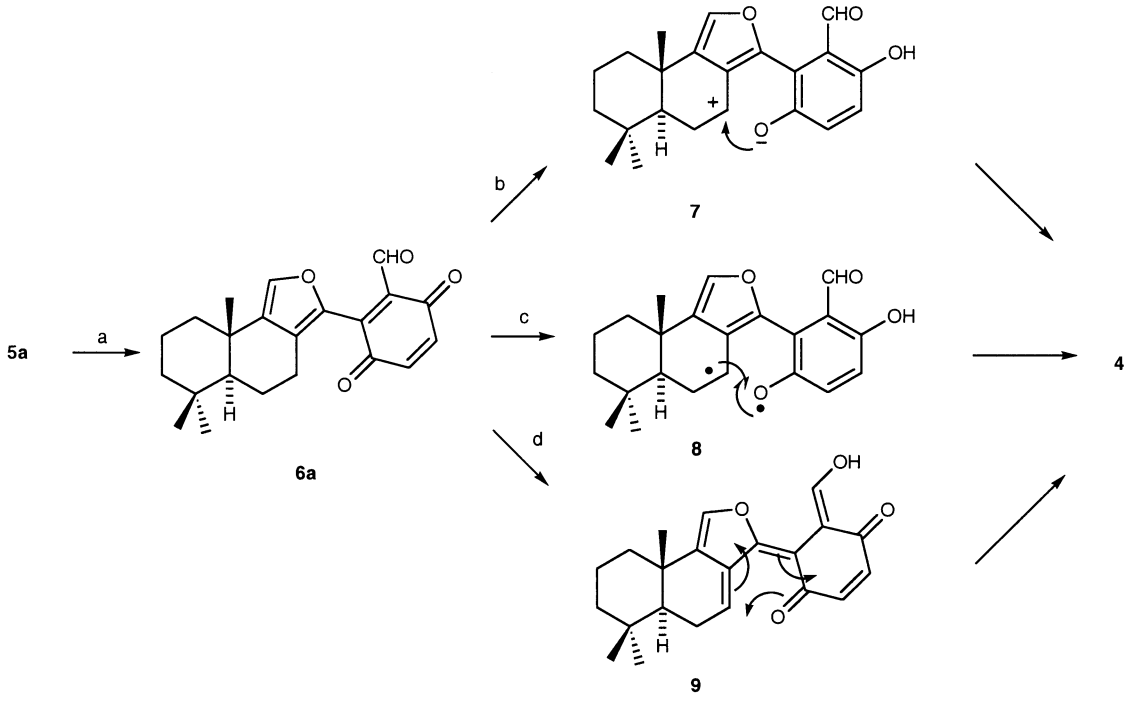
The reactions of furan **2** with activated quinones **3d** and **e** were studied by in situ generation from the corresponding hydroquinones and silver(I) oxide. The former provided a mixture of Michael adduct **5d** and the corresponding quinone **6d**, and the latter afforded the corresponding Michael adduct **5e**.

The absence of quinone **6a** in the reaction of furan **2** and quinone **3a** could be explained assuming its participation in the formation of furopyran **4**. Apparently, compound **4** could arise from **6a** via: (i) cyclisation of zwitterion intermediate **7** formed through an intramolecular hydride ion transfer process[17] (pathway b); (ii) intramolecular reaction of biradical intermediate **8** (pathway c) generated by hydrogen transfer,[18] or (iii) cyclisation of intermediate **9** formed by proton transfer (pathway d) (Scheme 1).

In order to shed some light on the course of the formation of **4**, compound **5a** was allowed to react with 1 equiv. of DDQ in anhydrous dioxane under nitrogen atmosphere at room temperature. The reaction progress was monitored by TLC analysis using compound **4** as reference.

The reaction proceeded rapidly to give furopyran **4** as the sole product. The same result was obtained when the reaction was carried out in darkness. These experiments support a stepwise ionic mechanism where compound **4** arises from the cyclisation of intermediates **7** or **9**.

Taking into account that the reaction of compound **2** with quinones **3a–e** were performed using the same conditions, it is reasonable to deduce that the absence of products type **4** in the reaction of **2** with **3b** and **c** could be attributed to the



Scheme 1. Probable mechanism formation of compound **4**. Reactions: (a) oxidation, (b) hydride transfer, (c) hydrogen transfer and (d) proton transfer.

redox potential of the quinone fragment in **6a** and **c**, which does not facilitate an intramolecular hydride transfer reaction. This assumption was verified through an experiment where furylquinone **6c** was recovered after being treated with DDQ in boiling toluene for 6 h. This result does not disregard the fact that formation of **4** could proceed through a proton transfer process. The resistance toward cyclisation of **6b–d** through this mechanism probably is due to the less electrophilic character of the acetyl, methoxycarbonyl, and nitro groups than the formyl group.

In view of the recent reported results[19] on the conversion of Diels–Alder adducts of furans into Michael adducts, we decided to monitor the reaction of **2** with **3c** in order to have evidence on the participation of possible Diels–Alder adduct intermediates on the formation of the Michael adducts. The reaction progress was followed by ^{1}H NMR analysis in $CDCl_3$ at room temperature. The signals of Diels–Alder adducts were not detected during the early stage of the reaction; after 90 min, increasing production of furylquinone **6c** and methyl gentisate were found.

These results indicate that the reaction of **2** with quinones **3a–e** are initiated by a Michael addition to give the corresponding adducts **5a–e** which, depending on the formation rate, undergo dehydrogenation reactions with the activated 1,4-benzoquinone **3** to give the corresponding furylquinones **6**.

It is reasonable to assume that regiospecific formation of the Michael adducts **5** is controlled by the nucleophilic attack of **2** to the activated quinones **3a–e** through the less hindered 12 position.

(+)-Euryfuran (**2**) and compounds **4–6** were tested in vitro against the intracellular *Leishmania amazonensis* amastigotes stage in mouse peritoneal macrophages. The results of the biological evaluation are indicated in Table 2.

Regarding the antileishmanial potential of the tested compounds, it is clear that all the euryfurylquinones and hydroquinones are more active than (+)-euryfuran (**2**) but less active than the references drugs. The ID50 values indicate that euryfurylhydroquinones **5a**, **b** and **e** display greater leishmanicidal activities than euryfurylquinones **6b–d**. Among the evaluated products, compound **5a** shows promising activity at non-cytotoxic concentration against macrophages.

Table 2. Inhibitory concentrations ID50 and cytotoxicity TC50 of euryfurylquinones and hydroquinones against *L. amazonensis*

Product	IC50 (μM)	TC50 (μM)
2	>25	>25
4	18	13
5a	9	25
5b	16	8
5e	13	13
6b	25	50
6c	33	33
6d	25	25
d.r. 1	2	>32
d.r. 2	5	0.1

d.r.: drug reference; 1: Chimanine B; 2: Amphoterecin B.

In conclusion, these results show the potential of the regiospecific Michael reaction of (+)-euryfuran (**2**) with activated 1,4-benzoquinones **3a–e** for the synthesis of a wide range of new quinones and hydroquinones containing the (+)-euryfuryl moiety. This new type of sesquiterpene quinone and hydroquinones can be considered as novel compounds for the development of new antiprotozoal agents. The access and antiprotozoal evaluation of new members of this series is currently under investigation.

3. Experimental

3.1. General methods

Melting points are uncorrected. Optical rotations were obtained for chloroform solutions on an Optical Activity Ltd. polarimeter and their concentrations are expressed in g/100 mL. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AM-200 spectrometer at 200 and 50 MHz, respectively, in $CDCl_3$. Chemical shifts (δ) are expressed in ppm downfield relative to TMS and the coupling constants (J) are reported in Hz. 2D NMR techniques and DEPT were used for signal assignment. IR spectra were recorded in KBr and frequencies are in cm^{-1}. The elemental analyses were performed in the Analytical Laboratory of our Faculty. Analytical and preparative TLCs were performed on Merck DC-Alufolien GF254. Euryfuran **1** was prepared according the procedure reported in the literature.[16] Substrates for the preparation of quinones **3a–c** were available from commercial sources. Precursors for quinones **3d** and **e** were prepared by following procedures reported in the literature.[12,20]

3.1.1. Reaction of furan 2 with in situ generated 2-formyl-1,4-benzoquinone (3a). A suspension of 2,5-dihydroxybenzaldehyde (177 mg, 1.28 mmol), euryfuran **2** (280 mg, 1.28 mmol), silver(I) oxide (350 mg, 1.52 mmol), and sodium sulfate (500 mg) in dichloromethane (35 mL) was vigorously stirred at room temperature for 1 min. The mixture was filtered through kieselguhr and the filtrate was evaporated under reduced pressure. The crude was column chromatographed on silica gel (dichloromethane). Evaporation of the less polar fraction afforded 11-hydroxy-2,6,6-trimethyl-2bS,3,4,5,6,6aS,7,7aS-octahydro-1,8-dioxacyclopenta[*fg*]naphthacene-12-carbaldehyde (**4**) (40 mg, 27%) as yellow crystals mp 143.5–144.5°C (Found: C, 75.23; H, 7.32. $C_{22}H_{24}O_4$ requires: C, 74.98; H, 6.86); $[\alpha]_D^{22}=-18.1$ (*c*, 16). IR ν 3146 (OH), 1646 (C=O). ^{1}H NMR δ 11.47 (s, 1H, 11-OH), 10.64 (s, 1H, CHO), 7.26 (s, 1H, H-2), 7.14 (d, 1H, J=8.9 Hz, H-9), 6.72 (d, 1H, J=8.9 Hz, H-10), 5.47 (d, 1H, J=7.6 Hz, H-7a$_{ax}$), 2.40–2.04 (m, 3H, H-7 and H-3), 1.81–1.26 (m, 6H), 1.18 (s, 3H, 2b-Me), 1.01 (s, 3H, 6-Me), 0.97 (s, 3H, 6-Me′). ^{13}C NMR δ 196.9, 157.17, 146.50, 143.47, 137.3, 136.3, 126.5, 121.0, 119.7, 116.7, 113.9, 69.3, 49.6, 42.5, 37.4, 34.3, 33.4, 33.3, 27.0, 22.0, 21.4, 18.5.

The more polar fraction gave 3,6-dihydroxy-2-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-benzaldehyde (**5a**) (158 mg, 35%) as yellow crystals mp 104–104.5°C (Found: C, 75.02; H, 7.67. $C_{22}H_{26}O_4$ requires: C, 74.55; H, 7.39); $[\alpha]_D^{22}=+102.4$ (*c*,

2.1). IR ν 3423 (O–H), 1650 (C=O). ^{1}H NMR δ 11.36 (s, 1H, 6-OH), 9.60 (s, 1H, CHO), 7.30 (s, 1H, H-1′), 7.20 (d, 1H, J=9.1 Hz, H-4), 6.94 (d, 1H, J=9.1 Hz, H-5), 5.72 (s, 1H, 3-OH), 2.70 (ddd, J=1.6, 6.5, 17.0 Hz, H-4′$_{eq}$), 2.42 (ddd, J=7.2, 11.3, 17.0 Hz, H-4′$_{ax}$), 2.01 (br d, 1H, J=10.8 Hz, H-9′$_{eq}$), 1.90–1.31 (m, 8H), 1.25 (s, 3H, 9′a-Me), 0.95 (s, 3H, 6′-Me), 0.92 (s, 3H, 6′-Me′). ^{13}C NMR δ 196.7, 156.7, 146.9, 139.3, 138.9, 136.7, 126.1, 123.3, 119.4, 118.5, 117.2, 51.2, 41.9, 39.2, 34.1, 33.5 33.2, 25.0, 21.6, 21.6, 18.9, 18.8.

3.1.2. Reaction of furan 2 with 2-formyl-1,4-benzoquinone (3a). A suspension of 2,5-dihydroxybenzaldehyde (177 mg, 1.28 mmol), silver(I) oxide (350 mg, 1.52 mmol) and sodium sulfate (500 mg) in benzene was stirred at 40°C for 2 h. The mixture was filtered and the filtrate was added to a solution of furan **2** (280 mg, 1.28 mmol) in benzene (25 mL) and the mixture was left at room temperature for 5 min. Evaporation of the solvent followed by column chromatography (dichloromethane) of the residue afforded **5a** (342 mg, 76%).

3.1.3. Reaction of furan 2 with 2-acetyl-1,4-benzoquinone (5b). A solution of furan **2** (218 mg, 1.0 mmol), and quinone **3b** (150 mg, 1.0 mmol) in dichloromethane (35 mL) was left at room temperature for 5 h. The solvent was removed under reduced pressure and the residue was column chromatographed on silica gel (dichloromethane). The less polar fraction gave pure 3,6-dihydroxy-2-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-acetophenone (**5b**) (61 mg, 17%) as yellow crystals mp 130.8–131.0°C (Found: C, 75.36; H, 7.56. $C_{23}H_{28}O_4$ requires: C, 74.97; H, 7.66); $[\alpha]_D^{31}$=−22.26 (*c*, 5.39). IR ν 3415 (OH), 2935 (C–H), 1635 (C=O). ^{1}H NMR δ 11.46 (s, 1H, 6-OH), 7.31 (s, 1H, H-1′), 7.13 (d, 1H, J=9.0 Hz, H-4), 6.97 (d, 1H, J=9.1 Hz, H-5), 5.41 (s, 1H, 3-OH), 2.63 (dd, J=6.0, 16.5 Hz, H-4′$_{eq}$), 2.36 (ddd, J=7.0, 11.3, 17.5 Hz, H-4′$_{ax}$), 2.02 (br d, 1H, J=10.9 Hz, H-9′$_{eq}$), 1.84 (s, 3H, COMe), 1.81–1.27 (m, 8H), 1.24 (s, 3H, 9′a-Me), 0.95 (s, 3H, 6′-Me), 0.92 (s, 3H, 6′-Me′). ^{13}C NMR δ 205.5, 155.5, 147.0, 140.6, 140.0, 136.5, 123.5, 122.0, 120.5, 120.4, 115.9, 51.5, 41.9, 39.3, 34.1, 33.5, 33.2, 28.3, 25.1, 21.6, 21.3, 18.9, 18.8.

From the more polar fraction 2-acetyl-3-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-1,4-benzoquinone (**6b**) (147 mg, 80%; referred to 0.5 equiv. of **3b**) was isolated as red crystals mp 145–146°C (Found: C, 75.24%; H, 7.33. $C_{23}H_{26}O_4$ requires: C, 75.38; H, 7.15); IR ν 1772 (C=O), 1713 (C=O), 1672 (C=O). ^{1}H NMR δ 7.23 (s, 1H, H-1′), 6.76 (s, 2H, H-5 and H-6), 2.92–2.73 (m, 2H, H-4′), 2.40 (s, 3H, MeCO), 1.97–1.23 (m, 8H), 1.20 (s, 3H, 9′a-Me), 0.94 (s, 3H, 6′-Me), 0.90 (s, 3H, 6′-Me′). ^{13}C NMR δ 200.7, 185.5, 185.4, 141.7, 139.5, 139.1, 138.2, 136.4, 136.0, 131.1, 130.6, 50.7, 41.7, 39.2, 34.1, 33.4, 33.1, 31.4, 24.9, 23.7, 21.5, 18.9, 18.8.

3.1.4. Reaction of furan 2 with 2-methoxycarbonyl-1,4-benzoquinone (3c). A solution of **2** (270 mg, 1.23 mmol) and quinone **3c** (205 mg, 1.23 mmol) in dichloromethane (35 mL) was left at room temperature for 20 h. The solvent was removed under reduced pressure and the residue was column chromatographed on silica gel (dichloromethane) to give 2-methoxycarbonyl-3-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-1,4-benzoquinone (**6c**) (181 mg, 77% referred to 0.5 equiv. of **3c**) as red crystals mp 119–120°C (Found: C, 72.51; H, 6.91. $C_{23}H_{26}O_5$ requires: C, 72.23; H, 6.85); IR ν 1744 (C=O), 1672 (C=O). ^{1}H NMR δ 7.25 (s, 1H, H-1′), 6.79 (s, 2H, H-5 and H-6), 3.88 (s, 3H, OMe), 2.86 (ddd, 1H, J=2.2, 6.9, 18.4 Hz, H-4′$_{eq}$), 2.75 (ddd, 1H, J=6.8, 11.0, 18.2 Hz, H-4′$_{ax}$), 1.95 (br d, 1H, J=12.1 Hz, H-9′$_{eq}$), 1.87–1.23 (m, 8H), 1.20 (s, 3H, 9′a-Me), 0.94 (s, 3H, 6′-Me), 0.90 (s, 3H, 6′-Me′). ^{13}C NMR δ 185.1, 183.7, 165.3, 141.7, 141.7, 139.6, 136.5, 136.0, 132.4, 131.1, 131.1, 52.5, 50.6, 41.7, 39.2, 34.0, 33.4, 33.0, 24.9, 24.0, 21.5, 18.9, 18.8.

3.1.5. Reaction of furan 2 with in situ generated 2-nitro-1,4-benzoquinone (3d). A suspension of 2-nitro-1,4-dihydroxybenzene (179 mg, 1.15 mmol), furan **2** (251 mg, 1.15 mmol), silver(I) oxide (300 mg, 1.3 mmol) and sodium sulfate (500 mg) in dichloromethane (30 mL) was stirred at rt for 15 min. The mixture was filtered through kieselguhr, the filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel (dichloromethane). Evaporation of the less polar fraction gave 2-nitro-3-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-1,4-benzoquinone (**6d**) (196 mg, 46%; respect to 0.5 equiv. of 2-nitro-1,4-dihydroxybenzene) as violet crystals mp 162–163°C (Found: C, 67.90; H, 6.24; N, 3.84. $C_{21}H_{23}NO_5$ requires: C, 68.28; H, 6.28; N, 3.79); IR ν 2929 (C–H), 1680 (C=O), 1661 (C=O), 1543 (NO_2). ^{1}H NMR δ 7.34 (s, 1H, H-1′), 6.88 (s, 2H, H-5 and H-6), 2.93 (ddd, 1H, J=2.5, 7.1, 18.1 Hz, H-4′$_{eq}$), 2.81 (ddd, 1H, J=6.7, 11.3, 18.9 Hz, H-4′$_{ax}$), 1.96 (br d, 1H, J=11.0 Hz, H-9′$_{eq}$), 1.89–1.23 (m, 8H), 1.20 (s, 3H, 9′a-Me), 0.95 (s, 3H, 6′-Me), 0.91 (s, 3H, 6′-Me′). ^{13}C NMR δ 184.3, 176.9, 142.4, 142.3, 142.2, 137.0, 136.7, 135.4, 134.9, 124.9, 50.6, 41.7, 39.1, 34.1, 33.3, 33.0, 24.8, 24.4, 21.5, 18.8, 18.7.

From the more polar fraction 3,6-dihydroxy-2-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-nitrobenzene (**5d**) (117 mg, 27%) was isolated as yellow crystals mp 104.0–104.5°C (Found: C, 67.83; H, 6.31; N, 3.88. $C_{21}H_{25}NO_5$ requires: C, 67.91; H, 6.78; N, 3.77); $[\alpha]_D^{25}$=+102.4 (*c*, 21). IR ν 3451 (OH), 2944 (C–H), 1532 (NO_2). ^{1}H NMR δ 9.56 (s, 1H, 6-OH), 7.25 (s, 1H, H-1′), 7.22 (d, 1H, J=9.5 Hz, H-4), 7.12 (d, 1H, J=9.5 Hz, H-5), 5.77 (s, 1H, 3-OH), 2.57 (ddd, 1H, J=1.6, 6.4, 16.9 Hz, H-4′$_{eq}$), 2.32 (ddd, 1H, J=7.1, 11.4, 17.3 Hz, H-4′$_{ax}$), 2.00 (br d, 1H, J=11.8 Hz, H-9′$_{eq}$), 1.97–1.28 (m, 8H), 1.25 (s, 3H, 9′a-Me), 0.94 (s, 3H, 6′-Me), 0.91 (s, 3H, 6′-Me′). ^{13}C NMR δ 148.7, 147.9, 139.2, 137.7, 136.8, 133.3, 124.6, 121.3, 121.1, 111.6, 51.2, 41.9, 39.2, 34.1, 33.5, 33.2, 25.1, 21.6, 21.1, 19.0, 18.8.

3.1.6. Reaction of furan 2 with in situ generated 2-cyano-1,4-benzoquinone (3e). A stirred suspension of 2,5-dihydroxybenzonitrile (100 mg, 0.74 mmol), furan **2** (162 mg, 0.74 mmol), silver(I) oxide (927 mg, 4.0 mmol), and sodium sulfate (500 mg) in dichloromethane (25 mL)

was refluxed for 4 h. The mixture was filtered through kieselguhr and the solids were washed with dichloromethane. The filtrate was evaporated under reduced pressure and chromatographed on silica gel (dichloromethane) to afford 3,6-dihydroxy-2-(6′,6′,9′a-trimethyl-4′,5′,5′aS,6′,7′,8′,9′,9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-benzonitrile (**5e**) (183.0 mg; 70% with respect to 2,5-dihydroxybenzonitrile) as pale brown crystals mp 94–96°C (Found: C, 75.50; H, 7.10; N, 3.76. $C_{22}H_{25}NO_3$ requires: C, 75.19; H, 7.17; N, 3.99); $[\alpha]_D^{25}=+17.94$ (*c*, 2.23; MeOH). IR ν 3384 (OH), 2949 (C–H). ^{1}H NMR δ 7.29 (s, 1H, H-1′), 7.10 (d, 1H, *J*=9.0 Hz, H-4), 6.91 (d, 1H, *J*=9.0 Hz, H-5), 5.97 (br s, 2H, 3-OH and 6-OH), 2.78 (dd, 1H, *J*=6.0, 16.7 Hz, H-4′$_{eq}$), 2.61 (ddd, 1H, *J*=7.2, 10.9, 17.2 Hz, H-4′$_{ax}$), 2.00 (br d, 1H, *J*=12.0 Hz, H-9′$_{eq}$), 1.93–1.30 (m, 8H), 1.26 (s, 3H, 9′a-Me), 0.95 (s, 3H, 6′-Me), 0.92 (s, 3H, 6′-Me′). ^{13}C NMR δ 153.9, 147.5, 139.8, 139.5, 136.5, 122.8, 122.7, 120.2, 117.5, 116.0, 98.7, 51.1, 41.9, 39.2, 34.1, 33.5, 33.1, 25.0, 22.0, 21.6, 19.0, 18.8.

3.2. In vitro cytotoxic screening

The isolation of macrophages and parasites (*L. amazonensis*, strains LV79) was described previously in full details.[21] 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 and the overall decrease in parasite number. Under the best conditions, complete clearance of amastigotes can be achieved.

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