Studies on Quinones. Part 38: Synthesis and Leishmanicidal Activity of Sesquiterpene 1,4-Quinones☆

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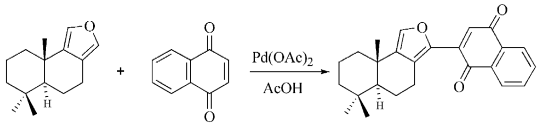
Abstract—The reaction of (+)-euryfuran **1** with several benzo-, naphtho- and benzo[*b*]thiophene-1,4-quinones in acetic acid yields the corresponding euryfuryl-1,4-quinones **3**, **5**, **7**, **8**, **10**, **12**, and **14**. The structure of compounds **7**, **8**, **12**, and **14** was assigned through 2D NMR ^{1}H–^{13}C HMBC experiments. The influence of the acidity of the solvent upon the reactivity and regioselectivity of the quinones to the oxidative coupling reaction, is discussed. The in vitro activity of the euryfurylquinones and their corresponding precursors against *Leishmania amazonensis* is described.

Introduction

Naturally occurring sesquiterpene quinones and hydroquinones are well-known substances that possess a variety of biological properties such as antitumor activity,[2] inhibition of the HIV 1 reverse transcriptase,[3] and immunomodulation.[4] Our interest in the synthesis and biological activity of quinones[5] led us to explore the preparation of sesquiterpene quinones and hydroquinones derived from (+)-euryfuran **1**, an antitumoral drimane sesquiterpene.[6]

Recently, we have described[7] the reaction of (+)-euryfuran **1** with highly electrophilic 1,4-benzoquinones (activated quinones) that produces antiprotozoal active euryfurylquinones and hydroquinones. Such reactions proceed in dichloromethane at room temperature to give the corresponding Michael adducts which, depending on the electron-withdrawing substituent on the quinone, undergo in situ redox reactions to the corresponding euryfurylquinones. The facile and regiospecific reactions of furan **1** with activated 2-substituted 1,4-benzoquinones probably is due to the electron-withdrawing effect of the substituent which induces a significant increase in the LUMO coefficient at the C-3 position.



1 **2** **3**

Scheme 1.

Our interest to prepare new members of the euryfurylquinone series for antiprotozoal evaluation led us to explore the reaction of furan **1** with quinones less reactive than activated quinones, such as 1,4-benzoquinone; 1,4-naphthoquinone and 5-hydroxy-1,4-naphthoquinone. Early precedents on the reactivity of these quinones with furans, reported by Eugster[8] and Kraus,[9] indicate that the reaction fails under a variety of conditions, including Lewis acid catalysis.

Bridson[10] has described the formation of 2-furyl-1,4-naphthoquinones by oxidative coupling of furans with 1,4-naphthoquinones using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), or an excess of the quinone

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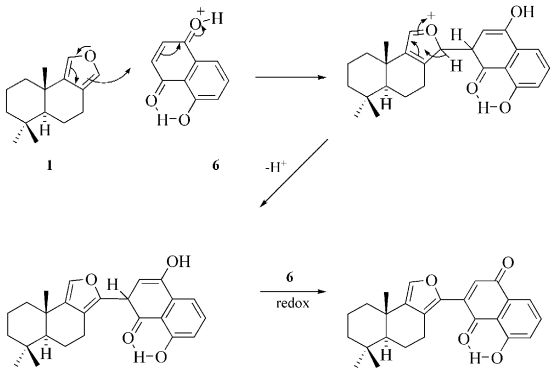
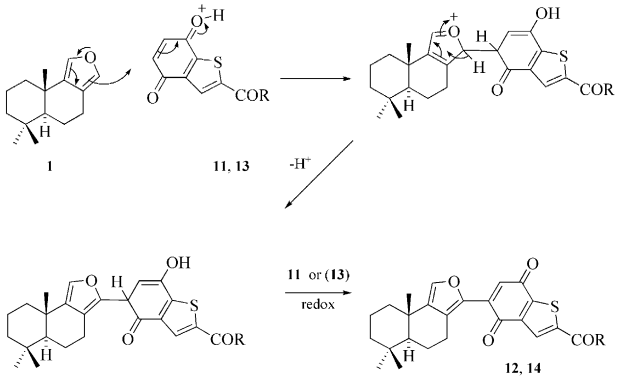


Scheme 2. Mechanism formation of quinone **7** by regioselective acid-induced furylation of quinone **6**.



Scheme 3. Mechanism formation of quinones **12** and **14** by regioselective acid-induced furylation of quinones **11** and **13**.

substrate. In this case, the success of the reaction apparently is due to the irreversible formation of the furylquinones by in situ oxidation of the furylhydroquinones, in equilibrium with the substrates.

Itahara has reported an interesting and general oxidative coupling method for the synthesis of aryl-1,4-quinones from arenes and quinones using palladium(II) acetate in acetic acid.[11,12] These results have been successfully extended to the synthesis of furyl-1,4-naphthoquinones.[13]

Here, we describe the preparation of euryfuryl-1,4-quinones resulting by oxidative coupling reaction of **1** with 1,4-quinones in acetic acid. The influence of the solvent to promote the Michael addition and the regioselectivity of the reaction with unsymmetrical quinones are important features that can be useful for the synthesis of new bioactive members of the euryfurylquinone series. Leishmanicidal activity is also described for quinones and their precursors.

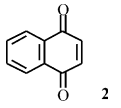
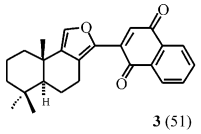
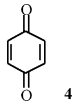
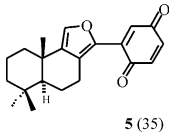
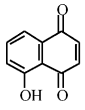
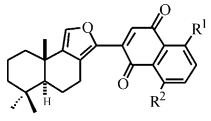
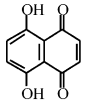
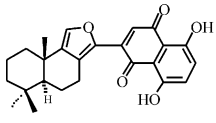
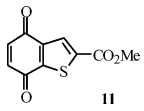
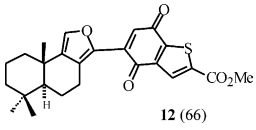
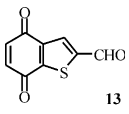
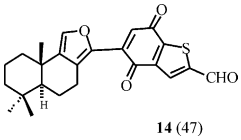
Results and Discussion

Chemistry

Preliminary attempts were made to prepare euryfurylquinone **3** by oxidative coupling reaction of furan **1** with 1,4-naphthoquinone **2** in different solvent media (e.g., dichloromethane, benzene, acetonitrile, dioxane, tetrahydrofuran) at reflux temperature. Nevertheless, the starting material was recovered in all of these trials. The oxidative coupling of euryfuran **1** with **2**, by using palladium(II) acetate according to Hitahara's procedure,[13] gave euryfurylquinone **3**, along with a complex mixture of polar products (Scheme 1). Compound **3** was isolated by column chromatography (35%) and characterized on the basis of its ^{1}H and ^{13}C NMR spectra and by comparison with those of eufurylquinones previously reported.[1,7]

It is noteworthy that formation of **3** was detected (TLC) when compounds **1** and **2** were left at room temperature in acetic acid in the absence of palladium(II) acetate. On this basis, the reaction of **1** with 2 equiv of **2** was carried out at reflux in acetic acid, under nitrogen atmosphere, monitoring the consumption of furan **1** (TLC). The treatment provided **3** in 51% isolated yield, and in this case, minor amounts of decomposition products were detected (TLC) with respect to the oxidative coupling using palladium(II) diacetate.

Table 1. Euryfurylquinones prepared by reaction of euryfuran **1** with1,4-quinones in acetic acid[a]

Entry	Quinone	*T* (°C)	Time[b] (h)	Products (yield%) [c,d,e]
1	 **2**	Reflux	24	 **3** (51)
2	 **4**	rt	12	 **5** (35)
3	 **6**	rt	72	 **7.** R^1 = H, R^2 = OH ; (39) **8.** R^1 = OH, R^2 = H; (10)
4	 **9**	Reflux	12	 **10** (46)
5	 **11**	rt	24	 **12** (66)
6	 **13**	rt	3	 **14** (47)

[a]All reactions were carried under nitrogen using 2 equiv of the quinone.
[b]Established by monitoring (TLC) euryfuran consumption.
[c]Yields based on quinones.
[d]Isolated.
[e]No additional efforts were made to improve yields.

Table 2. Inhibitory concentrations IC_{50} and cytotoxicity TC_{50} of euryfuryl-1,4-quinones and precursors against *Leishmania amazonensis*

Product	IC_{50} (μM)	TC_{50} (μM)	Precursor	IC_{50} (μM)	TC_{50} (μM)
3	33	67	**1**	> 100	> 100
5	> 80	> 80	**2**	5	2.5
7	8	16	**4**	8.5	8.5
8	24	24	**6**	7	7
10	23	23	**9**	6	6
12	30	> 60	**11**	nd[a]	nd[a]
14	28	> 57	**13**	22	22
Chimanine B	2	> 32	Amphotericin B	0.1	5

[a]nd, not determined.

In the light of these results, we examined the scope of the euryfurylation reaction with several 1,4-quinones. The reactions, carried out under nitrogen atmosphere, afforded in all cases the corresponding euryfuryl 1,4-quinones which were isolated by chromatography. Yields were calculated with respect to 1 equiv of the quinone substrate since 1 equiv is required for the oxidative coupling reaction. Further experiments using prolonged reaction times, replacing the acetic acid by formic acid or employing boron trifluoride diethyl etherate as Lewis catalyst were unsuccessful and extensive decomposition of the substrates was observed.

The results, summarised in Table 1, demonstrate that reaction of **1** with 1,4-quinones in acetic acid proceeds in a general manner to provide the corresponding euryfuryl-1,4-quinones.

The structures of euryfurylquinones **7**, **8**, **12**, and **14** (Table 1, entries 3, 5, and 6) were fully established from ^{1}H and ^{13}C NMR analysis. In the case of regioisomer **7** the presence of ^{3}JHMBC correlation between the carbon of the hydrogen bonded carbonyl group (C-4) at δ 189.1 ppm and the quinone proton (H-2) ppm, allows to assign its regiochemistry and that of regioisomer **8**. Concerning quinones **12** and **14**, their HMBC spectra show ^{3}JHMBC correlation between the carbon of the carbonyl groups (C-4) at δ 179.1 and 179.0 ppm, respectively, with the protons H-3 (8.14 and 8.16 ppm) and H-6 (6.98 and 7.02 ppm), thus providing evidences on the location of the euryfuryl moiety at the 5-position.

We can conclude that oxidative coupling reactions of furan **1** with quinones **2**, **4**, **6**, **9**, **11**, and **13** are induced by acetic acid which, probably acts as a Bronsted acid enhancing the electrophilicity of the quinone double bond. The regiochemistry of the reaction of furan **1** with quinone **6** in acid media could be explained in the light of other substitution reactions of **6** where the regioselectivity depends on the acid or basic nature of the nucleophile.[14] Thus, the reaction of quinone **6** with dimethylamine afforded the 2-substituted quinone as the unique regioisomer, whereas the reaction of **6** with thioglycolic acid afforded regiospecifically the 3-substituted quinone.[14] The direction of the addition of amine to **6** could be explained by the activation of the C-4 carbonyl group by the strong intramolecular hydrogen bonding with the 5-hydroxy group. In the case of the reaction with the sulfurated nucleophile, the regioselective attack to the 3-position probably is due to the activation of the C-1 carbonyl group by protonation with the carboxylic group of the thioglycolic acid. Protonation of the C-1 carbonyl group probably is more favorable than that of C-4 since, the electron donor capacity of the oxygen atom in the latter carbonyl is lowered by intramolecular hydrogen bonding to the 5-hydroxy group.

On the basis of these precedents it seems reasonable that the major regioisomer **7** resulting in the reaction of **1** with **6** is formed via an acid-induced Michael addition, as depicted in Scheme 2.

In the case of the reactions of **1** with benzo[*b*]thiophene-1,4-quinones **11** and **13** the regiochemistry of the addition could be explained by a favorable protonation of the C-7 carbonyl group which induces a regioselective attack of the furan **1** to the 5-position (Scheme 3). It is noteworthy that, according to the LUMO coefficient magnitudes for quinones **11** and **13**,[15] a nucleophilic attack to the quinone double bond is more favorable towards the 5- than the 6-position.

Biology

The leishmanicidal activity of furan **1**, euryfurylquinones **3**, **5**, **7**, **8**, **10**, **12**, **14**, and quinone precursors **2**, **4**, **6**, **9**, and **13** were tested in vitro on *Leishmania amazonensis* amastigotes in bone marrow-derived macrophages (Table 2). Although some euryfurylquinones and their quinone precursors showed a weak antileishmania activity, no one was as effective as the reference compounds (Chimanin B and Amphotericyn B) against *L. amazonensis* intracellular amastigotes (data not shown). However, most of these quinones have a weak selectivity (i.e., the ratio between cytotoxic and leishmanicidal effects). It was evident that the euryfuryl moiety is not an essential requirement for activity but, in some cases, appears to cause a slight increase of selectivity towards *Leishmania*. Further synthesis of new euryfurylquinones and in vitro screening against intracellular *Leishmania* would be necessary to find improved drug candidates for in vivo testing.

Conclusion

In conclusion, the results reported here demonstrate that oxidative coupling reactions of (+)-euryfuran (**1**) with low electrophilic quinones in acetic acid give

euryfuryl-1,4-quinones. The reaction show a remarkable regioselectivity which is controlled by the steric hindrance of the donor and protonation of the acceptor. Furthermore, this arylation method of 1,4-quinones proceeds cleanly and does not require an expensive catalyst, such as palladium(II) acetate, to promote the reaction. The promissory leishmanicidal activity of certain euryfuryl-1,4-quinones described here represents an advance in the search for novel antiprotozoal agents that belong to the series of synthetic sesquiterpene quinones. Efforts in the synthesis of a wide range of furyl-1,4-quinones using the present method for cytotoxic evaluation are currently under way.

Experimental

Chemical synthesis

Melting points (mp) are uncorrected. Optical rotations for compounds **3** and **7** were obtained for $CHCl_3$ solutions and their concentrations are expressed in g/100 mL on Optical Activity Ltd. polarimeter. Optical rotations for **5**, **8**, **12**, and **14** can not be measured due to strong absorption of the polarised light by the deep coloured chloroform solutions. ^{1}H NMR spectra were measured on a Bruker AM-200 in $CDCl_3$. Chemical shifts are expressed in ppm downfield relative to TMS (δ scale) and the coupling constants (*J*) are reported in Hz. ^{13}C NMR spectra were acquired in deuteriochloroform at 50 and 75 MHz on a Bruker AM-200 and a AM-300 spectrometers. 2D NMR techniques (COSY, HMBC 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 thin layer chromatography was performed on Merck DC-Alufolien GF254. Euryfuran **1** and quinone 11 were prepared following reported methods.[16,17] Substrates for Table 1, entries 1–4, were obtained from commercial sources. Substrates **2** and **4** were purified by column chromatography and sublimation, respectively.

4,7-Dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carbaldehyde (13). To a solution of (4,7-dimethoxybenzo[*b*]thiophen-2-yl)-methanol[17] (134 mg, 0.60 mmol) in dichloromethane (20 mL), pyridinium chlorochromate (193 mg, 0.90 mmol) was added while stirring at room temperature. After 3 h, the reaction mixture was filtered through silica gel. The filtrate was evaporated to afford 4,7-dimethoxybenzo[*b*]thiophene-2-carbaldehyde (109 mg, 83%) as yellow crystals (ether–hexane) mp 131–132 °C. IR ν 1667 (C=O). ^{1}H NMR (200 MHz) δ 10.00 (s, 1H, CHO), 8.08 (s, 1H, H-3), 6.77 (d, 1H, $^3J_{H,H}$ = 8.5 Hz, H-5 or H-6), 6.63 (d, 1H, $^3J_{H,H}$ = 8.5 Hz, H-6 or H-5), 3.92 (s, 3H, OMe), 3.90 (s, 3H, OMe). ^{13}C NMR (50 MHz) δ 184.57, 150.89, 148.48, 142.39, 133.38, 132.07, 131.01, 108.21, 104.79, 56.01, 55.74. Anal. calcd for $C_{11}H_{10}O_3S$: C, 59.44; H, 4.53; S 14.43. Found: C, 59,08; H, 4.47; S, 14.18.

A solution of CAN (1.19 g, 2.17 mmol) in water (3.4 mL) was added dropwise to a stirred solution of 4,7-dimethoxybenzo[*b*]thiophene-2-carbaldehyde (200 mg, 0.9 mmol) in acetonitrile (10 mL). The mixture was stirred at room temperature for 30 min, diluted with water and extracted with ethyl acetate (3×15 mL). The extract was dried ($MgSO_4$) and evaporated. The residue was column chromatographed to afford 4,7-dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carbaldehyde (**13**), (140 mg, 81%), yellow crystals (hexane–ethanol) mp 133–134 °C. IR ν 1678 (C=O), 1666 (C=O), 1657 (C=O). ^{1}H NMR (200 MHz) δ 10.08 (s, 1H, CHO), 8.17 (s, 1H, H-3), 7.00 (d, 1H, $^3J_{H,H}$ = 10.3 Hz, H-5 or H-6), 6.93 (d, 1H, $^3J_{H,H}$ = 10.3 Hz, H-6 or H-5). ^{13}C NMR (75 MHz) δ 183.1, 180.4, 179.84, 148.4, 147.7, 140.8, 138.4, 138.5, 132.5. Anal. calcd for $C_9H_4O_3S$: C, 56.24; H, 2.10; S,16.68. Found: C, 56.30; H, 2.09; S, 16.44.

General procedure for the preparation of euryfuryl-1,4-quinones

A solution of euryfuran **1** and the corresponding quinone in glacial acetic acid (10 mL) was treated in the conditions outlined in Table 1. The reaction mixture was poured into water (100 mL), neutralized with sodium hydrogencarbonate and extracted with dichlorometane (2×30 mL). The organic extract was washed with water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was column chromatographed on silica gel with hexane–ethyl acetate as eluent. All products were further purified by recrystallization.

2-(6′, 6′, 9′a-Trimethyl-4′, 5′, 5′aS, 6′, 7′, 8′, 9′, 9′aS-octahydronaphtho[1,2-*c*]furan-3-yl)-1,4-naphthoquinone (3). 50 mg, 51% from **1** (57 mg, 0.26 mmol) and quinone **2** (82.6 mg, 0.52 mmol), orange crystals (ether–methanol) mp 165–166 °C. $[\alpha]_D^{22} = -24.04°$ (*c* 2.08). IR ν 1676 (C=O), 1645 (C=O). ^{1}H NMR (200 MHz) δ 8.13–8.03 (m, 2H, H-8 and H-5), 7.78–7.69 (m, 2H, H-6 and H-7), 7.33 (s, 1H, H-1′), 7.09 (s, 1H, H-3), 3.05 (dd, 1H, $^3J_{H,H}$ = 5.1; $^2J_{H,H}$ = 18.3 Hz, H-4′eq), 2.85 (ddd, 1H, $^3J_{H,H}$ = 7.2, 11.2; $^2J_{H,H}$ = 18.3 Hz, H-4′ax), 2.00 (m, 1H, H-9′eq), 1.91–1.26 (m, 8H), 1.24 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me). ^{13}C NMR (50 MHz) δ 189.8, 183.6, 141.63, 141.1, 138.9, 138.7, 133.7, 133.4, 132.7, 132.1, 129.8, 128.7, 126.7, 125.8, 50.8, 41.8, 39.4, 34.0, 33.4, 33.06, 24.9, 24.6, 21.5, 19.1, 18.9. Anal. calcd for $C_{25}H_{26}O_3$: C, 80.18; H, 7.00. Found, C, 80.58; H, 6.91.

2-(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 (5). 100 mg, 35% from **1** (190 mg, 0.87 mmol) and 1,4-benzoquinone (**4**) (188.43 mg, 1.74 mmol), brown crystals (ether–methanol) mp 124–125 °C. IR ν 1678 (C=O), 1645 (C=O). ^{1}H NMR (200 MHz) δ 7.30 (s, 1H, H-1′), 6.85 (s, 1H, 3-H), 6.74 (s, 2H, H-5 and H-6), 2.97 (dd, 1H, $^3J_{H,H}$ = 6.5; $^2J_{H,H}$ = 18.2 Hz, H-4′eq), 2.76 (ddd, 1H, $^3J_{H,H}$ = 7.2, 11.1; $^2J_{H,H}$ = 18.3 Hz, H-4′ax), 1.99 (m, 1H, H-9′eq), 1.92–1.25 (m, 8H), 1.22 (s, 3H, Me), 0.95 (s, 3H, Me), 0.91 (s, 3H, Me). ^{13}C NMR (50 MHz) δ 187.3, 185.7, 141.1, 141.1, 139.0, 136.7, 136.6, 136.3, 128.7, 127.1, 50.7, 41.8, 39.3, 34.0, 33.4, 33.1, 24.9, 24.4, 21.5, 19.0, 18.9. Anal. calcd for $C_{21}H_{24}O_3$: C, 77.75; H, 7.46. Found: C, 77.27; H, 7.67.

5-Hydroxy-3-(6′a, 6′b, 9′ab-trimethyl-4′, 5′, 5′aS, 6′, 7′, 8′, 9′, 9′aS-octahydronaphtho[1,2-*c*]furan-3′-yl)-1,4-naphthoquinone (7) and 5-hydroxy-2-(6′a, 6′b, 9′ab-trimethyl-4′, 5′, 5′aS, 6′, 7′, 8′, 9′, 9′aS-octahydronaphtho[1,2-*c*]furan-3-yl)-1,4-naphthoquinone (8). In the general procedure, a solution of 1 (43.10 mg, 0.20 mmol), juglone **6** (68.9 mg, 0.39 mmol) in glacial acetic acid was stirred at room temperature for 3 days. Workup followed by preparative TLC provided compounds **7** and **8**.

Quinone 7. (30 mg, 39%), orange crystals (ether-methanol) mp 178–179 °C. $[\alpha]_D^{22} = -72.73°$ (*c* 3.85). IR ν 3444 (O-H), 1643 (C=O), 1593 (C=O). ^{1}H NMR (200 MHz) δ 12.12 (s, 1H, 5-OH), 7.67–7.58 (m, 2H, H-7 and H-8), 7.34 (s, 1H, H-1′), 7.30–7.21 (m, 1H, H-6), 7.03 (s, 1H, H-2), 3.06 (dd, 1H, $^3J_{H,H} = 6.1$; $^2J_{H,H} = 17.6$ Hz, H-4′eq), 2.84 (ddd, 1H, $^3J_{H,H} = 7.2$, 11.1; $^2J_{H,H} = 18.3$ Hz, H-4′ax), 2.01 (m, 1H, H-9′eq), 1.91–1.31 (m, 8H), 1.24 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me). ^{13}C NMR (75 MHz) δ 189.1, 184.1, 161.7, 141.3, 141.2, 139.1, 138.4, 136.5, 132.1, 130.9, 128.7, 124.1, 118.4, 115.4, 50.8, 41.8, 39.3, 34.0, 33.4, 33.1, 25.0, 24.7, 21.5, 19.1, 19.0. Anal. calcd for $C_{25}H_{26}O_4$: C, 76.90; H, 6.71. Found, C, 77.09; H, 6.75.

Quinone 8. 8 mg, 10%, violet crystals (ether–methanol) mp 166–167 °C. IR ν 3443 (O-H), 1629 (C=O), 1596 (C=O). δ ^{1}H NMR (200 MHz) δ 12.21 (s, 1H, 5-OH), 7.68–7.55 (m, 2H, H-7 and H-8), 7.34 (s, 1H, H-1′), 7.30–7.21 (m, 1H, H-6), 7.04 (s, 1H, H-3), 3.06 (dd: $^3J_{H,H} = 5.5$; $^2J_{H,H} = 18.2$ Hz, H-4′eq), 2.85 (ddd: $^3J_{H,H} = 7.2$, 11.2; $^2J_{H,H} = 18.4$ Hz, H-4′ax), 1.94 (m, 1H, H-9′eq), 1.88–1.25 (m, 8H), 1.24 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me). ^{13}C NMR (50 MHz) δ 190.0, 181.0, 160.9, 141.7, 141.4, 139.7, 139.4, 135.8, 132.7, 129.8, 129.1, 124.1, 119.3, 115.1, 50.8, 41.8, 39.3, 34.0, 33.4, 33.1, 24.9, 24.8, 21.5, 19.0, 19.0. Anal. calcd for $C_{25}H_{26}O_4$: C, 76.90; H, 6.71. Found, C, 76.50; H, 6.55.

5,8-Dihydroxy-2-(6′, 6′, 9′a-trimethyl-4′, 5′, 5′a, 6′, 7′, 8′, 9′, 9′aS-octahydronaphtho[1,2-c]furan-3′-yl)-1,4-naphthoquinone (10). 130 mg, 46% from **1** (151.1 mg, 0.69 mmol) and naphtazarin **9** (263.6 mg, 1.38 mmol); violet crystals (ether–methanol) mp 184–185 °C. IR ν 3443 (O–H), 1613 (C=O), 1585 (C=O). ^{1}H NMR (200 MHz) δ 12.71 (s, 1H, OH), 12.68 (s, 1H, OH), 7.33 (s, 1H, H-1′), 7.20 (s, 2H, H-6 and H-7), 7.08 (s, 1H, H-3), 3.06 (dd, 1H, $^3J_{H,H} = 5.3$; $^2J_{H,H} = 18.3$, H-4′eq), 2.82 (ddd, 1H, $^3J_{H,H} = 7.3$, 11.2; $^2J_{H,H} = 18.3$, H-4′ax), 2.00 (m, 1H, H-9′eq), 1.91–1.30 (m, 8H), 1.24 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me). ^{13}C NMR (50 MHz) δ 183.4, 182.5, 162.1, 161.0, 141.5, 141.2, 139.1, 138.5, 131.0, 130.3, 129.8, 128.8, 112.4, 111.8, 50.8, 41.8, 39.3, 34.0, 33.4, 33.1, 25.0, 24.8, 21.5, 19.1, 19.0. Anal. calcd for $C_{25}H_{26}O_5$: C, 73.87; H, 6.45. Found, C, 73.92; H, 6.36.

4,7-Dioxo-5-(6′, 6′,9′a-trimethyl-4′, 5′, 5′aS, 6′, 7′, 8′, 9′, 9′aS-octahydronaphtho[1,2-c]furan-3′-yl)-4,7-dihydrobenzo[*b*]-thiophene-2-carboxylic acid methyl ester (12). 85 mg, 66% from **1** (64.3 mg, 0.30 mmol) and quinone **11** (131 mg, 0.59 mmol), red crystals (ether–methanol) mp 188–189 °C IR ν 1727 (C=O), 1673 (C=O), 1635 (C=O). ^{1}H NMR (200 MHz) δ 8.14 (s, 1H, 3-H), 7.34 (s, 1H, H-1′), 6.98 (s, 1H, H-6), 3.96 (s, 3H, OMe), 3.03 (dd, 1H, $^3J_{H,H} = 6.0$; $^2J_{H,H} = 18.1$ Hz, H-4′eq), 2.82 (ddd, 1H, $^3J_{H,H} = 7.2$, 11.2; $^2J_{H,H}$ 18.3 Hz, H-4′ax), 2.00 (m, 1H, H-9′eq), 1.91–1.31 (m, 8H), 1.23 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me). ^{13}C NMR (75 MHz) δ 179.6, 179.1, 161.6, 147.1, 141.5, 141.4, 140.5, 139.5, 139.2, 138.3, 131.2, 129.7, 128.9, 53.0, 50.7, 41.9, 39.3, 34.0, 33.4, 33.1, 24.9, 24.7, 21.5, 19.0, 18.9. Anal. calcd for $C_{25}H_{26}O_5S$: C, 68.47; H, 5.98; S, 7.31. Found, C, 68.90; H, 5.99; S, 7.13.

4,7-Dioxo-5-(6′, 6′, 9′a-trimethyl-4′, 5′, 5′aS, 6′, 7′, 8′, 9′, 9′aS-octahydro-naphtho[1,2-c]furan-3′-yl)-4,7-dihydro-benzo[*b*]thiophene-2-carbaldehyde (14). 70 mg, 47% from **1** (80.3 mg, 0.37 mmol) and quinone 13 (129.54 mg, 0.74 mmol), violet crystals (ether–methanol) mp 189–190 °C. IR ν 1676 (C=O), 1627 (C=O). ^{1}H NMR (200 MHz) δ 10.05 (s, 1H, CHO), 8.16 (s, 1H, 3-H), 7.36 (s, 1H, H-1′), 7.02 (s, 1H, H-6), 3.04 (dd, 1H, $^3J_{H,H} = 6.1$; $^2J_{H,H} = 18.3$ Hz, H-4′eq), 2.83 (ddd, 1H, $^3J_{H,H} = 7.2$, 11.1; $^2J_{H,H} = 18.3$ Hz, H-4′ax), 2.00 (m, 1H, H-9′eq), 1.92–1.31 (m, 8H), 1.24 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me). ^{13}C NMR (75 MHz) δ 183.1, 179.4, 179.0, 148.6, 147.6, 141.6, 141.5, 140.7, 139.8, 138.4, 133.2, 130.1, 128.9, 50.7, 41.8, 39.3, 34.0, 33.4, 33.1, 24.9, 24.8, 21.5, 19.0, 18.9. Anal. calcd for $C_{24}H_{24}O_4S$: C, 70.56; H, 5.92; S, 7.85. Found, C, 70.94; H, 5.89; S, 7.49.

In vitro cytotoxic screening

The generation of macrophages from bone marrow precursors, the isolation of parasites (*L. amazonensis*, strains LV79) from mouse lesions and the protocol of infection were described previously in full details.[18] For all drugs, stock solutions were prepared in DMSO at a concentration of 500 μg/mL. Two-fold serial dilutions were made from 250 μg/mL in culture medium supplemented with 0.5% DMSO final. Twenty-four h after infection, freshly prepared drugs are added to the infected cultures in triplicate. The first final drug concentration is 50 μg/mL and the final DMSO concentration is 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 ×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 looking at the regression of parasitophorous vacuoles and the overall decrease in parasite number.

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