Synthesis, antimalarial and antileishmanial activity of novel 13-benzyl-15,16-bisnorlabdane derivatives

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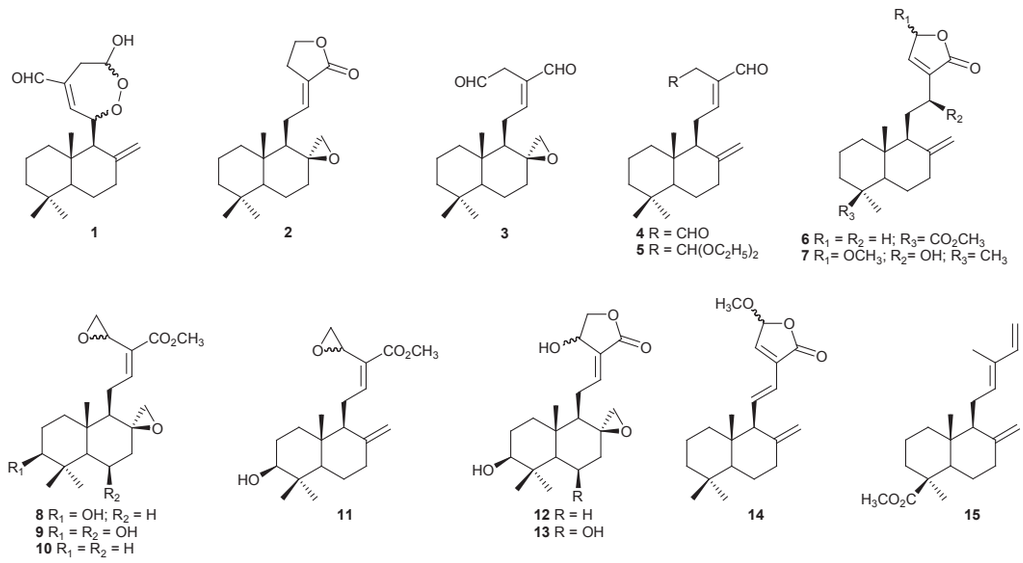
Twelve 13-benzyl-15,16-bisnorlabdanes in which the C-13 and C-14 substituents are varied have been prepared from the naturally occurring labdane diterpene (+)-manool. These synthesised compounds were evaluated for antimalarial activity, *in vitro* as hemozoin formation inhibitors and *in vivo* against *Plasmodium berghei*. These derivatives were also assayed for antileishmanial activity against *Leishmania mexicana*.

Keywords: bisnorlabdanes, hemozoin formation inhibitors, antimalarial, antileishmanial

Malaria and leishmaniasis are protozoan diseases with a large global distribution and which have a high mortality rate affecting millions of people every year. Globally, an estimated 3.3 billion people were at risk of malaria in 2011, and estimates by the World Health Organization (WHO) have indicated that approximately 80% of cases and 90% of deaths occur in Africa, with children less than five years of age and pregnant women most severely affected.[1] The *Plasmodium falciparum* parasite is responsible for most of the fatal cases of malaria. Leishmaniasis threatens about 350 million people in 98 countries or territories around the world, about 2 million estimated new cases occurring each year, and 12 million people are believed to be currently infected.[2] Malarial parasites have developed unacceptable levels of resistance towards existing treatment regimens[3–8] and drugs currently used against leishmaniasis show high toxicities, producing clinical resistance and requiring long-term treatment.[9] Therefore, there is an urgent need for a rapid search and discovery of new antimalarial and antileishmanial agents with a novel structural backbone.

Plants have traditionally been an excellent and reliable source for the discovery and development of new drugs. Although several synthetic drugs possess structures with little resemblance to those of natural products, these compounds are often superior as therapeutic agents to their natural counterparts. However, half the drugs on the market are direct descendants of natural products.[10] Labdanes diterpenoids are among the most common types of bicyclic diterpenes isolated from plants and sponges[11–15] and they have shown a large spectrum of interesting biological activity such as cytotoxic, antifungal, anti-inflammatory, antiparasitic and analgesic activity.[16] Several natural labdane-type diterpenes have shown good activity against chloroquine sensitive (compounds **1–6**, IC_{50} = 24.0–54.0 μM) and resistant (compounds **8–13**, IC_{50} = 5.0–39.9 μM) *Plasmodium falciparum* strain (3D7 and FcB1, respectively)[17–19] and against *Leishmania donovani* (compounds **7**, **14** and **15**, IC_{50} = 5.7–60.0 μM).[20–21]

In this context, we previously reported the synthesis of optically active labdane-related natural products showing antimalarial activity, which act by inhibiting the *β*-hematin formation and/or the globin proteolysis. Some of them showed a significant inhibition of one process or a moderate inhibition of both.[22] In continuation of this work, aimed at discovering compounds with antiparasitic activity, we report the preparation and, antimalarial and antileishmanial activity of twelve novel related-labdane 13-benzyl-15,16-bisnorlabdanes **21a**–**23d** in which the C-13 and C-14 substituents are varied.



Scheme 1

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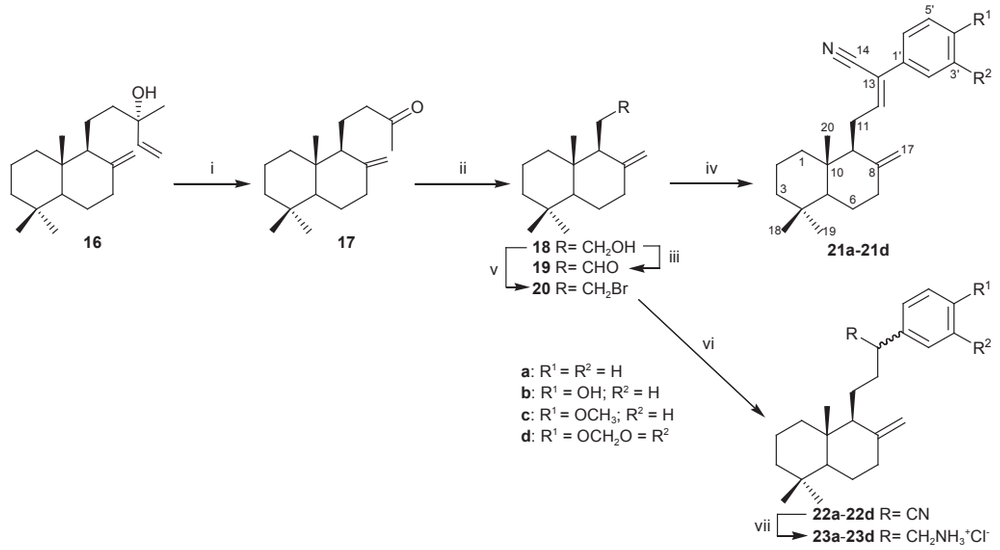

Scheme 2 (i) $KMnO_4$, Bu_4NBr, $CHCl_3$, 0 °C, 12 h; (ii) refs. 23,24; (iii) refs. 23,24; (iv) K_2CO_3, CH_3OH, γ-bicyclohomofarnesal **19**, (R^1,R^2)-benzylacetonitrile, rt, 4 h; (v) refs. 23,24; (vi) NaH, THF, (R^1,R^2)-benzylacetonitrile, rt → reflux, 90 min → bromide **20**, rt → reflux, 3 h; (vii) $LiAlH_4$, THF, 0 °C → reflux, 6 h → Et_2O•HCl, 0 °C, 1 h.

Results and discussion

13-Benzyl-15,16-bisnorlabdanes **21a**–**23d** were obtained from naturally occurring and commercially available (+)-manool **16**, a labdane diterpene commonly used as semi-synthetic precursor for the synthesis of others labdane diterpenes.[23] γ-Bicyclohomofarnesal **19** and the bromide **20** were prepared by removing the side chain of the starting material **16** using a procedure reported by Villamizar *et al.*[24–25] and used as key intermediaries in the preparation of derivatives **21a**–**23d**. Compound **16** was oxidised according to a slightly modified method of Costa *et al.*[26] to afford ketone **17** in 91% yield. γ-Bicyclohomofarnesal **19** was condensed with the appropriate benzylacetonitrile in the presence of K_2CO_3 and CH_3OH at room temperature for 4 h to obtain 13-benzyl-15,16-bisnorlabda-8(17),12(13)-dien-14-nitrile derivatives **21a**–**d** (Scheme 2). The new alkene (C12–C13) which was formed on compounds **21a**–**d** with an exclusive -*Z* configuration, was confirmed by NOESY through observing the spatial interaction of the olefinic proton (H-12) with the aromatic protons (H-2' and H-6'). Compound **20** was coupled with appropriate benzylacetonitrile in the presence of NaH and THF for 5 h at reflux temperature to afford an inseparable mixture (1:1, calculated from ^{1}H NMR integrals) of 13-*α* and 13-*β*-benzyl-15,16-bisnorlabda-8(17)-en-14-nitrile derivatives **22a**–**d**. The nitrile group of compounds **22a–d** was reduced with $LiAlH_4$ in THF refluxing for 6 h, then the respective amine chloride derivatives **23a**–**d** were formed by treating the crude product with a saturated solution of Et_2O•HCl at 0 °C.

The antimalarial and antileishmanial activity of compounds **21a**–**23d** are presented in Table 1. A possible relationship between the structure and the inhibition of *β*-haematin formation (IHF) of compounds containing nitrile double bond conjugation **23a**–**d** suggested that removing the conjugated double bond tended to improve inhibition and removal of the conjugated double bond and converting the nitrile to an amino group significantly enhanced inhibition. Among the compounds

Table 1 Antimalarial and antileishmanial evaluation of bisnorlabdanes **21a–23d**

Compound	*In vitro* antimalarial		*In vivo* antimalarial			Antileishmanial	
	IHF/%[a]	IC_{50}/mM[b]	P/%[c]	S/%[d]	ST/day[e]	PI/%[h]	IC_{50}/μM[i]
21a	<40	>25	ND[j]	–	–	0.09	>100
21b	<40	>25	ND	–	–	0.74	>100
21c	<40	>25	ND	–	–	2.31	84.26
21d	<40	>25	ND	–	–	72.27	26.3
22a	<40	>25	2.40±0.89	94.85	8.75±3.59	0	>100
22b	49.04±1.29	>25	11.15±2.21	76.07	10.40±3.20	0	>100
22c	69.37±0.69	6.12	2.40±1.14	94.85	23.80±4.32[m]	0	>100
22d	46.90±0.59	>25	18.16±5.67	61.03	9.20±4.32	0	>100
23a	88.69±0.74[k]	3.47	5.80±3.27	87.55	9.40±2.30	8.58	48.92
23b	88.53±1.06[k]	0.22	10.60±3.84	77.25	11.33±3.93	13.26	52.30
23c	73.00±4.57	2.36	3.40±1.67	92.70	8.50±1.73	5.87	38.66
23d	78.12±1.03	3.87	10.57±4.19	77.32	8.00±4.30	69.24	70.38
CQ[f]	88.03±0.77	0.48	0.95±0.75	97.96	28.00±1.34	–	–
NC[g]	–	–	46.60±1.94	–	8.60±1.42		–

[a]IHF, Inhibition of β-haematin formation, at 25 mM. [b]IC_{50}, Concentration required to inhibit β-haematin formation by 50%. [c]P, Parasitemia, at fourth day post-infection in infected mice with Plasmodium berghei. [d]S, Parasitemia suppression, at fourth day post-infection. [e]ST, Survival time of infected mice. [f]CQ, Chloroquine. [g]NC, Negative control, infected mice and only treated with saline-Tween solution. [h]PI, Promastigote inhibition, incubation by 54 h with drugs at 20 μM. [i]IC_{50}, Concentration required to inhibit L. mexicana promastigote by 50%. [j]ND, Not determined. [k]P>0.05 compared to IHF obtained with CQ. [m]P>0.05 compared to survival time of infected mice and treated with CQ.

that were tested, **22c**, **23c** and **23d** presented good inhibition and, **23a** and **23b** inhibited the β-haematin formation as much as chloroquine ($P > 0.05$). Special attention must be paid to **23b**, which exhibited an IC_{50} twice as potent as chloroquine. On *in vivo* antimalarial assay, compounds **22a**, **22c**, **23a** and **23c** gave good parasitemia suppression on the fourth day post-infection compared to the non-treated mice (87.55–94.85%). However, only compound **22c** increased the mean survival time of animals as significantly as chloroquine ($P > 0.05$).

On *in vitro* antileishmanial evaluation, compounds **21d**, **23a** and **23c** (IC_{50} = 26.3–48.92 μM) were found to be moderately active. Compounds **21c**, **23b** and **23d** showed weak activities (IC_{50} = 52.30–84.26 μM) and compounds **21a**, **21b** and **22a**–**d** were found to be completely inactive ($IC_{50} > 100$ μM). Possible SAR suggests that the decline in activities of compounds **22a**–**d** may be due to the removal of the conjugated double bond linked to the nitrile and aromatic groups. Compound **21d** having a nitrile group, conjugated double bond and 3,4-methylendioxy substituent on the aromatic ring was found to be the most active compound in the series.

This study reports the synthesis of novel 13-benzyl-15,16-bisnorlabda-8(17),12(13)-dien-14-nitrile **21a**–**d**, 13-benzyl-15,16-bisnorlabda-8(17)-en-14-nitrile **22a**–**d** and 13-benzyl-15,16-bisnorlabda-8(17)-en-14-amine **23a**–**d** derivatives. Several of the bisnorlabdanes which had been prepared exhibited *in vitro* and *in vivo* significant antimalarial activity and some displayed an *in vitro* moderate antileishmanial activity. Compound **22c** is promising and provides a useful model for further structural and biological optimisation to obtain new antimalarial agents.

Experimental

Melting points were measured with a Kofler hot-stage apparatus and were uncorrected. NMR spectra were recorded with Bruker Avance-300 and Avance-500 spectrometers. IR spectra were recorded using a Nicolet Magna 560 FT-IR spectrometer. Mass Spectra (MS) were obtained on a TSQ Quantum mass spectrometer. Elemental analyses were performed using a Fisons EA-1108 instrument. Optical rotations were obtained for $CHCl_3$ solutions on a Perkin-Elmer 341 polarimeter, and their concentrations are expressed in g/100 mL. Manool resin was purchased from Westchem Industries, Ltd. and purified to obtain (+)-manool **16**, $[\alpha]_D^{24}$ +28 (c 1.5, $CHCl_3$). THF and CH_3OH were purified before use. All other solvents and reagents were obtained from commercial suppliers and used without further purification. Merck silica gel (70–230 mesh ASTM) was used for column chromatography. TLC was performed on Analtech silica gel 60 G254 and the spots were observed either by exposure to iodine or by UV light. All organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure below 60 °C.

Synthesis of **21a–d**; *general procedure*

A mixture of aldehyde **19** (50.0 mg, 0.2133 mmol) and benzylnitrile, 4-hydroxybenzylnitrile, 4-methoxybenzylnitrile or 3,4-methylendioxybenzylnitrile (0.2133 mmol) in methanol (0.50 mL) was added to a suspension of K_2CO_3 (147.4 mg; 1.0666 mmol) in methanol (1.50 mL) under nitrogen at room temperature, the reaction mixture was stirred for 4 h. The reaction was diluted with water, neutralised with HCl (2 M) and extracted with diethyl ether. The organic extract was dried over $MgSO_4$, evaporated and the crude product was chromatographed over silica gel using mixtures of hexane and ethyl acetate.

13-Benzyl-15,16-bisnorlabda-8(17),12(13)-dien-14-nitrile (**21a**): Yield: 80%; white solid, m.p. 80–82 °C. $[\alpha]_D^{24}$ +4.1 (*c* 6.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.77, 0.82 and 0.88 (s, 3H each, CH_3), 4.56 and 4.87 (s, 1H each, H-17), 6.80 (t, 1H, *J* = 7.0 Hz, H-11), 7.34 (m, 3H, H-3′, H-4′, H-5′), 7.47 (m, 2H, H-2′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 148.38 (C-8), 148.15 (C-12), 133.38 (C-1′), 128.83 (C-3′, C-5′), 128.64 (C-4′), 125.48 (C-2′, C-6′), 116.74 (C-14), 115.51 (C-13), 107.75 (C-17), 56.70 (C-9), 55.44 (C-5), 42.01 (C-3), 39.70 (C-10), 39.15 (C-1), 37.98 (C-7), 33.59 (C-4, C-18), 27.33 (C-11), 24.19 (C-6), 21.71 (C-19), 19.31 (C-2), 14.41 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3081, 2933, 2210, 1638. MS (ESI): *m/z* = 334.10 $[M+H]^+$. Anal. Calcd for $C_{24}H_{31}N$ (333.25): C, 86.43; H, 9.37; N, 4.20. Found: C, 86.23; H, 9.31; N, 4.16%.

13-(4-Hydroxy)benzyl-15,16-bisnorlabda-8(17),12(13)-dien-14-nitrile (**21b**): Yield: 75%; white solid, m.p. 156–158 °C. $[\alpha]_D^{24}$ –3.9 (*c* 4.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.74, 0.81 and 0.87 (s, 3H each, CH_3), 4.54 and 4.86 (s, 1H each, H-17), 6.66 (t, 1H, *J* = 7.0 Hz, H-12), 6.82 (d, 2H, *J* = 8.2 Hz, H-2′, H-6′), 7.32 (d, 2H, *J* = 8.2 Hz, H-3′, H-5′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 156.43 (C-4′), 148.15 (C-8), 146.36 (C-12), 126.92 (C-2′, C-6′), 125.80(C-1′), 116.96 (C-14), 115.79 (C-3′, C-5′), 114.73 (C-13), 107.68 (C-17), 56.72 (C-9), 55.40 (C-5), 41.98 (C-3), 39.66 (C-10), 39.10 (C-1), 37.96 (C-7), 33.56 (C-4, C-18), 27.16 (C-11), 24.17 (C-6), 21.65 (C-19), 19.29 (C-2), 14.42 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3421, 3080, 2933, 2218, 1641. MS (ESI): *m/z* = 350.09 $[M+H]^+$. Anal. Calcd for $C_{24}H_{31}NO$ (349.24): C, 82.47; H, 8.94; N, 4.01. Found: C, 82.26; H, 8.87; N, 3.53%.

13-(4-Methoxy)benzyl-15,16-bisnorlabda-8(17),12(13)-dien-14-nitrile (**21c**)*:* Yield: 85%; white solid, m.p. 100–102 °C. $[\alpha]_D^{24}$ –4.7 (*c* 5.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.75, 0.81 and 0.87 (s, 3H each, CH_3), 3.79 (s, 3H, Ar–OCH_3), 4.55 and 4.86 (s, 1H each, H-17), 6.66 (t, 1H, *J* = 7.0 Hz, H-12), 6.86 (d, 2H, *J* = 8.6 Hz, H-3′, H-5′), 7.40 (d, 2H, *J* = 8.9 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 159.95 (C-4′), 148.17 (C-8), 146.06 (C-12), 126.74 (C-2′, C-6′), 126.03 (C-1′), 116.94 (C-14), 114.89 (C-13), 114.19 (C-3′, C-5′), 107.71 (C-17), 56.75 (C-9), 55.43 (C-5), 55.34 (Ar–OCH_3), 42.02 (C-3), 39.69 (C-10), 39.13 (C-1), 37.99 (C-7), 33.58 (C-4, C-18), 27.17 (C-11), 24.19 (C-6), 21.70 (C-19), 19.31(C-2), 14.39 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3081, 2938, 2214, 1642. MS (ESI): *m/z* = 364.18 $[M+H]^+$. Anal. Calcd for $C_{25}H_{33}NO$ (363.26): C, 82.60; H, 9.15; N, 3.85. Found: C, 82.41; H, 9.08; N, 3.49%.

13-(3,4-Methylendioxy)benzyl-15,16-bisnorlabda-8(17),12(13)-dien-14-nitrile (**21d**)*:* Yield: 90%; white solid, m.p. 21–123 °C. $[\alpha]_D^{24}$ –10.4 (*c* 6.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.75, 0.81 and 0.87 (s, 3H each, CH_3), 4.53 and 4.86 (s, 1H each, H-17), 5.95 (s, 2H, OCH_2O), 6.63 (t, 1H, *J* = 7.0 Hz, H-12), 6.77 (d, 1H, *J* = 7.9 Hz, H-5′), 6.92 (d, 1H, *J* = 1.6, H-2′), 6.98 (dd, 1H, *J* = 8.2, 1.6 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 148.24 (C-3′), 148.15 (C-8), 148.07 (C-4′), 146.66 (C-12), 127.70 (C-1′), 119.91 (C-6′), 116.80 (C-14), 114.99 (C-13), 108.41 (C-5′), 107.71 (C-2′), 105.50 (C-17), 101.44 (OCH_2O), 56.75 (C-9), 55.45 (C-5), 42.03 (C-3), 39.72 (C-10), 39.15 (C-1), 38.01 (C-7), 33.62 (C-4, C-18), 27.20 (C-11), 24.22 (C-6), 21.74 (C-19), 19.34 (C-2), 14.43 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3081, 2931, 2218, 1634. MS (ESI): *m/z* = 378.10 $[M+H]^+$. Anal. Calcd for $C_{25}H_{31}NO_2$ (377.24): C, 79.54; H, 8.28; N, 3.71. Found: C, 79.32; H, 8.20; N, 3.62%.

Synthesis of **22a–d**; *general procedure*

A solution of benzylnitrile, 4-hydroxybenzylnitrile, 4-methoxybenzylnitrile or 3,4-methylendioxybenzylnitrile (2.0047 mmol) in THF (2.0 mL) was added to a suspension of NaH (6.6824 mmol) in THF (6.0 mL) was added at room temperature. The reaction mixture was stirred and refluxed for 90 minutes. Then, a solution of the bromide **20** (435 mg, 1.4534 mmol) in THF (2.0 mL) was added and the reaction was stirred and refluxed for an additional 3 h. Finally, the reaction was diluted with water, neutralised with HCl (2 M) and extracted with diethyl ether. The organic extract was dried over $MgSO_4$, evaporated and the crude product was chromatographed over silica gel using mixtures of hexane and ethyl acetate.

13-Benzyl-15,16-bisnorlabda-8(17)-en-14-nitrile (**22a**): Yield: 93%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.64–0.66, 0.78–0.79 and 0.85 (s, 3H each, CH_3), 3.73 (m, 1H, H-13), 4.33–4.40 and 4.78–4.79 (s, 1H each, H-17), 7.33 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 148.23–147.83 (C-8), 136.03–135.92 (C-1′), 128.93 (C-3′, C-5′), 127.93–127.87 (C-4′), 127.21–127.10 (C-2′, C-6′), 120.98–120.75 (C-14), 106.60–106.23 (C-17), 56.57–56.16 (C-9), 55.53–55.47 (C-5), 42.06 (C-3), 39.76–39.69 (C-13), 38.99–38.93 (C-10), 38.21–38.17 (C-1), 37.82–37.59 (C-7), 35.03–34.88 (C-12), 33.56–33.51 (C-4, C-18), 24.34 (C-6), 21.65 (C-19), 21.30–21.18 (C-11), 19.30–19.27 (C-2), 14.38–

14.35 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3076, 2929, 2235, 1641. MS (ESI): *m/z* = 336.13 [M+H]$^+$. Anal. Calcd for $C_{24}H_{33}N$ (335.26): C, 85.91; H, 9.91; N, 4.17. Found: C, 85.70; H, 9.84; N, 4.04%.

13-(4-Hydroxy)benzyl-15,16-bisnorlabda-8(17)-en-14-nitrile (**22b**): Yield: 85%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.62–0.65, 0.77–0.78 and 0.84 (s, 3H each, CH_3), 3.66 (m, 1H, H-13), 4.31–4.39 and 4.76–4.78 (s, 1H each, H-17), 5.25 (bs, 1H, Ar–OH), 6.79–6.81 (d, 2H, *J* = 8.9 Hz, H-3′, H-5′), 7.13–7.15 (d, 2H, *J* = 8.8 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 155.40–155.34 (C-4′), 148.28–147.91 (C-8), 128.56–128.44 (C-2′, C-6′), 128.01–127.89 (C-1′), 121.35–121.11 (C-14), 115.83 (C-3′, C-5′), 106.60–106.26 (C-17), 56.57–56.19 (C-9), 55.55–55.50 (C-5), 42.08 (C-3), 39.79–39.72 (C-13), 39.02–38.98 (C-10), 38.23–38.19 (C-1), 37.01–36.82 (C-7), 35.00–34.87 (C-12), 33.55 (C-4, C-18), 24.36 (C-6), 21.67 (C-19), 21.23–21.14 (C-11), 19.30 (C-2), 14.37 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3342, 3068, 2932, 2253, 1640. MS (ESI): *m/z* = 352.16 [M+H]$^+$. Anal. Calcd for $C_{24}H_{33}NO$ (351.26): C, 82.00; H, 9.46; N, 3.98. Found: C, 81.81; H, 9.41; N, 3.86%.

13-(4-Methoxy)benzyl-15,16-bisnorlabda-8(17))-en-14-nitrile (**22c**): Yield: 94%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.62–0.65, 0.77 and 0.84 (s, 3H each, CH_3), 3.67 (m, 1H, H-13), 4.31–4.40 and 4.76–4.78 (s, 1H each, H-17), 6.85–6.88 (dd, 2H, *J* = 8.7, 1.5 Hz, H-3′, H-5′), 7.19–7.22 (dd, 2H, *J* = 8.7, 1.5 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 159.20–159.15 (C-4′), 148.24–147.89 (C-8), 128.34–128.22 (C-2′, C-6′), 128.03–127.92 (C-1′), 121.29–121.05 (C-14), 114.31 (C-3′, C-5′), 106.61–106.26 (C-17), 56.56–56.18 (C-9), 55.52–55.47 (C-5), 55.29 (Ar–OCH_3), 42.07 (C-3), 39.78–39.69 (C-13), 38.99–38.95 (C-10), 38.21–38.18 (C-1), 36.98–36.79 (C-7), 35.03–34.90 (C-12), 33.56–33.52 (C-4, C-18), 24.33 (C-6), 21.66 (C-19), 21.22–21.13 (C-11), 19.31 (C-2), 14.38 (C-20). IR (KBr) $_{max}$/cm^{-1}: 3081, 2934, 2235, 1642. MS (ESI): *m/z* = 366.19 [M+H]$^+$. Anal. Calcd for $C_{25}H_{35}NO$ (365.27): C, 82.14; H, 9.65; N, 3.83. Found: C, 81.94; H, 9.56; N, 3.79%.

13-(3,4-Methylendioxy)benzyl-15,16-bisnorlabda-8(17)-en-14-nitrile (**22d**): Yield: 98%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.63–0.65, 0.77–0.78 and 0.85 (s, 3H each, CH_3), 3.63 (m, 1H, H-13), 4.33–4.41 and 4.77–4.80 (s, 1H each, H-17), 5.95 (s, 2H, OCH_2O), 6.76 (m, 3H, H-2′, H-5′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 148.26–148.13 (C-3′), 147.89 (C-8), 147.33–147.28 (C-4′), 129.75–129.61 (C-1′), 121.07–120.84 (C-14), 120.69–120.53 (C-6′), 108.51 (C-5′), 107.64–107.59 (C-2′), 106.64–106.27 (C-17), 101.32 (OCH_2O), 56.59–56.19 (C-9), 55.56–55.50 (C-5), 42.09 (C-3), 39.79–39.72 (C-13), 39.03–38.99 (C-10), 38.23 (C-1), 37.50–37.30 (C-7), 35.07–34.96 (C-12), 33.57 (C-4, C-18), 24.36 (C-6), 21.67 (C-19), 21.26–21.18 (C-11), 19.32 (C-2), 14.40–14.37 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3079, 2937, 2239, 1641. MS (ESI): *m/z* = 380.15 [M+H]$^+$. Anal. Calcd for $C_{25}H_{33}NO_2$ (379.25): C, 79.11; H, 8.76; N, 3.69. Found: C, 78.90; H, 8.70; N, 3.60%.

Synthesis of **23a**–**d***; general procedure*

A solution of compound **22a**, **22b**, **22c** or **22d** (0.1639 mmol) in dry THF (1.0 mL) was added to a suspension of $LiAlH_4$ (0.6557 mmol) in dry THF (2.0 mL) under nitrogen and with stirring at 0 °C. The reaction mixture was then refluxed for 6 h. Then water was added, neutralised with HCl (2 M) and extracted with ethyl acetate. A saturated solution of Et_2O•HCl at 0 °C was added dropwise to a solution of crude product in ether (0.5 mL).

13-Benzyl-15,16-bisnorlabda-8(17)-en-14-amine (**23a**): Yield: 87%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.54–0.58, 0.77–0.78 and 0.84–0.86 (s, 3H each, CH_3), 2.84 (m, 1H, H-13), 3.11 (m, 1H, H-14), 4.10–4.50 (d, 1H, *J* = 1.6–1.9 Hz, H-17), 4.68–4.82 (d, 1H, *J* = 2.6–2.5 Hz, H-17), 7.34 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 149.87–149.55 (C-8), 141.61–141.37 (C-1′), 130.20–130.17 (C-3′, C-5′), 129.21–129.07 (C-4′), 128.83–128.73 (C-2′, C-6′), 106.99 (C-17), 58.45–57.79 (C-9), 56.90–56.81 (C-5), 46.04–45.88 (C-14), 43.30–43.24 (C-3), 40.72–40.68 (C-10), 40.16–39.95 (C-1), 39.39–39.32 (C-7), 34.47–34.42 (C-12), 34.19–34.09 (C-13), 34.05–33.82 (C-4, C-18), 25.59–25.52 (C-6), 22.42 (C-19), 22.19–22.10 (C-11), 20.37–20.28 (C-2), 14.84 (C-20). IR (KBr) ν_{max}/cm^{-1}: 2929, 1982. MS (ESI): *m/z* = 340.20 [M−Cl]$^+$. Anal. Calcd for $C_{24}H_{38}ClN$ (375.27): C, 76.66; H, 10.19; N, 3.73. Found: C, 76.45; H, 10.12; N, 3.60%.

13-(4-Hydroxy)benzyl-15,16-bisnorlabda-8(17)-en-14-amine (**23b**): Yield: 83%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.56–0.59, 0.77–0.78, 0.84–0.86 (s, 3H each, CH_3), 2.74 (m, 1H, H-13), 3.08 (m, 1H, H-14), 4.14–4.49, 4.69–4.82 (d, 1H each, *J* = 2.2–2.2 Hz, H-17), 6.80–6.83 (d, 1H, *J* = 13.8 Hz, H-3′, H-5′), 7.07–7.10 (d, 1H, *J* = 14.1 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 158.13–158.07 (C-4′), 149.96–149.56 (C-8), 131.90–131.62 (C-2′, C-6′), 130.16–130.02 (C-1′), 116.92–116.88 (C-3′, C-5′), 107.05–106.92 (C-17), 58.46–57.75 (C-9), 56.93–56.82 (C-5), 45.56–45.15 (C-14), 43.32–43.27 (C-3), 40.72–40.68 (C-10), 40.17–39.98 (C-1), 39.41–39.34 (C-7), 34.47–34.43 (C-12), 34.33–34.09 (C-13), 34.06–33.94 (C4, C18), 25.60–25.53 (C-6), 22.45 (C-19), 22.16–22.11 (C-11), 20.38–20.30 (C-2), 14.86 (C-20). IR (KBr) ν_{max}/cm^{-1}: 3277, 2927, 1957. MS (ESI): *m/z* = 356.41 [M−Cl]$^+$. Anal. Calcd for $C_{24}H_{38}ClNO$ (391.26): C, 73.53; H, 9.77; N, 3.57. Found: C, 73.31; H, 9.71; N, 3.37%.

13-(4-Methoxy)benzyl-15,16-bisnorlabda-8(17)-en-14-amine (**23c**): Yield: 98%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.56–0.59, 0.77–0.78, 0.84–0.86 (s, 3H each, CH_3), 2.74 (m, 1H, H-13), 3.05 (m, 1H, H-14), 3.80 (s, 3H, Ar–OCH_3), 4.14–4.49, 4.69–4.82 (s, 1H each, H-17), 6.95–6.98 (dd, 2H, *J* = 8.9, 2.2 Hz, H-3′, H-5′), 7.28–7.31 (dd, 2H, *J* = 8.9, 2.2 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): 160.20–160.09 (C-4′), 149.61–149.51 (C-8), 134.90–134.79 (C2′, C6′), 128.98–128.91 (C1′), 115.26 (C3′, C5′), 106.99–106.90 (C17), 57.69–56.98 (C-9), 55.92–55.82 (C-5), 55.10 (Ar–OCH_3), 45.57–45.15 (C-14), 43.32–43.29 (C-3), 40.71–40.68 (C-10), 40.17–39.98 (C-1), 39.42–39.34 (C-7), 34.49–34.47 (C-12), 34.33–34.06 (C-13), 34.09–33.93 (C-4, C-18), 25.60–25.53 (C-6), 22.47 (C-9), 22.13–22.00 (C-11), 20.41–20.30 (C-2), 14.85 (C-20). IR (KBr) ν_{max}/cm^{-1}: 2940, 1998. MS (ESI): *m/z* = 370.43 [M−Cl]$^+$. Anal. Calcd for $C_{25}H_{40}ClNO$ (405.28): C, 73.95; H, 9.93; N, 3.45. Found: C, 73.71; H, 9.84; N, 3.27%.

13-(3,4-Methylendioxy)benzyl-15,16-bisnorlabda-8(17)-en-14-amine (**23d**): Yield: 98%; white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.57–0.61, 0.78, 0.84–0.86 (s, 3H each, CH_3), 2.78 (m, 1H, H-13), 3.07 (m, 1H, H-14), 4.18–4.50 (d, 1H, *J* = 1.3–1.6 Hz, H-17), 4.72–4.82 (d, 1H, *J* = 2.2–2.6 Hz, H-17), 5.95–5.96 (s, 2H, OCH_2O), 6.78 (m, 3H, H-2′, H-5′, H-6′). ^{13}C NMR ($CDCl_3$, 75.45 MHz): δ 149.91 (C-3′), 149.61 (C-8), 148.66–148.59 (C-4′), 135.17–134.88 (C-1′), 122.81–122.64 (C-6′), 109.54 (C-5′), 108.69–108.63 (C-2′), 106.99 (C-17), 102.55 (OCH_2O), 58.43–57.76 (C-9), 56.92 (C-5), 45.71 (C-14), 43.31 (C-3), 40.71 (C-10), 40.20 (C-1), 39.40–39.34 (C-7), 34.48–34.43 (C-12), 34.23–34.09 (C-13), 33.84 (C-4, C-18), 25.60 (C-6), 22.44 (C-9), 22.17–22.12 (C-11), 20.39–20.32 (C-2), 14.88 (C-20). IR (KBr) ν_{max}/cm^{-1}: 2926, 1953. MS (ESI): *m/z* = 384.14 [M−Cl]$^+$. Anal. Calcd for $C_{25}H_{38}ClNO_2$ (419.26): C, 71.49; H, 9.12; N, 3.33. Found: C, 71.30; H, 9.07; N, 3.25%.

Antimalarial evaluation

In vitro *inhibition of β-haematin formation*: The β-haematin formation assay was performed according to a slightly modified method of Baelmans *et al.*[26] Briefly, 50 µL of a fresh solution of haematin chloride (5.2 mg mL^{-1}, 4 mM) in dimethyl sulfoxide (DMSO), was distributed in 96-well micro plates. The tested compounds **21a**–**23d** (50 µL, 0.1–100 mM) in DMSO, positive control CQ (50 µL, 0.1–100 mM) in water, 50 µL of the solvents as negative controls (water and DMSO) and 100 µL of acetate buffer (0.2 M, pH 4.4), were added in triplicate to test wells. The final concentration of CQ and tested compounds ranged from 0.025 to 25 mM/well. Plates were incubated at 37 °C for 48 h to allow completion of the reaction and centrifuged at 4000 RPM for 15 min. After discarding the supernatant, the pellet was washed twice with DMSO (200 µL) and finally dissolved in NaOH (200 µL, 0.2 N). The solubilised aggregates were further diluted 1:2 with NaOH (0.1 N) and the absorbance recorded at 405 nm by using Microplate Reader (BIORAD-550).

In vivo *antimalarial assay:* The compounds were also tested in a malaria murine model according to Peters and Robinson.[27] Male albino mice of Hygiene National Institute (HNI) strain, weighing 18–23 g were maintained on a commercial pellet diet and housed under conditions approved by the Ethics Committee of the Faculty of Pharmacy, Central University of Venezuela. *Plasmodium berghei* (ANKA strain), a rodent malarial parasite, was used for infection. Briefly, mice (*n* = 6) were infected by IP passage of 10^6 infected erythrocytes diluted in phosphate

buffered saline solution (PBS 10 mM, pH 7.4, 0.1 mL). The compounds were dissolved in DMSO (0.1 M), diluted with Saline-Tween 20 solutions (2%). Two hours after infection, each compound was administered once IP (20 mg kg^{-1} day^{-1}) for four consecutive days. On day 4, the parasitemia was monitored by microscopic examination of Giemsa-stained smears. Chloroquine (20 mg kg^{-1} day^{-1}) was used as a positive control. The results were expressed as the percentage of parasitemia at the fourth day post-infection and were compared to the values reported by non-treated *Plasmodium berghei* infected mice. The survival time beyond the control group (without drug treatment) was recorded. Data were statistically analysed using unpaired *t*-tests for specific group comparisons; assuming 95% confidence according GraphPad Prism 5.0.

Antileishmanial evaluation

Promastigotes of the *L. mexicana* strain AZV were maintained at 28 °C on a Schneider medium supplemented with 20% foetal bovine serum (FBS). Hamster footpad lesions were used as sources of amastigotes for isolation and maintenance of the parasites. Briefly, *L. mexicana* promastigotes were seeded in 96-well micro plates to a final density of 10^6 promastigote mL^{-1}. One row was left with medium and without compounds (negative controls). Bisnorlabdane derivatives **21a**–**23d** in triplicate, were added to the culture at different concentrations (20, 40, 60, 80 and 120 μM). After incubation for 54 h at 28 °C, the remaining parasites were counted in a Neubauer's chamber and compared with the controls.

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References

1 World Health Organization, *World malaria report 2012*, WHO Press, Switzerland, Geneva, 2012. pp. 88.
2 World Health Organization, *Research priorities for Chagas disease, human African Trypanosomiasis and Leishmaniasis (technical report series, No. 975)*, WHO Press, Switzerland, Geneva, 2012. pp. 100.
3 World Health Organization, *Global report on antimalarial drug efficacy and drug resistance: 2000–2010*, WHO Press, Switzerland, Geneva, 2010. pp. 115.
4 U. Farooq and R.J. Mahajan, *Vect. Borne. Dis*., 2004, **41**, 45.
5 C. Amaratunga, S. Sreng, S. Suon *et al*., *Lancet Infect. Dis*., 2012, **12**, 851.
6 A.P. Phyo, S. Nkhoma, K. Stepniewska *et al*., *The Lancet*, 2012, **379**, 1960.
7 Q. Cheng, D.E. Kyle and M.L. Gatton, *Int. J. Parasitol. Drugs Drug Resist*., 2012, **2**, 249.
8 A.M. Dondorp, F. Nosten, P. Yi *et al*., *N. Engl. J. Med*., 2009, **361**, 455.
9 H.W. Murray, *Am. J. Trop. Med. Hyg.*, 2004, **71**, 787.
10 D. Kinghorn, J.N. Pan and C.F. Heebyung, *J. Nat. Prod*., 2011, **74**, 1539.
11 J.R. Hanson, *Nat. Prod. Rep*., 2012, **29**, 890.
12 J.R. Hanson, *Nat. Prod. Rep*., 2011, **28**, 1755.
13 J.R. Hanson, *Nat. Prod. Rep*., 2009, **26**, 1156.
14 J.R. Hanson, *Nat. Prod. Rep*., 2007, **24**, 1332.
15 I.S. Marcos, L. Castañeda, P. Basabe, D. Díez and J.G. Urones, *Mini-Rev. Org. Chem*., 2012, **9**, 54.
16 I. Chinou, *Curr. Med. Chem*., 2005, **12**, 1295.
17 G. Duker-Eshun, J.W. Jaroszewski, W.A. Asomaning *et al*., *Planta Med*., 2002, **68**, 642.
18 J. Asili, M. Lambert, H.L. Ziegler *et al*., *J. Nat. Prod*., 2004, **67**, 631.
19 M. Kenmogne, E. Prost, D. Harakat *et al*., *Phytochemistry*, 2006, **67**, 433.
20 Z. Cheikh-Ali, T. Okpekon, F. Roblot *et al*., *Phytochemistry Lett.*, 2011, **4**, 240.
21 V. Samoylenko, D.C. Dunbar, M.A. Gafur *et al*., *Phytother. Res*., 2008, **22**, 1570.
22 J.E. Villamizar, J.P. Pittelaud, J.R. Rodrigues *et al*., *Nat. Prod. Res*., 2009, **23**, 891.
23 F.J. Salazar and J.E. Villamizar, *J. Chem. Res.*, 2013, **37**, 63.
24 J.E. Villamizar, J. Fuentes, F. Salazar, E. Tropper and R. Alonso, *J. Nat. Prod*., 2003, **66**, 1623.
25 J.E. Villamizar, F.J. Salazar, J. Fuentes, E. Tropper and R. Alonso, *J. Chem. Res*., 2003, 504.
26 M. Costa, R. Tavares, W.B. Motherwell and M.J. Curto, *Tetrahedron Lett.*, 1994, **35**, 8839.
27 R. Baelmans, E. Deharo, E. Muñoz, M. Sauvain and H. Gisnburg, *Exp. Parasitol.*, 2000, **96**, 243.
28 W. Peters and B.L. Robinson, *Handbook of animal models of infection*, eds O. Zack and M. Sande, Academic Press, San Diego, CA, 1999. Malaria, pp. 757–773.