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A New Antimalarial Quassinoid from *Simaba orinocensis*

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A new antimalarial quassinoid, namely, orinocinolide (**1**), was isolated from the root bark of *Simaba orinocensis*, together with the previously reported simalikalactone D (**2**). The structure of **1** was determined primarily from 1D and 2D NMR analysis, as well as by chemical derivatization. Compound **1** was found to be as equally potent as **2** against *Plasmodium falciparum* clones D6 and W2 (IC_{50} 3.27 and 8.53 ng/mL vs 3.0 and 3.67 ng/mL, respectively), but was 4- and 28-fold less toxic than **2** against VERO cells (IC_{50} 10 vs 2.3 μg/mL) and HL-60 (IC_{50} 0.7 vs 0.025 μg/mL), respectively. In addition, **2** was >46- and >31-fold more potent than pentamidine and amphotericin B (IC_{50} 0.035 vs 1.6 and 1.1 μg/mL) against *Leishmania donovani*, while **1** was inactive. Orinocinolide (**1**) inhibited growth of human cancer cells SK-MEL, KB, BT-549, and SK-OV-3, but was less potent than **2** (IC_{50} 0.8−1.9 vs 0.3−1.0 μg/mL) against these cells.

Plants of the family Simaroubaceae are widely used in traditional medicine for the treatment of cancer, malaria, dysentery, and other diseases in countries around the world, e.g., *Brucea antidysenterica* (J. F. Mill.) in Africa,[1] *B. javanica* (L.) Merr. and *Ailanthus altissima* (Mill.) Swingle in China,[2] *Simaba guianen*sis Aubl. in Brazil,[3] and *Castela texana* (Torr. & A. Gray) Rose in Mexico.[4] Purple, an American physician, reported the antimalarial activity of *S. cedron* as early as 1854 and suggested it could be used as a substitute for quinine.[5,6] The plant family Simaroubaceae consists of six subfamilies with 32 genera, and over 170 arborous or shrubby species mostly grow in the tropics. On the basis of morphological and anatomic evidence, five of these six subfamilies have been removed from this family at one time or another. This would leave only the Simarouboideae (22 genera) in the Simaroubaceae.[7]

Simaba orinocensis Kunth (synonyms: *S. angustifolia* Benth., *S. foetida* Benth., and *S. multiflora* A. Juss.) is a native tree found in the Amazonian riversides and seasonally inundated areas of South America. Earlier investigations on *S. orinocensis* resulted in the isolation of alkaloids, quassinoids, triterpenes, coumarins, and coumarino lignans.[8−12] The quassinoids have been the subject of extensive investigations on their antitumor, antimalarial, anti-inflammatory, amoebicide, antifeedant, and herbicidal activities.[1] An EtOH extract of *S. orinocensis* root bark and leaves exhibited considerable in vitro antimalarial activity to warrant fractionation. On the basis of the initial activity of crude extracts, we focused our attention on the bioactivity-guided fractionation of the EtOH extract of the root bark, which resulted in the isolation of a new antimalarial quassinoid, **1**, and the previously reported simalikalactone D (**2**).[3]

Results and Discussion

The HRESIMS of **1** exhibited the $[M + Na]^+$ ion at *m/z* 503.2360, attributed to the molecular formula $C_{25}H_{36}O_9$. The ^1H NMR spectrum of **1** showed three tertiary methyls (δ 1.61, 1.39, and 1.20; Me-18, Me-21, and Me-19, respectively), a secondary methyl group (δ 1.18 d, J = 7.0 Hz; Me-5′), a primary methyl group (δ 0.96, t, J = 7.5 Hz; Me-4′), and the proton of a trisubstituted double bond (δ 5.40, br s, H-3). The ^{13}C NMR spectrum of **1** displayed 25 signals, five of which were in accordance with the presence of a 2-methylbutane-2-carboxylic acid side chain. After subtraction of the five-carbon side chain, the remaining 20 resonances were attributable to a quassinoid skeleton.[3,7,8,11] The ^1H and ^{13}C NMR signals for the quassinoid moiety were consistent with a $C_{20}H_{28}O_8$ framework, indicating the presence of 7 degrees of unsaturation, i.e., two double bonds [δ_{C-16} 167.7 (C=O); δ_{C-3} 123.6, and δ_{C-4} 135.4] and a five-ring system.

The covalent connectivities of **1** were unambiguously established by analysis of the G-DQF-COSY and G-HMQC experiments, which revealed the presence of four isolated spin systems in the quassinoid framework, as observed for **2**:[3,16] H-1 → H-7, H-9 → H-12, H-14 → H-15, and H-20a → H-20b. A G-HMBC experiment showed ^3J correlation between $\delta_{C-1'}$ 175.7, H-15 (δ 6.20), H-3′ (δ 1.51, 1.75), and H_3-5′ (δ 1.18), thus confirming the butane-2 carboxylic acid moiety at C-15 (Figure 1). In addition, the HMBC showed key ^3J correlation between δ_{C-4} 135.4, H-2 (δ 4.11) and H-6 (δ 1.66), δ_{C-5} 43.0, H-3 (δ 5.40), H_3-18 (δ 1.61), and H_3-19 (δ 1.20), and δ_{C-13} 80.9, H-11 (δ 4.70), H_2-20 (δ 3.52), and H_3-21 (δ 4.64), thus allowing the assignments of a hydroxyl group at C-2, a double bond at C-3(4), and an oxymethylene bridge between C-13 and C-20. The complete HMBC

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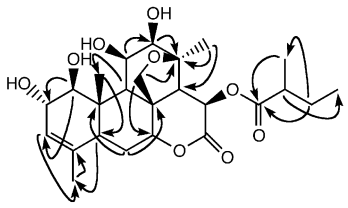
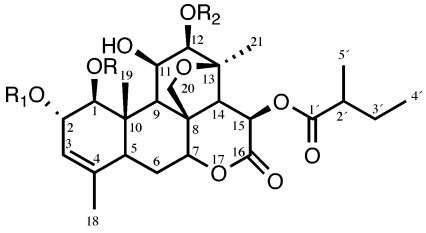
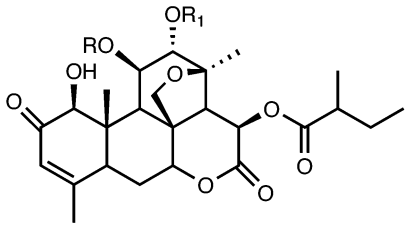


Figure 1. Key HMBC (solid lines) correlations of **1**.

correlation is depicted in Figure 1. On the basis of the foregoing data, the gross structure of **1** was established as shown.



1 R=R_1=R_2=H
1a R=H R_1=R_2=Ac
1b R=R_1=R_2=Ac



2 R=R_1=H
2a R=R_1=Ac

Acetylation of **1** yielded the corresponding acetates, **1a** and **1b**. The IR spectra of **1a** and **1b** displayed absorption bands at ~3450−3460 cm^{-1}, indicating the presence of free hydroxyl group(s). ^{1}H NMR and COSY studies of **1a** distinguished the H-2 (δ 5.13, dd, J = 1.5 and 6.5 Hz) and H-12 (δ 4.86, br s) protons, showing the expected downfield shifts in comparison to **1**. Furthermore, inspection of the ^{1}H NMR spectrum of **1b** showed downfield shifts for H-1, H-2, and H-12 [δ 5.23, d, J = 7.5 Hz, δ 5.41, and 4.62 (br s), respectively]. Complete assignments of each acetylated oxymethine proton of **1a** and **1b** were achieved by analyzing G-DQF-COSY, while the carbons of **1b** were assigned from G-HMQC and G-HMBC spectra. The C-11(O) position was interestingly not acetylated in both **1a** and **1b**, suggesting that the *cis*-oriented C-11(O) and C-12(O) may inhibit the acetylation of C-11(O) due to steric hindrance.

The relative stereochemistry of **1** was resolved by 2D NMR NOESY data. It showed correlation between H-1, H-11, H-9, and H-5 and between H-12, H-11, H-9, and Me-21, as well as between H-15 and H-9, suggesting that these protons were α-cofacial. On the other hand, observation of the key NOESY correlations between Me-19, H-2, and H-20b, and between H-20a and H-20b, as well as between H-7 and H-14 revealed that these protons occupy the β-face of the molecule. These results also suggested that **1** has the usual A/B *trans* and B/C *trans* ring junctions, and the C-12 hydroxyl group is β-cofacial (Figure 2). The NOESY spectrum of **2**[3,13] showed correlations between H-1, H-5, H-9, and H-11, as observed for **1**, but revealed the absence of correlation between H-11 (δ 4.61) and H-12 (δ 3.75), suggesting the β-orientation of H-12.

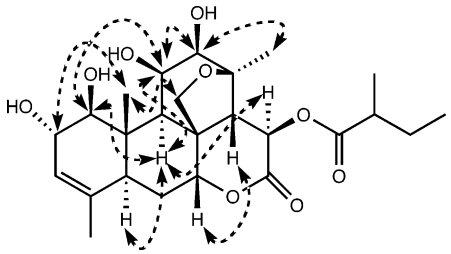


Figure 2. Key NOESY (dashed lines) correlations of **1**.

Compound **1** demonstrated strong in vitro antimalarial activity against chloroquine-sensitive and chloroquine-resistant *P. falciparum* clones D6 and W2 (IC_{50} 3.27 and 8.53 ng/mL, respectively), while **2** (IC_{50} 3.0 and 3.67 ng/mL, respectively) was equally potent as **1**, and **2a** showed very weak activity against these clones (Table 1). Table 1 also demonstrates that the hydroxyl group at C-2 (**1**) significantly reduced the mammalian (VERO) toxicity compared to **2** (IC_{50} 10 vs 2.3 μg/mL). Antileishmanial activity evaluation revealed that **1** and acetylated products **1b** and **2a** were inactive against *L. donovani* (Table 1), while **2** was 46- and 31-fold more potent than the standard antileishmanial agents pentamidine and amphotericin B, respectively (IC_{50} 0.035 vs 1.6 and 1.1 μg/mL). When tested for in vitro cytotoxic activity against human cancer cells SK-MEL, KB, BT-549, and SK-OV-3, **1** was found to be less potent than **2** (IC_{50} 1.9, 1.9, 1.7, and 0.8 μg/mL vs and 0.28, 1.0, 0.3, and 0.3 μg/mL), but as potent as doxorubicin (IC_{50} 1.57, 1.7, 1.0, and 1.53 μg/mL) (Table 2). The antiviral,[13] antileishmanial,[14] antimalarial,[3,15] and anticancer[16] activity of **2** had previously been reported, and various analogues were synthesized to improve its biological activity.[15−17]

Cell adhesion molecules such as lymphocyte function-associated molecule 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) are important not only in the regulation of immunity and inflammation[18] but also in survival for the parasites under infectious condition.[19] Recently, nitric oxide (NO) was shown to inhibit *P. falciparum* infection by inhibiting cytoadherence; therefore, regulation of NO may be a new target for antimalarial therapy.[20] On the basis of these observations, compounds **1**, **2**, and **2a** were evaluated for their in vitro effect on LFA-1/ICAM-1-mediated cell adhesion and nitric oxide synthase for antimalarial activity, as well as tumor inhibitory and anti-inflammatory activities (Table 3). Compound **2** inhibited cell aggregation (MIC 0.01 μg/mL) significantly when compared to **1** and cytochalasin B (MIC 0.26 and 2.3 μg/mL, respectively). Both **1** and **2** were inactive in the secondary cell adhesion assay, while they were >57- and >1600-fold more potent than cytochalasin B (IC_{50} 0.7, 0.025, and 40.0 μg/mL, respectively) against HL-60 cells. Furthermore, **1**, **2**, and **2a** showed inhibition of inducible nitric oxide synthase (iNOS), which is implicated in increased blood flow to the tumor site, resulting in tumor growth. The cytotoxic effects of **1** and **2** were also observed against RAW264.7 cells (Table 3); thus, they were considered to be nonspecific iNOS inhibitors. The cell aggregation and iNOS inhibition might be due to toxicity of these compounds.

Quassinoids **1** and **2** inhibited protein biosynthesis in vitro in a translation system derived from the Krebs cells, while the acetylated derivatives **1b** and **2a** were found to be inactive (Table 4). At 2 μM, **1** and **2** inhibited protein synthesis in vivo (96.5% and 98.5% inhibition, respectively) as measured by metabolic labeling with ^{35}S-methionine

Table 1. Antiparasitic Activities of Compounds **1**, **1b**, **2**, and **2a**[a]

	L. donovani		*P. falciparum* D6[b]		*P. falciparum* W2[c]		VERO
extract/compound	IC_{50} (μg/mL)	IC_{90} (μg/mL)	IC_{50} (ng/mL)	SI[d]	IC_{50} (ng/mL)	SI[d]	IC_{50} (μg/mL)
1	25.0 ± 1.33	>50	3.27 ± 1.16	3058	8.53 ± 0.18	1176	10.0 ± 0.5
1b	>50[e]	>50[e]	>4760[e]		>4760[e]		10.0 ± 0.5
2	0.035 ± 0.012	0.2 ± 1.3	3.00 ± 0.47	750	3.67 ± 0.31	613	2.3 ± 0.2
2a	>50[e]	>50[e]	3200 ± 400	>7.4	1700 ± 333	>14	>23.8
chloroquinine	NT	NT	18.0 ± 2	>556	176.7 ± 25.6	>56	>10[f]
artemisinin	36.0 ± 2.0	>50	14.3 ± 1.8	>699	8.50 ± 1.5	>1176	>10[f]
pentamidine	1.6 ± 0.1	3.5 ± 0.3	NT		NT		NT
amphotericin B	1.1 ± 0.01	2.1 ± 0.02	NT		NT		NT

[a] The IC_{50} and IC_{90} values are expressed as mean ± SEM of three determinations. [b] Chloroquine-sensitive clone. [c] Chloroquine-resistant clone. [d] Selectivity index = IC_{50} VERO cells/IC_{50} *P. falciparum*. [e] Not active. [f] Not cytotoxic up to the maximum dose (10 μg/mL) tested.

Table 2. Cytotoxic Activities of Compounds **1**, **2**, and **2a**[a]

	IC_{50} (μg/mL)			
compound	SK-MEL[b]	KB[c]	BT-549[d]	SK-OV-3[e]
1	1.9 ± 0.1	1.9 ± 0.2	1.7 ± 0.1	0.8 ± 0.07
2	0.28 ± 0.07	1.0 ± 0.08	0.3 ± 0.01	0.3 ± 0.01
2a	>10[f]	>10[f]	>10[f]	>10[f]
doxorubicin	1.57 ± 0.11	1.7 ± 0.13	1.0 ± 0.13	1.53 ± 0.04

[a] The IC_{50} values are expressed as mean ± SEM of three determinations. [b] SK-MEL: human malignant melanoma. [c] KB: human epidermal carcinoma. [d] BT-549: human ductal carcinoma. [e] SK-OV-3: human ovary carcinoma. [f] Not active up to the maximum dose (10 μg/mL) tested.

(Table 4). The reduced toxicity of **1** relative to **2** (Tables 2 and 4) is likely due to its reduced inhibitory properties as assessed in the in vitro Krebs translation system (compared relative luciferase values at 10.0 and 0.4 μM). The in vitro inhibition of protein synthesis of a chloroquine-resistant strain of *P. falciparum* by quassinoids has been reported previously.[21] During intraerythrocytic proliferation, the malaria parasite makes its own ribosomes, and stronger binding of quassinoids on the parasite ribosomes than the binding for the compound to the host cell ribosomes may be responsible for selective antimalarial action of quassinoids. This may explain the specificity of quassinoids, as well as why other inhibitors of protein synthesis do not demonstrate antimalarial activity.

Our findings have revealed the importance of oxygenation at C-2 for antimalarial activity of **1** and **2**. Although the structural differences between **1** and **2** are the presence of hydroxyl groups at C-2α and C-12β for **1**, instead of a ketone at C-2 and a hydroxyl group at C-12α for **2**, compound **1** was 714-fold less active than **2** against *L. donovani* (IC_{50} 25 vs 0.035 μg/mL), but equally potent against *P. falciparum*. These indicate that the carbonyl group at C-2 and/or hydroxyl group at C-12α is critical for the antileishmanial activity of compound **2**, while **1** is selectively active against malaria. The antiparasitic, cytotoxic, and cell proliferation activities and inhibition of translation of **1** and **2** were insignificant after acetylation, which indicated the importance of free hydroxyl groups. Because of the selective and potent activity against the *P. falciparum* clones W2 and D6, and the relatively lower toxicity toward mammalian cells, compound **1** warrants further investigation as a promising antimalarial lead compound.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were recorded on an Electrothermal 9100 instrument. Optical rotations were measured using a JASCO DIP-370 digital polarimeter in MeOH at ambient temperature. UV spectra were obtained in MeOH, using a Hewlett-Packard 8452A spectrophotometer. IR spectra were taken as KBr disks on an Ati Mattson (Genesis Series) FTIR spectrophotometer. The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$ or C_5D_5N, using TMS as internal standard. Multiplicity determinations (DEPT) and 2D NMR spectra (COSY, HMQC, HMBC) were run using standard Bruker pulse programs. The HRMS were obtained by direct injection using Bruker Bioapex-FTMS with electrospray ionization (ESI). TLC was carried out on Si gel F254 plates, with the solvent system CH_2Cl_2−MeOH (95:5). For flash column chromatography, Si gel G (J. T. Baker, 40 μm flash) was used, with CH_2Cl_2−MeOH mixtures as solvents. Centrifugal preparative TLC (using a Chromatotron instrument, Harrison Research Inc. model 8924) was carried out on 4 mm Si gel GF Chromatotron rotors (Analtech, Inc.), with the solvent system $CHCl_3$−MeOH (95:5). The isolated compounds were visualized using short wave under UV light (254 nm), followed by spraying with anisaldehyde/H_2SO_4 reagent.

Plant Material. The root bark and leaves of *Simaba orionensis* (Simaroubaceae) were collected from Loreto (Maynas), Peru, in January 1993 and June 1997, respectively, and identified by Dr. Sidney T. McDaniel. Voucher specimens for roots (IBE 10347) and leaves (IBE 11932) were deposited at the Herbarium of Mississippi State University.

Extraction and Isolation. The powdered air-dried root bark and leaves (1 kg each) were extracted by percolation with 95% EtOH (6 L × 3). The combined extracts were evaporated separately under reduced pressure and then freeze-dried (yield 55 and 70 g, respectively). Antimalarial screening of the EtOH extracts of root and leaves showed activity against *P. falciparum* clone D6 (IC_{50} 220 and 737 ng/mL, respectively). The crude EtOH extract of root bark (30 g) was dissolved in 10% MeOH−H_2O and partitioned with $CHCl_3$ (500 mL × 4), and the combined $CHCl_3$ fraction was dried over anhydrous Na_2SO_4 and evaporated under a vacuum to yield 29 g of residue.

The $CHCl_3$ residue (20 g) was flash chromatographed on silica gel (500 g), using $CHCl_3$−MeOH (95:5; 1 L) as eluant to afford **2** as plates [1.5 g, mp 238 °C (recrystallized from MeOH); $[\alpha]_D$ +50° (*c* 0.1, MeOH); lit.[3] mp 230 °C; $[\alpha]_D$ +53°]. Further elution with $CHCl_3$−MeOH (93:7) yielded crude **1** (500 mg), which was subjected to centrifugal preparative TLC (using a Chromatotron; 4 mm Si gel rotor, flow rate: 4 mL min^{-1}), using $CHCl_3$−MeOH (97:3) as solvent, to afford **1** (400 mg). The spectral data (UV, IR, NMR, and HRMS) of **2** were generally in agreement with those reported in the literature.[3,16]

(+)-Oricinolide (1): off-white granules, $[\alpha]_D$ +44.6° (*c* 0.095, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.25) nm; IR (KBr) ν_{max} 3440, 2985−2820, 1760 (C=O), 1725 (C=O), 1650, 1480, 1440, 1375, 1350, 1330, 1280, 1265, 1130, 1070, 860, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.20 (1H, brs, H-15), 5.40 (1H, brs, H-3), 4.70 (1H, brs, H-11), 4.64 (1H, d, J = 7.5 Hz, H-20b), 4.57 (1H, brs, H-7), 4.11 (1H, brs, H-2), 3.72 (1H, d, J = 7.0 Hz, H-1), 3.66 (1H, brs, H-12), 3.52 (1H, d, J = 7.5 Hz, H-20a), 2.45[a] (1H, m, H-2′), 2.43[a] (1H, m, H-5), 2.32 (1H, d, J = 13.0

Table 3. Cell Proliferation, Aggregation, Adhesion,[a] and Inducible Nitric Oxide Synthase (iNOS) Activities of Compounds **1**, **2**, and **2a**

compound	cell aggregation MIC (μg/mL) A	XTT IC_{50} (μg/mL) B	specific index B/A	cell adhesion IC_{50} (μg/mL) C	MTT[b] IC_{50} (μg/mL) D	specific index D/C	iNOSIC_{50} (μg/mL) E	MTT[c] IC_{50} (μg/mL) F	specific index F/E
1	0.26	0.7 ± 0.3	2.7	>25	2.5 ± 1.0		6.5 ± 0.9	4.2 ± 1.4	0.6
2	0.01	0.025 ± 0.01	2.5	>25	0.032 ± 0.01		0.76 ± 0.02	0.08 ± 0.003	
2a	20.8	40 ± 10.8	1.9	>25	39.0 ± 3.1		23.0 ± 5.3	24 ± 4.2	1.0
cytochalasin[d]	2.3	40 ± 8.1	17.2	7.1 ± 1.3	0.26 ± 0.1	0.04	5.6 ± 2.5	6.5 ± 2.1	1.1
L-NMMA[e]	NT	NT		NT	NT		5.0 ± 1.9	>25.0	>5.0
APDC[f]	NT	NT		NT	NT		21.0 ± 8.2	<0.4	<0.02
dexamethasone[g]	NT	NT		NT	NT		36.0 ± 7.8	>25	>0.69

[a] Assay system for inhibitors of LFA-1/ICAM-1-mediated aggregation combined with XTT assay as a primary assay. Following LFA-1/ICAM-1-mediated adhesion assay was performed with HL-60 cells and CHO-ICAM-1 cells as a secondary assay. [b] Tested against CHO-ICAM-1. [c] Tested against RAW 264.7. [d] Cytochalasin B was used for the standard of cell adhesion assay. [e] L-NMMA, N^G-monomethyl-L-arginine acetate (iNOS selective inhibitor). [f] APDC, ammonium pyrrolidinedithiocarbamate (NOS inhibitor, inhibitor of NF-κB). [g] Dexamethasone, glucocorticoid (iNOS inhibitor). The IC_{50} values are expressed as mean ± SEM of three determinations.

Table 4. In Vitro[a] and in Vivo[b] Inhibition of Protein Synthesis of Compounds **1**, **1b**, **2**, and **2a**

	in vitro		in vivo			
compound (test conc)	average relative firefly luciferase	average relative renila luciferase	inhibition of translation	test conc	% translation	% inhibition
1 (10 μM)	0.05	0.09	+	2 μM	3.5	96.5
(2 μM)	0.35	0.57	−			
1b (10 μM)	1.02	1.03	−	2 μM	100	0
(2 μM)	0.87	1.14	−			
2 (2 μM)	0.05	0.09	+	2 μM	1.5	98.5
(0.4 μM)	0.17	0.25	+			
2a (10 μM)	0.42	0.52	−	2 μM	100	0
(2 μM)	1.34	0.87	−			
anisomycin (10 μM)	<0.01	<0.01	+	10 μM	0	98.5
DMSO (0.5%)	1	1	−	0.1%	100	0

[a] The firefly or relative renila luciferase values relative to the DMSO (vehicle) control are presented. [b] In vivo labeling of HeLa cells with ^{35}S-methionine in the presence of compound. The % translation was determined relative to the DMSO (vehicle) control.

Hz, H-14), 2.20 (1H, s, H-9), 1.75 (1H, m, H-3′b), 1.66 (1H, m, H-6), 1.61 (3H, s, H-18), 1.51 (1H, m, H-3′a), 1.39 (3H, s, H-21), 1.20 (3H, s, H-19), 1.18 (3H, d, J = 7.0 Hz, H-5′), 0.96 (3H, t, J = 7.5 Hz, H-4′) ([a]overlapped signals); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 175.7 (C, C-1′), 167.7 (C, C-16), 135.4 (C, C-4), 123.6 (CH, C-3), 84.3 (C, C-7), 81.5 (CH, C-1), 80.9 (C, C-13), 78.9 (CH, C-12), 74.8 (CH, C-11), 73.1 (CH, C-2), 72.7 (CH_2, C-20), 67.9 (CH, C-15), 52.6 (CH, C-14), 46.5 (C, C-10), 44.1 (C, C-8), 43.0 (CH, C-5), 42.2 (CH, C-9), 41.3 (CH, C-2′), 28.3 (CH_2, C-6), 27.0 (CH_2, C-3′), 23.4 (CH_3, C-21), 21.1 (CH_3, C-18), 16.7 (CH_3, C-5′), 12.0 (CH_3, C-19), 11.9 (CH_3, C-4′); HRESIMS m/z 503.2360 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_9$, 503.2386) (100%).

Acetylation of 1. Compound **1** (50 mg) was dissolved in pyridine and treated with Ac_2O at room temperature for 24 h. Regular workup of the reaction mixture (45 mg) afforded two products, which were purified by CPTLC (1 mm silica gel GF disk, solvent: CH_2Cl_2−MeOH, 96:4) to give **1a** (20 mg) and **1b** (15 mg) as colorless solids. Compound **1a**: $[\alpha]_D$ +35.7° (*c* 0.013, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.35) nm; IR (KBr) ν_{max} 3460, 2985−2820, 1775 (br), 1750 (C═O), 1720, 1655, 1470, 1450, 1370, 1340, 1320, 1285, 1260, 1120, 1060, 865, 825 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.02 (1H, brs, H-15), 5.32 (1H, d, J = 1.5 Hz, H-3), 5.13 (1H, dd, J = 1.5, 6.5 Hz, H-2), 4.46 (1H, d, J = 5.0 Hz, H-11), 4.86 (1H, brs, H-12), 4.74 (1H, d, J = 7.5 Hz, H-20b), 4.59 (1H, brs, H-7), 3.77 (1H, d, J = 6.5 Hz, H-1), 3.52 (1H, d, J = 7.5 Hz, H-20a), 2.45[a] (1H, m, H-2′), 2.44[a] (1H, m, H-5), 2.38 (1H, d, J = 13.0 Hz, H-14), 1.91 (1H, d, J = 4.0 Hz, H-9), 2.19 (1H, dt, J = 2.5, 3.0 Hz, H-6), 1.74 (1H, m, H-3′), 1.64 (3H, s, H-18), 1.49 (1H, m, H-3′), 1.27 (3H, s, H-21), 1.21 (3H, s, H-19), 1.15 (3H, d, J = 7.0 Hz, H-5′), 0.95 (3H, t, J = 7.5 Hz, H-4′), 1.99, 2.11 (each 3H, s, 2 × OAc) ([a]overlapped signals); HRESIMS m/z 587.2460 $[M + Na]^+$ (calcd for $C_{29}H_{40}O_{11}$, 587.2496) (100%). Compound **1b**: $[\alpha]_D$ +67.5° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (4.35) nm; IR (KBr) ν_{max} 3450, 2985−2820, 1770 (br), 1755 (C═O), 1650, 1480, 1440, 1375, 1350, 1330, 1280, 1265, 1130, 1070, 860, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.10 (1H, brs, H-15), 5.41[a] (1H, brs, H-2), 5.40[a] (1H, brs, H-3), 5.23 (1H, d, J = 7.5 Hz, H-1), 4.73 (1H, d, J = 7.5 Hz, H-20b), 4.62[b] (1H, brs, H-7), 4.62[b] (1H, brs, H-12), 3.91 (1H, brs, H-11), 3.59 (1H, d, J = 7.5 Hz, H-20a), 2.49 (1H, m, H-2′), 2.69 (1H, d, J = 13.0 Hz, H-5), 2.48 (1H, d, J = 13.0 Hz, H-14), 2.06, 2.06, 2.00[c] (each 3H, s, 3 × OAc), 2.00[c] (1H, s, H-9), 1.79 (1H, m, H-3′B), 1.66 (1H, m, H-6), 1.69 (3H, s, H-18), 1.55 (1H, m, H-3′A), 1.45 (3H, s, H-19), 1.34 (3H, s, H-21), 1.20 (3H, d, J = 7.0 Hz, H-5′), 0.99 (3H, t, J = 7.5 Hz, H-4′) ([a−c]overlapped signals); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 174.6 (C, C-1′), 170.9, 170.7, 169.6 (3 × CH_3CO−O), 166.7 (C, C-16), 137.6 (C, C-4), 120.2 (CH, C-3), 83.3 (C, C-7), 78.9 (CH, C-1), 80.0 (C, C-13), 80.1 (CH, C-12), 73.5 (CH, C-11), 70.1 (CH, C-2), 73.0 (CH_2, C-20), 63.1 (CH, C-15), 53.0 (CH, C-14), 45.7 (C, C-10), 45.7 (C, C-8), 44.0 (CH, C-5), 42.0 (CH, C-9), 41.2 (CH, C-2′), 27.7 (CH_2, C-6), 27.1 (CH_2, C-3′), 23.0 (CH_3, C-21), 21.1 (CH_3, C-18), 16.9 (CH_3, C-5′), 12.5 (CH_3, C-19), 12.0 (CH_3, C-4′), 21.5, 21.4, 21.3 (3 x CH_3CO−O); HRESIMS m/z 629.2570 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{12}$, 629.2596) (100%).

Acetylation of 2. Compound **2** (50 mg) was acetylated with pyridine−acetic anhydride at room temperature for 24 h, using the above procedure, to yield **2a** (40 mg). Its physical and spectral data were in agreement with those published in the literature[3,13] [mp 278 °C (recrystallized from EtOAc); $[\alpha]_D$ +66° (*c* 0.1, MeOH); lit.[3] mp 285−87 °C; $[\alpha]_D$ unreported].

Antimalarial/Parasite LDH Assay. The in vitro antimalarial assay procedure[22] utilized was an adaptation of the parasite lactate dehydrogenase (pLDH) assay developed by Makler et al.[23,24] The assay was performed in a 96-well microplate and included two *P. falciparum* clones [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. In primary screening the crude plant extracts were tested, in duplicate, at a single concentration of 15.9 μg/mL only on the chloroquine-sensitive (D6) strain of *P. falciparum*. The extract showing >50% growth inhibition of

the parasite was subjected to screening. For bioassay-guided fractionation, the column fractions were also tested only at single concentration. The pure compounds were subjected to additional testing for determination of IC_{50} values. The standard antimalarial agents chloroquine and artemisinin were used as positive controls, with DMSO as the negative (vehicle) control. The selectivity indices (SI) were determined by measuring the cytotoxicity of samples on mammalian cells (VERO; monkey kidney fibroblast).

Antileishmanial Assay. Antileishmanial activity of the compounds was tested in vitro against a culture of *Leishmania donovani* promastigotes, grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS, Gibco Chem. Co.) at 26 °C. A 3-day-old culture was diluted to 5×10^5 promastigotes/mL. Drug dilutions (50−3.1 μg/mL) were prepared directly in cell suspension in 96-well plates. Plates were incubated at 26 °C for 48 h, and growth of leishmania promastigotes was determined by Alamar Blue assay.[25] Standard fluorescence was measured on a FLUOstar Galaxy plate reader (BMG LabTechnologies) at an excitation wavelength of 544 nm and emission wavelength of 590 nm. Pentamidine and amphotericin B were used as the standard antileishmanial agents. Percent growth was calculated and plotted versus test concentration for computing the IC_{50} and IC_{90} values.

Cytotoxicity Assay. The in vitro cytotoxic activity was determined against four human cancer cell lines, SK-MEL, KB, BT-549, and SK-OV-3 (Table 2), obtained from the American Type Culture Collection (ATCC, Rockville, MD). For initial (primary) evaluation, extracts and fractions were screened at a single concentration (100 and 10 μg/mL, respectively). Follow-up secondary assays were then conducted at three concentrations (10, 3.3, and 1.1 μg/mL). The assay is performed in 96-well tissue culture-treated microplates. Cells (25 000 cells/well) were seeded to the wells of the plate and incubated for 24 h. Samples were added and plates were again incubated for 48 h. The number of viable cells was determined using neutral red according to a modification of the procedure of Borenfreund et al. (1990).[26] IC_{50} values were determined from logarithmic graphs of growth inhibition versus concentration. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

Cell Aggregation, XTT, and Cell Adhesion Assay Procedures. These assays were carried out as described previously.[18,27]

Determination of NO Production. Mouse monocyte macrophage RAW 264.7 cells (2×10^5 cells) in the D-MEM (10% FCS, antibiotics, Phenol Red-free) medium of 200 μL were plated into a 96-well microtiterplate. Four hours after incubation at 37 °C under a 5% CO_2−95% air atmosphere, 25 μL of each sample and lipopolysaccharide (Sigma, final 10 μg/mL) solution were added to each well. After the plate was reincubated for 20 h, NO production of RAW 264.7 cells was determined by using Griess reagent according to the method described by Green et al.[28] Aliquots of the medium (100 μL) were mixed with an equal volume of Griess reagent (0.05% w/v *N*-1-(naphthyl)ethylenediamine dihydrochloride, 0.5% w/v sulfanilamide in 2.5% v/v phosphoric acid). The absorbance at 540 nm was measured using an EL 312e microplate reader (Bio-Tek Instruments). All assays were performed in duplicate and repeated in at least three independent experiments.

MTT Assay for Cytotoxicity of RAW264.7 Cells.[29] RAW 264.7 cells (2×10^4 cells) in 225 μL were plated into a 96-well microplate. Four hours after incubation, 25 μL of each sample solution was added to each well and the plate was reincubated for 2 days. After cells were exposed to the test materials, 25 μL of MTT stock solution (5 mg/mL in PBS) was added to each well and the plate was incubated at 37 °C for 4 h. After aspiration of the medium, 100 μL of 0.04 N HCl−2-propanol was added to each well and mixed for 20 min at room temperature, and the plate was read on a microplate reader using a test wavelength of 570 nm (reference wavelength at 630 nm).

In Vitro Translations. In vitro translation assays were performed with a bicistronic mRNA reporter, in which the first cistron encodes the firefly (FF) luciferase (luc) protein and the second cistron encodes the renilla (Ren) luc protein. Expression of the second cistron is driven by hepatitis C virus 5′ untranslated region. In vitro transcriptions were performed as previously described[30] using BamH I linearized templates. Translations were performed in Krebs extracts at 30 °C for 1 h as previously reported.[31] Since the final concentration of DMSO in the compound additions was 0.5%, control translation reactions contained 0.5% DMSO. Anisomycin (10 μM), a known inhibitor of protein synthesis, was used as the positive control. Firefly and renilla luciferase activity (RLU) were measured on a Berthold Lumat LB 9507 luminometer as previously reported.[32] The obtained luciferase activities were normalized to the activity obtained in the control translations (which were set at one). Each data point represents the average of two translations.

In Vivo Metabolic Labeling Studies. For metabolic labeling studies, 7×10^4 HeLa cells were seeded into 2 cm^2 plates in duplicate the day prior to the experiment. Cells were incubated in the presence of compound, or vehicle alone, and ^{35}S-methionine (150−225 uCi/mL) [Perkin-Elmer Life Sciences] was added to cells 10 min before harvesting. ^{35}S-Methionine was added in methionine-free media supplemented with 10% dialyzed FCS. After 20 min, cells were washed once with phosphate-buffered saline, trypsinized, and harvested by centrifugation. The supernatant was removed by aspiration, and the cell pellet was lysed in RIPA buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). Forty percent of the lysate was used for TCA precipitation. The lysate was spotted onto Whatman 3 MM paper (that had been preblocked with 0.1% methionine), dried, and placed in cold 10% TCA for 20 min. Filters were transferred to 5% TCA, boiled for 15 min, washed once with 5% TCA and once with 95% ethanol, and dried. Radioactivity was determined by scintillation counting. The obtained counts were normalized to protein concentration in each sample which had been determined using a modified Lowry assay (DC Protein Assay; Bio-Rad).

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