Caffeic Acid Esters and Lignans from *Piper sanguineispicum*

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Three new caffeic acid esters (**1**−**3**), four new lignans (**4**−**7**), and the known compounds (7′*S*)-parabenzlactone (**8**), dihydrocubebin (**9**), and justiflorinol (**10**) have been isolated from leaves of *Piper sanguineispicum*. Their structures were determined by spectroscopic methods, including 1D and 2D NMR, HRCIMS, CD experiments, and chemical methods. Compounds **1**−**10** were assessed for their antileishmanial potential against axenic amastigote forms of *Leishmania amazonensis*. Caffeic acid esters **1** and **3** exhibited the best antileishmanial activity (IC_{50} 2.0 and 1.8 μM, respectively) with moderate cytotoxicity on murine macrophages.

Leishmaniasis is a tropical disease caused by Trypanosomatidae of the genus *Leishmania*. It has spread to 88 countries over Africa, Asia, Europe, and America and exhibits a wide range of clinical symptoms. The World Health Organization (WHO) has estimated two million new cases every year, and 350 million people are considered to be at risk.[1] Chemotherapy is based largely on antimony compounds such as Pentostam and Glucantime; however, renal and cardiac toxicity[2,3] together with clinical resistance against these commonly used antimonial agents[4] have prompted a search for new chemicals in order to overcome these problems.[5] Since the availability of effective pharmaceuticals in remote places is also a problem, the use of folk remedies based on medicinal plants for the treatment of leishmaniasis is a common practice.[6]

In this context, our ethnopharmacological surveys[7,8] of the Chayahuitas ethnic group, located in an endemic zone of *Leishmania* in Peru (Loreto Department), allowed us to select various plant species with antileishmanial potential. Among them, leaves of *Piper sanguineispicum* Trel. (Piperaceae), which are used by this community as an anti-inflammatory remedy, displayed one of the most promising in vitro leishmanicidal activity against axenic amastigote forms of *Leishmania amazonensis*.

Phytochemical investigation of the genus *Piper*,[9] which is widely distributed in tropical and subtropical regions of the world, has revealed several classes of antiprotozoal compounds including alkaloids,[10,11] lignans,[12] chalcones, and dihydrochalcones.[13] Compounds from *Piper* species have also shown interesting leishmanicidal activity. For example, piperine, an alkaloid present in several species, has activity similar to Pentostam against promastigotes of *L. donovani*.[6] Flavokavain, isolated from leaves of *P. rusbyi*, is active against *L. amazonensis*, *L. braziliensis*, and *L. donovani* promastigotes.[14] In addition, chalcones and dihydrochalcones isolated from *P. aduncum* and *P. elongatum* have been led to moderate activity against *Leishmania* spp.[15,16]

In the present investigation, a bioassay-guided fractionation of a 90% EtOH leaf extract led to the isolation of three new caffeic acid esters (**1**−**3**), four new lignans (**4**−**7**), and the known lignans (7′*S*)-parabenzlactone (**8**), dihydrocubebin (**9**), and justiflorinol (**10**). In vitro activity of all the compounds was assessed against axenic amastigote forms of *L. amazonensis*. Moreover, in vitro cytotoxicity on macrophages, human breast cancer cells (MCF-7), and monkey kidney cells (VERO) was also determined in order to probe the chemotherapeutic potential of most of the leishmanicidal compounds isolated.

Results and Discussion

Compound **1** was isolated as a white, amorphous solid with a positive optical rotation, $[\alpha]^{20}_{D}$ +43.3. The molecular formula $C_{14}H_{18}O_4$ was established on the basis of HRCIMS (*m/z* 251.1275 $[M + H]^+$; calcd 251.1283) and from its ^{1}H and ^{13}C NMR spectra (Table 1). The IR spectrum of **1** showed the presence of OH (3331 cm^{-1}), ester (1681 cm^{-1}), and trisubstituted aromatic (1441 cm^{-1}) groups. The ^{1}H NMR spectrum exhibited three aromatic proton resonances [δ_H 6.88 (1H, d, J = 8.2 Hz, H-5), 6.98 (1H, dd, J = 8.2, 2.0 Hz, H-6), and 7.11 (1H, d, J = 2.0 Hz, H-2)], indicating a 1,3,4-trisubstituted aromatic ring. The ^{1}H NMR showed signals of two *trans* double-bond protons [δ_H 7.56 (1H, d, J = 15.9 Hz, H-7) and 6.23 (1H, d, J = 15.9 Hz, H-8)], suggesting a *trans*-caffeoyl moiety in compound **1**. The complex spin system at high field δ_H 1.27−1.72 suggested the presence of an alkyl chain in the molecule. The intense doublet at δ_H 1.28 (3H, d, J = 6.27 Hz, H-5′) and the triplet at δ_H 0.92 (3H, t, J = 7.24 Hz, H-4′) corresponding to two methyl groups are indicative of a branched alkyl chain. The carbon signals in the ^{13}C NMR spectrum of **1** confirmed the caffeic acid and branched alkyl side chain moieties. The linkage of a caffeoyl moiety with the alkyl chain was solved by analysis of the HMBC spectrum, which revealed a cross-peak between H-1′ at δ_H 5.03 and the carbonyl C-9 at δ_C 168.4. Compound **1** was therefore elucidated as 1-methylbutyl caffeate. Total assignments of protons and carbons of **1** were based on the results of the ^{1}H−^{1}H COSY, HSQC, and HMBC experiments (Table 1). The absolute configuration at C-1′ was determined to be *S* by measurement of the optical rotation of the corresponding aliphatic alcohol obtained after alkaline hydrolysis of **1** and by comparison of the value with the $[\alpha]_D$ of (*S*)-(+)-2-pentanol.

The molecular formula $C_{16}H_{22}O_4$ (*m/z* 279.1586 $[M + H]^+$; calcd 279.1596) was assigned for compound **2** by HRCIMS and corresponded to 28 Da more than compound **1**. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those observed for **1** (Table 1) with two more methylenes in **2**. Compound **2** was therefore elucidated as 1′-methylhexyl caffeate. As for **1**, the absolute configuration at C-1′ was deduced to be *S*.

In the same way, the molecular formula $C_{18}H_{26}O_4$ (*m/z* 307.1903 $[M + H]^+$; calcd 307.1909) was assigned for compound **3** by HRCIMS, corresponding to two more methylenes compared to **2**. The structure of **3** was therefore deduced to be 1′-methyloctyl

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Chart 1

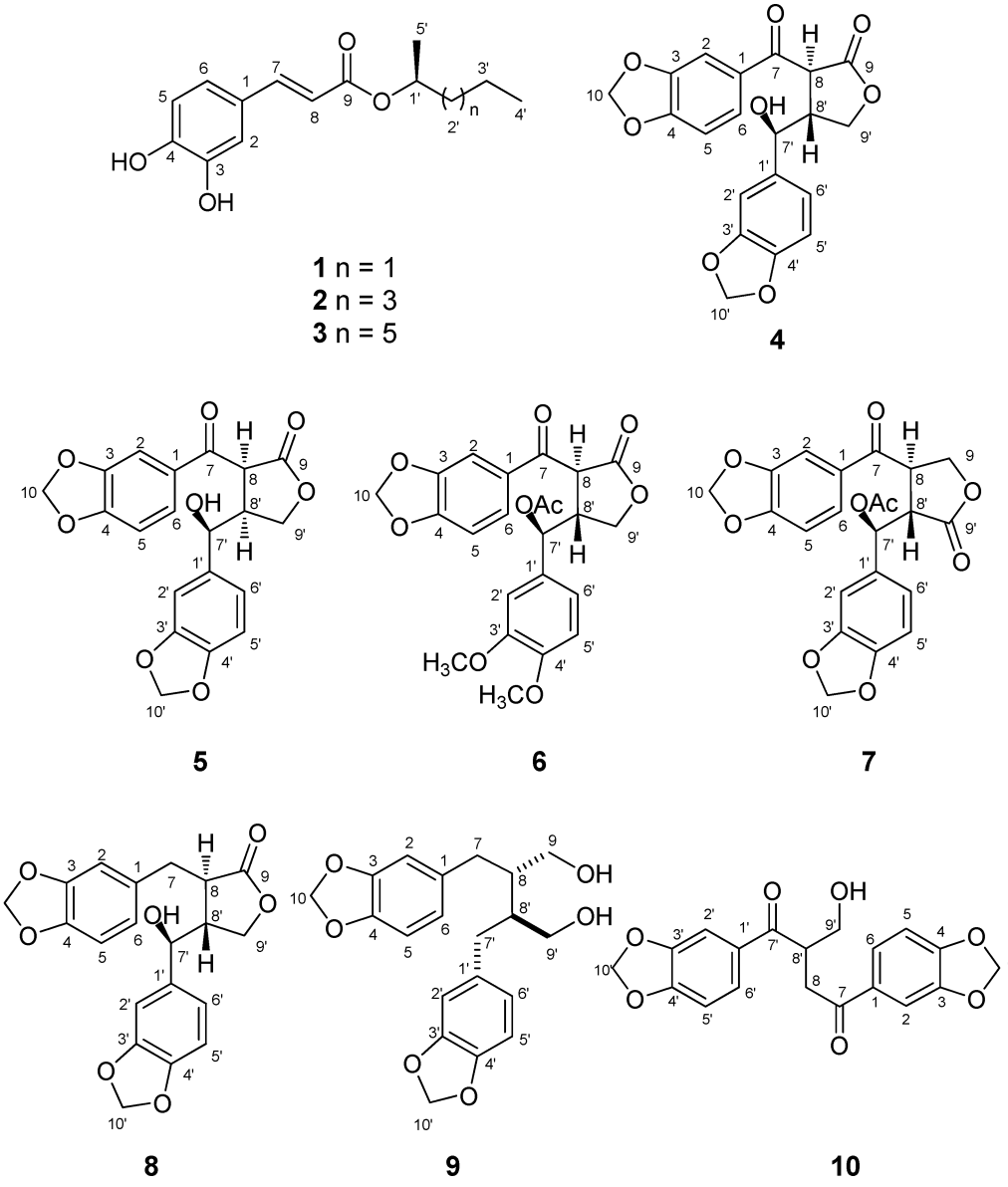
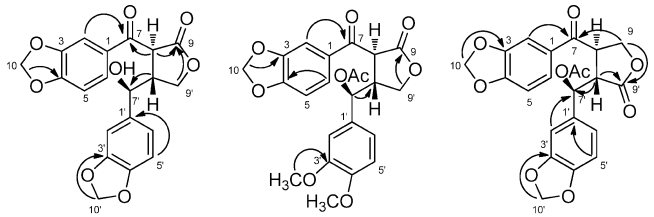


Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**3** in $CDCl_3$

	1[a]			**2**[b]			**3**[b]		
position	δ_H (*J* in Hz)	δ_C	HMBC	δ_H (*J* in Hz)	δ_C	HMBC	δ_H (*J* in Hz)	δ_C	HMBC
1		127.3			127.5			127.5	
2	7.11, d (2.0)	114.4	3,4,6	7.12, d (2.0)	114.5	3,4,6,7	7.12, d (2.0)	114.5	3,4,6,7
3		144.1			143.8			143.8	
4		146.8			146.4			146.4	
5	6.88, d (8.2)	115.5	1,3,4	6.88, d (8.2)	115.5	1,3,4	6.88, d (8.2)	115.5	1,3,4
6	6.98, dd (8.2, 2.0)	122.4	2,4	7.01, dd (8.2, 2.0)	122.3	2,4,7	7.01, dd (8.2, 2.0)	122.3	2,4,7
7	7.56, d (15.8)	145.3	1,2,6,9	7.57, d (15.9)	144.7	2,6,8,9	7.57, d (15.9)	144.8	1,2,8,9
8	6.23,d (15.8)	115.6	1,7,9	6.26, d (15.9)	116.1	1,7,9	6.26, d (15.9)	116.1	1,7,9
9		168.4			167.7			167.7	
1′	5.03, m	71.7	2′,3′,9′	5.03, m	71.6	9	5.03, m	71.6	9
2′	1.50−1.72, m	38.1	1′,3′,4′	1.50−1.70, m	36.0	3′	1.50−1.70, m	36.0	3′
3′	1.27−1.41, m	18.7	4′	1.24−1.40, m	25.1		1.24−1.40, m	22.6	
4′	0.92, t (7.2)	13.9	2′,3′	1.24−1.40, m	31.7		1.24−1.40, m	29.4	
5′	1.28, d (6.2)	20.0	1′,2′	1.24−1.40, m	20.1		1.24−1.40, m	29.2	
6′				0.88, t (7.0)	14.0	4′, 5′	1.24−1.40, m	31.8	
7′				1.28, d (6.2)	20.1	1′, 2′	1.24−1.40, m	22.6	
8′							0.87, t (7.0)	14.1	6′,7′
9′							1.28, d (6.2)	20.1	1′,2′
3-OH				5.92, br			5.92, br		
4-OH				6.22, br			6.24, br		

[a] Measured at 300 MHz. [b] Measured at 400 MHz.



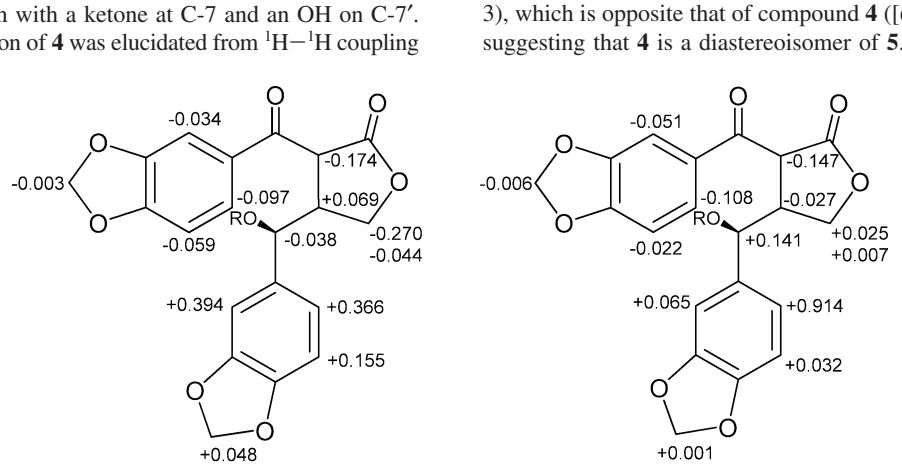
4 **6** **7**

Figure 1. Key HMBC correlations for compounds **4**, **6**, and **7**.

caffeate. As for **1** and **2**, the *S* configuration was deduced for C-1′ after alkaline hydrolysis.

Compound **4** was a white, amorphous solid, with a negative specific optical rotation, $[\alpha]^{20}_{D}$ −24. The molecular formula $C_{20}H_{16}O_8$ was determined by HRESIMS (*m*/*z* 407.0758 $[M + Na]^+$; calcd 407.0743). The IR spectrum showed absorption bands at 3418, 1775, and 1660 cm^{-1}, suggesting the presence of OH, ester, and carbonyl functions, respectively, and the UV spectrum featured absorption maxima at 283 and 317 nm due to the presence of separate aromatic rings. The ^1H NMR spectrum displayed signals for two sets of ABX systems of aromatic protons at δ_H 6.76−7.56 (6H), two methylenedioxy groups at δ_H 5.97 (2H, dd, J = 5.2, 1.1 Hz, H-10′) and 6.13 (2H, s, H-10), an oxygenated methylene at δ_H 4.29 (1H, dd, J = 9.0, 8.9 Hz, H-9′a) and 4.38 (1H, dd, J = 9.0, 8.0 Hz, H-9′b), and three methine protons at δ_H 3.34−3.39 (1H, m, H-8′), 4.76 (1H, d, J = 6.1 Hz, H-8), and 4.89 (1H, d, J = 5.9 Hz, H-7′). The 20 carbon signals observed in the DEPT and HSQC experiments confirmed the presence of a carbonyl at δ_C 193.5 (C-7), an ester group at δ_C 174.0 (C-9), two trisubstituted aromatic rings, and two methylenedioxy groups at δ_C 102.0 (C-10′) and 103.2 (C-10), which were indicative of two 3′,4′-methylenedioxyphenyl units, a methylene at δ_C 70.2 (C-9′), and three methines. The aforementioned data strongly suggested that compound **4** was a dibenzylbutyrolactone lignan with an additional ketone function. Analysis of the COSY spectrum showed correlations of H-8′/H-8, H-8′/H-9′a/H-9′b, and H-8′/H-7′. The HMBC spectrum also indicated correlations of H-9′ and H-8 with the carbonyl at δ_C 174.0 (C-9), supporting the partial structure of a disubstituted γ-lactone ring. In addition, long-range HMBC correlations of signals at δ_H 7.29 (H-2) and 7.54 (H-6) with the carbonyl C-7 at δ_C 193.5, and the signals at δ_H 6.91 (H-2′) and 6.92 (H-6′) with the oxygenated carbon C-7′ at δ_C 73.8, indicated connection of those carbons with the corresponding aromatic unit. These data and the other COSY and HMBC correlations (Figure 1) indicated that **4** was a dibenzylbutyrolactone lignan with a ketone at C-7 and an OH on C-7′. The relative configuration of **4** was elucidated from $^1H-^1H$ coupling constants between H-8′/H-7′ (J = 5.9 Hz) and H-8′/H-8 (J = 6.1 Hz) and from the nonequivalence of H-9′a and H-9′b in the ^1H NMR spectrum. Indeed, according to Corrie et al.[17] and Lopes et al.,[18] a nonequivalence of H-9′a and H-9′b indicated a *trans*-dibenzylbutyrolactone, while they are almost equivalent in the *cis* derivative. No significant correlations were observed in the NOESY experiment. Thus, an all-*trans* relationship of H-7′, H-8′, and H-8 was deduced for compound **4**. To solve the absolute configuration at C-7′, compound **4** was treated with (*S*)- and (*R*)-MPA[19] according to Mosher's ester method described by Latypov et al.[20] Each of the two sites showed a clear pattern of $\Delta\delta_{R-S}$ values with negative values on one side and positive values on the other (Figure 2), following the expected pattern for Mosher's method[21] and allowing the assignment of the *S* configuration at C-7′. The absolute configuration was therefore determined as 8*S*,7′*S*,8′*R*. On the basis of the above findings and according to IUPAC rules,[22] compound **4** was identified as (8*S*,7′*S*,8′*R*)-3,3′,4,4′-bis(methylenedioxy)-7′-hydroxy-7-oxolignano-9,9′-lactone, and it was named (−)-sanguinolignan A.

Compound **5** gave a molecular formula similar to compound **4**, $C_{20}H_{16}O_8$, by HRESIMS (*m*/*z* 407.0758 $[M + Na]^+$; calcd 407.0743). Its IR spectrum showed the same typical signals (3466, 1778, and 1667 cm^{-1}), and its UV spectrum exhibited absorption maxima at 284 and 318 nm, which matched those of **4**. The ^1H and ^{13}C NMR spectra of **5** revealed the same framework showing the characteristic signals of a dibenzylbutyrolactone lignan. The main differences were observed for the protons and carbons of the butyrolactone moiety and C-7′ (Tables 2 and 3). Indeed, protons H-9′ were shielded and almost equivalent at δ_H 3.70 (2H, t, J = 4.8 Hz), while the protons H-8 at δ_H 5.07 (1H, d, J = 10.4 Hz) and H-7′ at δ_H 5.38 (1H, d, J = 9.4 Hz) were deshielded. The connectivity of the carbon framework in **4** was established on the basis of combined COSY and HMBC correlations, and the planar structure of **5** was determined to be identical to that of compound **4**. However, the upfield shift and the equivalence of the H-9′ protons of compound **5** relative to that of compound **4** were consistent with a *cis* arrangement of H-8 and H-8′. The increase in the $^1H-^1H$ coupling constants between H-8′/H-8 (J = 10.4 Hz) and H-8′/H-7′ (J = 9.4 Hz) confirmed the H-8′/H-8 arrangement and also suggested a *cis* relationship of H-8′ and H-7′. Mosher's esterification with MPA was also applied to compound **5**, allowing the assignment of the *S* configuration to C-7′. The absolute configuration of compound **5** was therefore determined to be 8*S*,7′*S*,8′*S*. Moreover, the CD spectrum of **5** ($[\alpha]^{20}_{D}$ −34.3, CH_2Cl_2) showed a negative absorption peak at 282 nm and a positive absorption peak at 334 nm (Figure 3), which is opposite that of compound **4** ($[\alpha]^{20}_{D}$ −24, $(CH_3)_2CO$), suggesting that **4** is a diastereoisomer of **5**. Thus, the structure of



4*R* R = *(R)*-(+)-MPA
4*S* R = *(S)*-(-)-MPA

5*R* R = *(R)*-(+)-MPA
5*S* R = *(S)*-(-)-MPA

Figure 2. $\Delta\delta_{R-S}$ ($\delta_R - \delta_S$) values for the (*R*)- and (*S*)-MPA esters of **4** and **5**.

Table 2. ^{1}H NMR Data of Lignans **4**−**7** (300 MHz) in $(CD_3)_2CO$

H	4	5	6	7
1				
2	7.29, d (1.8)	7.55, d (1.8)	7.41, d (1.8)	7.20, d (1.8)
3				
4				
5	6.93, dd (7.5, 1.1)	7.02, d (8.0)	6.97, d (8.2)	6.91, d (8.0)
6	7.54, dd, (8.2, 1.8)	7.8, dd (8.0, 1.8)	7.62, dd (8.1, 1.8)	7.46, dd (8.0, 1.8)
7				
8	4.76, d (6.1)	5.07, d (10.4)	4.90, d (8.0)	4.65−4.75, m
9-a				4.09−4.17, m
9-b				4.65−4.75, m
10	6.13, s	6.16, s	6.16, s	6.13, s
1′				
2′	6.91, d (1.8)	7.01, d (1.8)	6.99, d (1.8)	6.84, d (1.8)
3′				
4′				
5′	6.77, d (8.2)	6.90, d (8.0)	6.90, d (8.1)	6.67, d (8.0)
6′	6.92, dd (8.1, 1.8)	6.98, d (8.0, 1.8)	6.94, dd (8.2, 1.8)	6.82, ddd (8.0, 1.8, 1.0)
7′	4.89, d (5.9)	5.38, d (9.4)	5.92, d (7.8)	6.20, d (3.8)
8′	3.34−3.39, m	3.17−3.27, m	3.75−3.80, m	3.69, dd (7.4, 3.8)
9′a	4.29, dd (9.0, 5.9)	3.70, dd (4.8)	4.17, dd (9.0, 8.0)	
9′b	4.38, dd (9.0, 8.0)		4.29, dd (9.0, 8.3)	
10′	5.97, dd (5.2, 1.1)	6.05, s		5.91, dd (12.9, 1.0)
Ac-CH_3			1.8, s	2.10, s
3′-OCH_3			3.79, s	
4′-OCH_3			3.77, s	

Table 3. ^{13}C NMR Data (δ) of Lignans **4**−**7** (75 MHz) in $(CD_3)_2CO$

C	4	5	6	7
1	131.6	132.3	131.8	131.2
2	108.8	108.8	108.9	108.5
3	149.2	149.3	149.4	149.3
4	153.3	153.5	153.5	153.3
5	108.6	109.3	112.5	108.8
6	126.8	127.2	126.8	125.9
7	193.5	192.8	192.8	195.7
8	50.8	51.7	51.4	44.1
9	174.0	172.7	173.4	69.5
10	103.2	103.3	103.3	103.3
1′	137.7	133.2	131.1	132.8
2′	107.1	107.7	111.0	107.1
3′	148.0	149.1	150.5	148.8
4′	148.8	149.1	150.5	148.2
5′	108.5	109.0	108.7	108.7
6′	120.3	121.7	120.0	119.9
7′	73.8	82.1	76.4	73.4
8′	49.0	51.5	46.8	50.6
9′	70.2	59.3	69.1	175.0
10′	102.0	102.4		102.2
3′-OCH_3			56.0	
4′-OCH_3			56.0	
Ac-CH_3			20.7	20.7
Ac-CO			169.9	169.5

5 was elucidated as (8*S*,7′*S*,8′*S*)-3,3′,4,4′-bis(methylenedioxy)-7′-hydroxy-7-oxolignano-9,9′-lactone and was named (−)-sanguinolignan B.

The HRESIMS of compound **6** (*m*/*z* 465.1158 $[M + Na]^+$; calcd 465.1162) gave the molecular formula $C_{23}H_{22}O_9$. Its UV spectrum showed the same typical absorption maxima (282 and 320 nm), but its IR spectrum showed the absence of hydroxyl functions and the presence of two ester functions and a carbonyl (1777, 1757, and 1677 cm^{-1}). The ^{1}H and ^{13}C NMR spectra exhibited features characteristic of a dibenzylbutyrolactone lignan. In comparison to **4**, the most prominent changes concerned the lactone substituents. The ^{1}H NMR spectrum exhibited only one methylenedioxy group at δ_H 6.16 (2H, s, H-10), two aromatic methoxy singlets at δ_H 3.77 and 3.79, and one methyl singlet at δ_H 1.80. Moreover, ^{13}C NMR confirmed the presence of an additional carbonyl at δ_C 169.9. From the HMBC spectrum, it was deduced that the two methoxy groups were connected to C-3′ and C-4′, and the methyl singlet and the carbonyl at δ_C 169.9 were assigned to an acetoxy unit attached to C-7′. The downfield shift of H-7′ at δ_H 5.92 (1H, d, J = 7.8 Hz) relative to that of compound **4** is also consistent with the OH substitution. Finally, the connectivity between the atoms in the γ-lactone and the aromatic ring was identical to the previous compounds, on the basis of COSY and HMBC correlations (Figure 1). From the H-9′ signals at δ_H 4.17 (1H, dd, J = 9.0, 8.0 Hz) and 4.29 (1H, dd, J = 9.0, 8.3 Hz) and the coupling constants between H-8′/H-7′ (J = 7.8 Hz) and H-8′/H-8 (J = 8.0 Hz), an all-*trans* relationship of H-7′/H-8′/H-8 was suggested for compound **6**. Since the CD spectrum of **6** ($[\alpha]^{20}_D$ −1.2, CH_2Cl_2) exhibited the same positive and negative absorption peaks as compound **4**, the same absolute configuration was proposed. Thus, compound **6** was identified as (8*S*,7′*S*,8′*R*)-7′-acetoxy-3,4-methylenedioxy-3′,4′-dimethoxy-7-oxolignano-9,9′-lactone, and it was named (−)-sanguinolignan C.

The molecular formula of compound **7** was established as $C_{22}H_{18}O_9$ by HRESIMS. The UV and IR spectra matched those of sanguinolignan C. ^{1}H and ^{13}C NMR features indicated that the structure of **7** was also very similar to that of **6**, except that a methylenedioxy group at δ_H 5.91 (2H, dd, J = 12.9, 1.0 Hz) was present instead of the two aromatic methoxy singlets. Furthermore, in the HMBC spectrum, H-7′ at δ_H 6.20 (1H, d, J = 3.8 Hz) showed a cross-peak with a carbonyl carbon at δ_C 175 (C-9′), the H-8′ at δ_H 4.65−4.75 (1H, m) showed cross-peaks with both C-7′ and C-9′, and the oxymethylene protons H-9 showed cross-peaks with both carbonyl C-9′ and C-7 (δ_C 195.7). These data allowed unambiguous assignment of a 7-oxolignano-9′,9-lactone. The downfield shift of C-8 (δ_C 44.1) confirmed that the lactone moiety was reversed in comparison to that of the other sanguinolignans. As demonstrated above, an all-*trans* relationship of H-7′/H-8′/H-8 for compound **7** was deduced from the ^{1}H NMR (Table 2). Moreover, the CD spectrum (Figure 3) displayed a similar profile, suggesting that the three-dimensional arrangement of substituents of the chiral centers C-8, C-8′, and C-7′ was analogous to that of **4** and **6**. Therefore, compound **7** was identified as (8*R*,7′*S*,8′*S*)-7′-acetoxy-3,3′,4,4′-bis(methylenedioxy)-7-oxolignano-9′,9-lactone, and it was named (−)-sanguinolignan D.

The known compounds (7′*S*)-parabenzlactone (**8**), dihydrocubebin (**9**), and justiflorinol (**10**) exhibited spectral data consistent with that reported.[23−25]

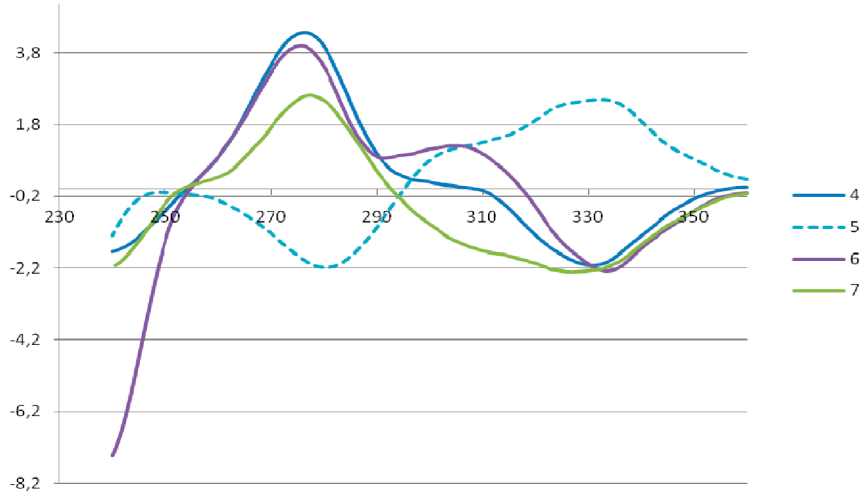


Figure 3. CD spectra for compounds **4**−**7**.

Table 4. IC_{50} Values for Compounds Isolated from *P. sanguineispicum*

compound	IC_{50} *L. amazonensis* axe. amas. in μM	IC_{50} cytotoxicity on macrophages in μM[a]	CAR[b]	IC_{50} on MCF7 cells in μM	IC_{50} on Vero cells in μM
1	2.0 ± 0.11	72.0 ± 1.8	36	62.0	14.0
2	10.0 ± 0.37	16.1 ± 1.1	1.6	89.8	10.6
3	1.8 ± 0.08	12.7 ± 0.4	7	31.0	3.2
4	36.7 ± 3.08	>260	>7	109.4	145.8
5	>130	NT		>260	249.9
6	105.4 ± 2.4	NT		>226	79.2
7	69.7 ± 2.8	NT		82.1	17.1
8	79.4 ± 0.68	NT		113.5	151.3
9	>140	NT		83.8	89.4
10	>140	NT		>281	140.4
caffeic acid	17.8 ± 0.15	>550	>31	>550	>550
amphotericin B	0.11 ± 0.01	3.6 ± 0.3	33		

[a] NT: not tested. [b] Cytotoxicity on macrophage/antileishmanial activity ratio.

Activities of the isolated compounds against axenic amastigote forms of *L. amazonensis*, as well as their cytotoxicity on mouse peritoneal macrophages and two mammalian cell lines (VERO and MCF-7), are summarized in Table 4. The most interesting compounds responsible for the leishmanicidal activity of *P. sanguineispicum* were found to be the caffeic acid derivatives, particularly 1-methylbutyl caffeate (**1**), exhibiting a cytotoxicity on macrophage/activity ratio similar to that of amphotericin B (about 35). Concerning the lignans, only (−)-sanguinolignan A (**4**) showed a moderate antileishmanial activity, but interestingly it had a very low cytotoxicity on macrophage and cancer or normal cell lines. Hemisynthesis of caffeic acid derivatives is being performed currently in an attempt to improve leishmanicidal activity of these compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The CD spectra were obtained on a JASCO J-815 spectrometer. UV spectra were recorded on a Specord 205. IR spectra were obtained on a Perkin-Elmer FT-IR Parabon 100. ^{1}H and ^{13}C NMR spectra were recorded on Brüker Avance 300, 400, or 500 instruments. HRCIMS and HRESI spectra were recorded on Waters GTC Premier and Waters LCT spectrometers. MPLC was carried out with a Büchi C-605 pump and a Büchi C-615 pump manager. Silica gel (Sigma-Aldrich, 230−400 mesh) and RP-18 (EM Science, LiChroprep, 40−63 μm) were used for column chromatography (CC) separations, and silica gel 60 PF_{254} (Merck) was used for analytical TLC (0.5 mm).

Plant Material. The leaves of *P. sanguineispicum* were collected in May 2007 from Loreto department in Peru and identified by Ricardo Callejas (Universidad de Antioquia, Medellín, Colombia). Voucher specimens (GO138) are deposited at the Herbarium of the Natural History Museum of Mayor de San Marcos University, Lima, Peru.

Extraction and Isolation. Dried leaves of *P. sanguinespicum* (870 g) were extracted with 90% EtOH (9 L) at room temperature. The filtrates were combined and concentrated under vacuum to afford 100 g of crude extract, which was then partitioned between H_2O−CH_2Cl_2 (1:1) (2 × 2 L). After evaporation, the CH_2Cl_2 extract was dissolved in MeOH and partitioned with petroleum ether (2 × 2 L). The solvent was removed under reduced pressure to provide 17.2 and 51.5 g of petroleum ether and MeOH fractions, respectively. The fractions were tested on axenic amastigotes of *L. amazonensis*, and the best activity was shown by the methanolic fraction (IC_{50} 2.4 μg/mL). One part of this extract (25 g) was chromatographed on silica gel by MPLC and eluted with a petroleum ether−AcOEt gradient system to give five fractions (A1−A5). The active fraction A2 (11.96 g, IC_{50} 1.7 μg/mL) was subjected to CC on RP-18 using a CH_3CN−H_2O gradient, providing six fractions (A2A−A2F). Final purification of A2C and A2E with CH_2Cl_2−AcOEt (95:5) on Sephadex LH-20 afforded compounds **2** (1.7 g) and **3** (3.0 g). Fraction A2B was further separated on a silica gel column using a C_6H_{12}−AcOEt gradient to yield five fractions (A2B1−A2B5). Compound **1** (60 mg) was obtained from fraction A2B2 by CC on Sephadex LH-20 using a CH_2Cl_2−AcOEt gradient. Fraction A2B4 was chromatographed on a RP-18 column eluted with MeOH−H_2O to give three fractions (A2B4A−A2B4C). Purification of fraction A2B4B on a silica gel column eluted with CH_2Cl_2−AcOEt (98:2) afforded **8** (30 mg). Fraction A2C was fractionated by CC on RP-18 using a MeOH−H_2O gradient to give five fractions, A2C1−A2C5. Fraction A2C2 was separated into four fractions (A2C2A−A2C2D) on Sephadex LH-20 eluted with a CH_2Cl_2−AcOEt gradient system. Fraction A2C2B gave **9** (18 mg) after purification over a silica gel column with CH_2Cl_2−AcOEt (95:5). Compound **4** (60 mg) was obtained as a precipitate after adding CH_2Cl_2 to fraction A2C3. After solvent evaporation, the soluble part was subjected to CC on a silica

gel column eluted with a CH_2Cl_2−AcOEt gradient to give four fractions (A2C3A−A2C3D). Purification of fractions A2C3A, A2C3B, and A2C3C on a Sephadex LH-20 column using CH_2Cl_2−AcOEt (95:5) gave **7** (24 mg), **5** (14 mg), **6** (13 mg), and **10** (7 mg).

(*S*)-1′-Methylbutyl caffeate (1): white powder; $[\alpha]^{20}_D$ +43.3 (*c* 0.53, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 294, 319 nm; IR (film) ν_{max} 3331, 2916, 1681, 1601, 1275, 1181 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRCIMS *m*/*z* 251.1275 [M + H]$^+$ (calcd for $C_{14}H_{19}O_4$, 251.1283).

(*S*)-1′-Methylhexyl caffeate (2): white powder; $[\alpha]^{20}_D$ +43 (*c* 1.06, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 293, 319 nm; IR (film) ν_{max} 3320, 2926, 1682, 1601, 1275, 1186 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRCIMS *m*/*z* 279.1586 [M + H]$^+$ (calcd for $C_{16}H_{23}O_4$, 279.1596).

(*S*)-1′-Methyloctyl caffeate (3): white powder; $[\alpha]^{20}_D$ +47.8 (*c* 1.07, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 294, 319 nm; IR (film) ν_{max} 3341, 2957, 1674, 1601, 1275, 1186 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRCIMS *m*/*z* 307.1903 [M + H]$^+$ (calcd for $C_{18}H_{27}O_4$, 307.1909).

Alkaline Hydrolysis of 1−3. To 20 mg of ester was added 2 mL of 0.1 N NaOH. The mixture was stirred in a boiling water bath for 1 h. After this time, the reaction mixture was diluted with water (10 mL) and extracted with $CHCl_3$ (10 mL × 3). The organic layers were combined and dried over anhydrous $MgSO_4$ and evaporated to dryness to give approximately 6−7 mg of the corresponding alcohol. The specific optical rotations, $[\alpha]^{20}_D$, were +10.3, +10.3, and +7.8 for the corresponding alcohols obtained from **1**, **2**, and **3**, respectively. They were compared with $[\alpha]^{20}_D$ published in ref 26, giving +10.25, +10.21, and +7.96 for (*S*)-(+)-2-pentanol, (*S*)-(+)-2-heptanol, and (*S*)-(+)-2-nonanol, respectively.

Sanguinolignan A (4): white powder; $[\alpha]^{20}_D$ −24 (*c* 1.0, $(CH_3)_2CO$); UV (CH_3OH) λ_{max} 283, 317 nm; IR (film) ν_{max} 3418, 1775, 1660, 1600, 1443, 1252, 1036 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 2 and 3; HRESIMS *m*/*z* 407.0758 [M + Na]$^+$ (calcd for $C_{20}H_{17}O_8Na$, 407.0743).

Preparation of (*R*)-(−)-α-Methoxyphenyl Acetate of 4 (4R). To a solution of 20 mg of *N*-(3-dimethyllaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) (0.12 mmol) in acetone (1 mL) and CH_2Cl_2 (0.5 mL) were added a catalytic amount of pyrrolidinopyridine and 17 mg of (*R*)-MPA (0.12 mmol). After complete dissolution, 4.5 mg of the alcohol **4** (0.012 mmol) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 10 mL of AcOEt and extracted with 0.1 N HCl (10 mL × 2), 0.1 N $NaHCO_3$ (10 mL × 2), and water (10 mL × 2). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica SPE cartridge (cyclohexane−AcOEt, 70:30) to give 5.4 mg (98%) of (*R*)-MPA ester: ^{1}H NMR ($(CD_3)_2CO$, 300 MHz) δ 3.66−3.78 (1H, m, H-8′), 3.94 (1H, dd, *J* = 9.0, 7.5, Hz, H-9′a), 4.27 (1H, dd, *J* = 9.0, 8.4 Hz, H-9′b), 4.69 (1H, d, *J* = 7.4 Hz, H-8), 5.86 (1H, d, *J* = 7.0 Hz, H-7′), 5.97 (2H, dd, *J* = 6.6, 1.0 Hz, H-10′), 6.18 (2H, dd, *J* = 2.0, 1.0 Hz, H-10), 6.72 (1H, d, *J* = 8.5 Hz, H-5), 6.81 (1H, dd, *J* = 6.4, 1.8 Hz, H-6), 6.82 (1H, overlapped, H-2′), 6.96 (1H, d, *J* = 8.2 Hz, H-5′), 7.37 (1H, overlapped, H-2), 7.53 (1H, dd, *J* = 8.3, 1.8 Hz, H-6). The α-MPA part had δ 3.25 (3H, s, CH_3O), 4.84 (1H, s), 7.27−7.36 (5H, m, aromatic protons).

Preparation of the (*S*)-(+)-α-Methoxyphenyl Acetate of 4 (4S). The (*S*)-MPA ester was obtained from 4 mg (0.0104 mmol) of **4** by the procedure described above. The reaction was stirred for 6 h. The residue was chromatographed on a RP-18 SPE cartridge (MeCN−H_2O, 70:30) to give 5.1 mg (93%) of (*S*)-MPA ester: ^{1}H NMR ($(CD_3)_2CO$, 300 MHz) δ 3.61−3.71 (1H, m, H-8′), 4.21 (1H, dd, *J* = 9.0, 8.2 Hz, H-9′a), 4.31 (1H, dd, *J* = 9.0, 8.3 Hz, H-9′b), 4.87 (1H, d, *J* = 8.2 Hz, H-8), 5.90 (1H, d, *J* = 7.5 Hz, H-7′), 5.92 (2H, dd, *J* = 5.1, 1.0 Hz, H-10′), 6.19 (1H, s, H-10), 6.43 (1H, overlapped, H-2′), 6.45 (1H, dd, *J* = 7.3, 1.8 Hz, H-6), 6.57 (1H, dd, *J* = 7.2, 1.1 Hz, H-5′), 7.02 (1H, d, *J* = 8.2 Hz, H-5), 7.41 (1H, *J* = 1.8 Hz, H-2), 7.63 (1H, dd, *J* = 8.2, 1.8 Hz, H-6). The α-MPA part had δ 3.21 (3H, s, CH_3O), 4.58 (1H, s), 7.27−7.36 (5H, m, aromatic protons).

Sanguinolignan B (5): white powder; $[\alpha]^{20}_D$ −34.3 (*c* 1.18, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 284, 318 nm; IR (film) ν_{max} 3466, 1778, 1667, 1602, 1446, 1249, 1036 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 2 and 3; HRESIMS *m*/*z* 407.0739 [M + Na]$^+$ (calcd for $C_{20}H_{17}O_8Na$, 407.0743).

Preparation of (*R*)-(−)-α-Methoxyphenyl Acetate of 5 (5*R*). The (*R*)-MPA ester was obtained from 4 mg (0.0104 mmol) of **5** by the procedure described above. The reaction was stirred for 4 h. Purification was carried out on a silica SPE cartridge using a gradient of cyclohexane−AcOEt (from 80:20 to 70:30) to obtain 5.4 mg (98%) of (*R*)-MPA ester: ^{1}H NMR ($(CD_3)_2CO$, 300 MHz) δ 3.40−3.50 (1H, m, H-8′), 4.21 (1H, dd, *J* = 4.7, 11.7 Hz, H-9′a), 4.32 (1H, dd, *J* = 5.5, 11.7 Hz, H-9′b), 4.80 (1H, d, *J* = 10.6 Hz, H-8), 5.30 (1H, d, *J* = 9.5 Hz, H-7′), 6.06 (2H, s, H-10′), 6.20 (2H, s, H-10), 6.90 (1H, d, *J* = 8.0 Hz, H-5′), 6.96 (1H, dd, *J* = 8.0, 1.6 Hz, H-6′), 7.01 (1H, d, *J* = 1.6 Hz, H-2′), 7.01 (1H, d, *J* = 8.2 Hz, H-5), 7.45 (1H, d, *J* = 1.8 Hz, H-2), 7.61 (1H, dd, *J* = 8.2, 1.8 Hz, H-6). The α-MPA part had δ 3.28 (3H, s, CH_3O), 4.73 (1H, s), 7.32−7.39 (5H, m, aromatic protons).

Preparation of (*S*)-(+)-α-Methoxyphenyl Acetate of 5 (5*S*). The (*S*)-MPA ester was obtained from 4 mg (0.0104 mmol) of **5** by the procedure described above. The reaction was stirred for 20 h. Purification was carried out on a silica SPE cartridge using a gradient of cyclohexane−AcOEt (from 80:20 to 70:30) to obtain 4.5 mg (83%) of (*S*)-MPA ester: ^{1}H NMR ($(CD_3)_2CO$, 300 MHz) δ 3.43−3.53 (1H, m, H-8′), 4.18 (1H, dd, *J* = 11.7, 5.8 Hz, H-9′a), 4.31 (1H, dd, *J* = 11.7, 4.8 Hz, H-9′b), 4.95 (1H, d, *J* = 10.7 Hz, H-8), 5.16 (1H, d, *J* = 9.7 Hz, H-7′), 6.06 (2H, s, H-10′), 6.20 (2H, s, H-10), 6.85 (1H, dd, *J* = 8.0, 1.7 Hz, H-6′), 6.89 (1H, d, *J* = 8.0 Hz, H-5′), 6.94 (1H, d, *J* = 1.7 Hz, H-2′), 7.03 (1H, d, *J* = 8.2 Hz, H-5), 7.50 (1H, d, *J* = 1.8 Hz, H-2), 7.71 (1H, dd, *J* = 8.2,1.8 Hz, H-6). The α-MPA part had δ 3.32 (3H, s, CH_3O), 4.73 (1H, s), 7.30−7.34 (5H, m, aromatic protons).

Sanguinolignan C (6): white powder; $[\alpha]^{20}_D$ −1.2 (*c* 0.85, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 282, 319 nm; IR (film) ν_{max} 2912, 1777, 1742, 1677, 1604, 1442, 1261, 1033 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 2 and 3; HRESIMS *m*/*z* 465.1158 [M + Na]$^+$ (calcd for $C_{23}H_{22}O_9Na$, 465.1162).

Sanguinolignan D (7): white powder; $[\alpha]^{20}_D$ −47.4 (*c* 2.15, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 283, 317 nm; IR (film) ν_{max} 2908, 1778, 1765, 1678, 1444, 1256, 1036 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 2 and 3; HRESIMS *m*/*z* 449.0855 [M + Na]$^+$ (calcd for $C_{22}H_{18}O_9Na$, 449.0849).

(7′*S*)-Parabenzlactone (8): white powder; $[\alpha]^{20}_D$ −16.5 (*c* 2.31, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 290 nm; IR (film) ν_{max} 3466, 1757, 1492, 1244, 1036 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.52−2.61 (1H, m, H-8′), 2.86−3.0 (3H, overlapped, H-7, H-8), 3.94 (2H, d, *J* = 7.3 Hz, H-9′), 4.61 (1H, d, *J* = 6.6 Hz, H-7′), 5.92 (2H, dd, *J* = 5.9, 1.4 Hz, H-10′), 5.96 (2H, dd, *J* = 3.9, 1.4 Hz, H-10), 6.58 (1H, dd, *J* = 7.9, 1.6 Hz, H-6′), 6.61 (1H, d, *J* = 1.6 Hz, H-2′), 6.65−6.75 (4H, overlapped, H-5′, H-2, H-5, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 35.3 (C-7), 43.9 (C-8′), 45.2 (C-8), 68.6 (C-9′), 75.6 (C-7′), 101.1 (C-10′), 101.4 (C-10), 106.3 (C-2′), 108.3 (C-5), 108.4 (C-5′), 110.1 (C-2), 119.5 (C-6′), 122.9 (C-6), 131.3 (C-1), 135.5 (C-1′), 146.4 (C-4′), 147.7 (C-4), 147.8 (C-3′), 148.2 (C-3), 179.1 (C-9); HRESIMS *m*/*z* 393.0965 [M + Na]$^+$ (calcd for $C_{20}H_{18}O_7Na$, 393.0950).

Dihydrocubebin (9): white powder; UV (CH_2Cl_2) λ_{max} 290 nm; IR (film) ν_{max} 3310, 1503, 1441, 1243, 1036 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.79−1.88 (1H, m, H-8), 2.61 (1H, dd, *J* = 13.9, 6.0 Hz, H-7a), 2.75 (1H, dd, *J* = 13.9, 8.6 Hz, H-7b), 3.50 (1H, dd, *J* = 11.4, 4.4 Hz, H-9a), 3.78 (1H, dd, *J* = 11.4, 4.7, H-9b), 5.92 (2H, s, H-10), 6.60 (1H, dd, *J* = 7.8, 1.5 Hz, H-6), 6.63 (1H, d, *J* = 1.5 Hz), 6.71 (1H, d, *J* = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.1 (C-7), 44.4 (C-8), 60.4 (C-9), 100.9 (C-10), 108.3 (C-5), 109.5 (C-2), 122.0 (C-6), 134.6 (C-1), 145.9 (C-4), 147.8 (C-3); HRESIMS *m*/*z* 739.2732 [2 M + Na]$^+$ (calcd for $C_{40}H_{44}O_{12}Na$, 739.2730).

Justiflorinol (10): white powder; $[\alpha]^{20}_D$ −50.4 (*c* 0.55, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 279, 313 nm; IR (film) ν_{max} 3436, 1666, 1443, 1037 cm^{-1}; ^{1}H NMR ($(CD_3)_2CO$, 500 MHz) δ 3.29 (1H, dd, *J* = 18.0, 3.6 Hz, H-8a), 3.65 (1H, dd, *J* = 18.0, 9.8 Hz, H-8b), 3.70−3.75 (1H, m, H-9′a), 3.85−3.89 (1H, m, H-9′b), 4.19−4.29 (1H, m, H-8′), 6.12 (2H, s, OCH_2O), 6.13 (2H, s, OCH_2O), 6.96 (1H, d, *J* = 8.3 Hz, H-5′), 6.98 (1H, d, *J* = 8.3 Hz, H-5), 7.41 (1H, d, *J* = 1.7 Hz, H-2′), 7.48 (1H, d, *J* = 1.7 Hz, H-2), 7.65 (1H, dd, *J* = 8.2, 1.7 Hz, H-6′), 7.69 (1H, dd, *J* = 8.2, 1.7 Hz, H-6); ^{13}C NMR ($(CD_3)_2CO$, 125 MHz) δ 38.9 (C-8), 45.7 (C-8′), 64.3 (C-9′), 102.9 (OCH_2O), 103.0 (OCH_2O), 108.1 (C-2), 108.6 (C-5″), 108.7 (C-2′), 125.1 (C-6), 125.5 (C-6′), 132.6 (C-1) 133.1 (C-1″), 149.0 (C-3), 149.1 (C-3″), 152.4 (C-4), 152.7 (C-4″), 196.9 (C-7), 199.9 (C-7′); HRESIMS *m*/*z* 379.0790 [M + Na]$^+$ (calcd for $C_{19}H_6O_7Na$, 379.0794).

Antileishmanial Assay. Experiments were conducted on axenic amastigotes of *Leishmania amazonensis* (strain MHOM/BR/76/LTB-012). Axenically grown amastigotes were maintained by weekly subpassages in MAA/20 medium at 32 ± 1 °C with 5% CO_2 in 25 cm^2 tissue culture flasks. Cultures were initiated with 5 × 10^5 amastigotes in 25 cm^2 tissue culture flasks with 5 mL of medium. To determine the activity of the extracts, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) micromethod was used as previously described.[27] Briefly, 100 μL of axenically grown amastigotes were seeded in 96-well flat-bottom microtiter plates. Crude extracts were tested at 100, 50, and 1 μg/mL of DMSO. Pure compounds were

tested at four concentrations in DMSO: 50, 5, 0.5, and 0.05 μg/mL for **1** to **4** and 50, 10, 2, and 0.2 μg/mL for **5** to **10**. After 72 h of incubation, 10 μL of MTT (10 mg/mL) was added to each well and plates were further incubated for 4 h. The enzymatic reaction was then stopped with 100 μL of 50% 2-propanol−10% sodium dodecyl sulfate and incubated for an additional 30 min under agitation at room temperature. Finally, the optical density (OD) was read at 570 nm with a 96-well scanner (Bio-Rad). All experiments were performed in triplicate, and standard deviations were calculated using Excel software.

Percent growth inhibition of the parasite was calculated by the following formula:

$$\% \text{ of inhibition} = \frac{(\text{OD control} - \text{OD drugs}) \times 100}{\text{OD control}}$$

The concentration inhibiting 50% of the parasite growth (IC_{50}) was calculated after evaluating percent growth inhibition at different concentrations (Excel software). Reference compound was amphotericin B.

Cytotoxicity Assay. Vero cells (a monkey kidney cell line) and MCF7 cells (a human breast cancer cell line) were cultured in RPMI (Cambrex), supplemented with 10% fetal calf serum (Boehringer). Cell growth was measured by [^{3}H]-hypoxanthine (Perkin-Elmer, France) incorporation after a 48 h incubation with serial drug dilutions. The amount of [^{3}H]-hypoxanthine incorporated in the presence of test compounds was compared with that of control cultures without the test compounds.[28] Inhibition percentages were plotted versus concentration, and IC_{50} values were evaluated graphically.

Murine peritoneal macrophages were treated with appropriate dilutions of tested compounds, and the trypan blue dye exclusion method was used.[29] Dilutions of 10, 1, and 0.1 μM in complete medium were then added to achieve a final volume of 100 μL. The culture was continued for another 48 h at 37 °C in 5% CO_2. After this incubation, the number of viable cells was scored by hematocytometer using 0.4% trypan blue solution in PBS. The half-maximal cytotoxic dose for each cell type was determined. All experiments were repeated three times.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) World Health Organization. *Report of the Fifth Consultative Meeting on Leishmania/HIV Coinfection*, 2007.
(2) Davies, C. R.; Reithinger, R.; Campbell-Lendrum, D.; Feliciangeli, D.; Borges, R.; Rodriguez, N. *Cad. Saude Publica* **2000**, *16*, 925–950.
(3) Croft, S. L.; Barrett, M. P.; Urbina, J. A. *Trends Parasitol.* **2005**, *21*, 508–512.
(4) Grogl, M.; Thomason, T. N.; Franke, E. D. *Am. J. Trop. Med. Hyg.* **1992**, *47*, 117–126.
(5) Arango, V.; Robledo, S.; Séon-Méniel, B.; Figadère, B.; Cardona, W.; Sáez, J.; Otálvaro, F. *J. Nat. Prod.* **2010**, *73*, 1012–1014.
(6) Chan-Bacab, M. J.; Pena-Rodriguez, L. M. *Nat. Prod. Rep.* **2001**, *18*, 674–688.
(7) Estevez, Y.; Castillo, D.; Tangoa Pisango, M.; Arevalo, J.; Rojas, R.; Alban, J.; Deharo, E.; Bourdy, G.; Sauvain, M. *J. Ethnopharmacol.* **2007**, *114*, 254–259.
(8) Odonne, G.; Bourdy, G.; Castillo, D.; Estevez, Y.; Lancha-Tangoa, A.; Alban-Castillo, J.; Deharo, E.; Rojas, R.; Stien, D.; Sauvain, M. *J. Ethnopharmacol.* **2009**, *126*, 149–158.
(9) Parmar, V.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, A. J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 591–673.
(10) Rukachaisirikul, T.; Prabpai, S.; Champung, P.; Suksamram, A. *Planta Med.* **2002**, *68*, 853–855.
(11) Rukachaisirikul, T.; Siriwattanakit, P.; Sukcharoenphol, K.; Wongvein, C.; Ruttanaweang, P.; Wongwattanavuch, P.; Suksamrarn, A. *J. Ethnopharmacol.* **2004**, *93*, 173–176.
(12) Lee, A. L.; Ley, S. V. *Org. Biomol. Chem.* **2003**, *1*, 3957–3966.
(13) Jenett-Siems, K.; Mockenhaupt, F. P.; Bienzle, U.; Gupta, M. P.; Eich, E. *Trop. Med. Int. Health* **1999**, *4*, 611–615.
(14) Flores, N.; Cabrera, G.; Jimenez, I. A.; Pinero, J.; Gimenez, A.; Bourdy, G.; Cortes-Selva, F.; Bazzocchi, I. L. *Planta Med.* **2007**, *73*, 206–11.
(15) Torres-Santos, E. C.; Moreira, D. L.; Kaplan, M. A. C.; Meirelles, M. N.; Rossi-Bergmann, B. *Antimicrob. Agents Chemother.* **1999**, *43*, 1234–1241.
(16) Hermoso, A.; Jimenez, I. A.; Mamani, Z. A.; Bazzocchi, I. L.; Pinero, J. E.; Ravelo, A. G.; Valladares, B. *Bioorg. Med. Chem.* **2003**, *11*, 3975–80.
(17) Corrie, J. E. T.; Green, G. H.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1970**, *23*, 133–145.
(18) Lopes, L. M. X.; Yoshida, M.; Gottlieb, O. R. *Phytochemistry* **1983**, *22*, 1515–1518.
(19) Sai Prakash, C. V.; Hoch, J. M.; Kingston, D. G. I. *J. Nat. Prod.* **2002**, *65*, 100–107.
(20) Latypov, S. K.; Seco, J. M.; Quinoa, E.; Riguera, R. *J. Org. Chem.* **1996**, *61*, 8569–8577.
(21) Seco, J. M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–118.
(22) Moss, G. P. *Pure Appl. Chem.* **2000**, *72*, 1493–1523.
(23) Raffaelli, B.; Wähälä, K.; Hase, T. *Org. Biomol. Chem.* **2006**, *4*, 331–341.
(24) De Carvalho, M. G.; Yoshida, M.; Gottlieb, O. R.; Gottlieb, H. E. *Phytochemistry* **1987**, *26*, 265–267.
(25) Susplugas, S.; Nguyen Van, H.; Bignon, J.; Thoison, O.; Kruczynski, A.; Sévenet, T.; Guéritte, F. *J. Nat. Prod.* **2005**, *68*, 734–738.
(26) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162–169.
(27) Sereno, D.; Lemesre, J. L. *Parasitol. Res.* **1997**, *83*, 401–403.
(28) Roumy, V.; Garcia-Pizango, G.; Gutierrez-Choquevilca, A. L.; Ruiz, L.; Jullian, V.; Winterton, P.; Fabre, N.; Moulis, C.; Valentin, A. *J. Ethnopharmacol.* **2007**, *112*, 482–489.
(29) Castillo, D.; Arevalo, J.; Herrera, F.; Ruiz, C.; Rojas, R.; Rengifo, E.; Vaisberg, A.; Lock, O.; Lemesre, J. L.; Gornitzka, H.; Sauvain, M. *J. Ethnopharmacol.* **2007**, *112*, 410–414.

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