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7,7-Dimethylaporphine and Other Alkaloids from the Bark of *Guatteria friesiana*

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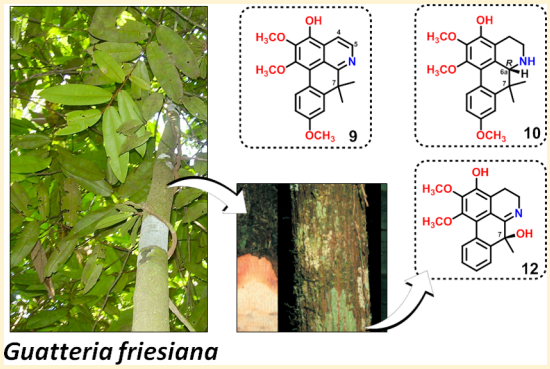
Supporting Information

ABSTRACT: Phytochemical investigation of the bark of *Guatteria friesiana* afforded 12 new aporphines (**1**−**12**), along with nine known alkaloids (**13**−**21**). The structures of the new alkaloids were determined on the basis of spectroscopic data interpretation. The cytotoxic activity of the isolated compounds against a small panel of tumor cell lines was assessed using the Alamar blue assay.



Guatteria Ruiz & Pav., the largest genus in the plant family Annonaceae, comprises approximately 310 species, all distributed through neotropical regions.[1] In Brazil, 385 identified species are catalogued, with most occurring in the Amazonian domain.[1] Some of them are known for their medicinal properties,[2] as supported by previous investigations revealing bioactive compounds showing potential anticancer,[3−6] antimicrobial,[4,5,7−10] antioxidant,[7] and antiparasitic activities.[11,12] *Guatteria friesiana* (W.A. Rodrigues) Erkens & Maas is known popularly as "envireira" or "envira", and previous phytochemical investigations have described the chemical constituents of its essential oil[6,9,13] as well as aporphine alkaloids found in its leaves and stem.[3,8] In a continuing search for bioactive compounds from Amazonian annonaceous plants, 12 new (**1**−**12**) and nine previously described (**13**−**21**) alkaloids were obtained in a systematic activity-guided investigation of the bark of *G. friesiana*.

RESULTS AND DISCUSSION

Compound **1** was obtained as light yellow oil. Its molecular formula of $C_{20}H_{19}NO_3$ was determined from its HRESIMS (*m*/*z* 322.1443 $[M + H]^+$, calcd 322.1438) and ^{13}C NMR data. The IR, UV, and NMR spectra were similar to those reported for demethoxyguadiscine (**14**).[8] Analysis of the NMR data (Tables 1 and 2) for **1** and for **14**[8] confirmed that these compounds are very similar, except for the presence of a methoxy group at C-3 (s, δ_H 4.04). This was supported by the HMBC correlations of H-4 with C-3 and CH_3O-3 (Tables 1 and 2). Therefore, structure **1** (3-methoxy-demethoxyguadiscine) was established, as shown.

Compound **2** was obtained as a light yellow oil. Its molecular formula was determined to be $C_{21}H_{23}NO_3$ from the ^{13}C NMR (Table 2) and HRESIMS data (*m*/*z* 338.1749 $[M + H]^+$, calcd 338.1756). Its IR, UV, and NMR spectra (Tables 1 and 2) were similar to those of **1**. The main differences in the NMR data were related to the absence of the methylenedioxy bridge signal at δ_H 6.10 (s) present in **1**, which was replaced by two resonances for methoxy groups for **2** at δ_H 3.81 (s, CH_3O-1)

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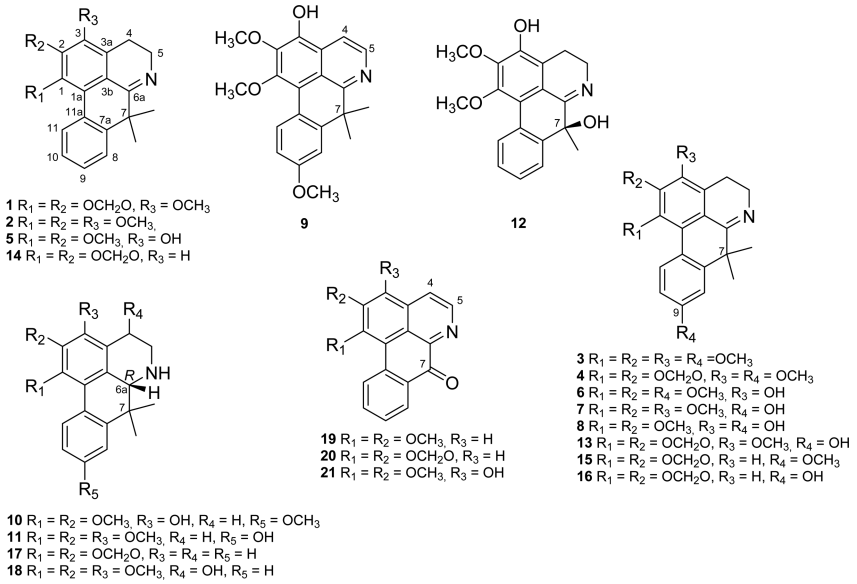
Chart 1



and δ_H 3.91 (s, CH_3O-2). On the basis of the spectroscopic data obtained, compound **2** (guatteriscine) was assigned as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **3** was isolated as a light yellow oil. Its HRESIMS showed a protonated molecular ion peak at m/z 368.1862 $[M + H]^+$, indicating a molecular formula of $C_{22}H_{25}NO_4$ (calcd 368.1862). A comparative analysis of the NMR data (Tables 1 and 2) for compounds **2** and **3** indicated many similarities between these substances. A difference was the presence of a methoxy group at C-9 (δ_H 3.86, s, CH_3O-9) in **3** instead of an aromatic hydrogen, as observed for **2**. The assignment of the substitution at C-9 was obtained from the HMBC correlations between H-11 and C-9, with a further correlation from the methoxy protons at CH_3O-9 with C-9 (Tables 1 and 2). Additionally, a spin system comprising the three protons at δ_H 7.06 (d, J = 2.7 Hz), δ_H 6.82 (dd, J = 8.8 and 2.7 Hz), and H-11 (δ_H 8.43, d, J = 8.8 Hz) was in accordance with the pattern of substitution proposed for **3** (Tables 1 and 2). Therefore, compound **3** (9-methoxyguatteriscine) was established as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **4** was obtained as a light yellow oil. Its molecular formula of $C_{21}H_{21}NO_4$ was determined from the HRESIMS (m/z 352.1549 $[M + H]^+$, calcd 352.1548) and ^{13}C NMR data. Its IR and UV spectra were closely comparable to those reported for guadiscine (**15**).[14] A comparative analysis of the NMR data (Tables 1 and 2) for **4** and guadiscine showed a signal for a methoxy group at C-3 (s, δ_H 4.01) in **4** instead of an aromatic proton observed for guadiscine.[14] This structural proposal was supported by the HMBC correlations of H-4 with C-3 and CH_3O-3 (Tables 1 and 2). Thus, compound **4** (3-methoxyguadiscine) was established as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **5** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{20}H_{21}NO_3$ from the ^{13}C NMR (Table 2) and HRESIMS data (m/z 324.1609 $[M + H]^+$, calcd 324.1599). The IR spectrum indicated a hydroxy group (3392 cm^{-1}). A comparative analysis of the NMR data (Tables 1 and 2) for compounds **2** and **5** indicated several similarities between these substances. The main difference apparent was for a C-3-hydroxy substituent in **5** that replaced the methoxy group present in **2**. This substitution was confirmed through the HMBC correlations of the H-4 protons with C-3. Therefore, compound **5** (guatterfriesine) was established as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **6** was obtained as a yellow needle-like solid. Its molecular formula of $C_{21}H_{23}NO_4$ was determined from its HRESIMS (m/z 354.1706 $[M + H]^+$, calcd 354.1705) and ^{13}C NMR data. A comparison of the IR, UV, and NMR data (Tables 1 and 2) between compounds **5** and **6** showed that these two substances are closely comparable, except for the presence of a methoxy group at C-9 for **6**. Thus, compound **6** (9-methoxyguatterfriesine) was established, as shown.

Compound **7** was isolated as a yellow amorphous powder. Its HRESIMS showed a protonated molecular ion peak at m/z 354.1707 $[M + H]^+$, indicating a molecular formula of $C_{21}H_{23}NO_4$ (calcd 354.1705), which was corroborated by the ^{13}C NMR data (Table 2). The IR spectrum indicated the presence of a hydroxy group (3436 cm^{-1}). A comparative analysis of the NMR data (Tables 1 and 2) for compounds **3** and **7** indicated the similar structures of these substances. The main difference was a C-9-hydroxy substituent in **7** replacing the aromatic proton in **3**. This was supported by the HMBC correlation between H-11 and C-9. Therefore, compound **7** (9-hydroxyguattescine) was determined as a new regioisomer of 9-methoxyguatterfriesine, as shown.

Compound **8** was obtained as a yellow amorphous powder. Its molecular formula of $C_{20}H_{21}NO_4$ was determined from its HRESIMS (m/z 340.1544 $[M + H]^+$, calcd 340.1549) and ^{13}C NMR data. Its IR, UV, and NMR spectra were similar to those of **5**. This was supported by the HMBC correlations of H-8, H-10, and H-11 protons with C-9. Therefore, compound **8** (9-hydroxyguatterfriesine) was assigned as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **9** was obtained as a brown amorphous powder. Its molecular formula was determined to be $C_{21}H_{21}NO_4$ from the ^{13}C NMR (Table 2) and HRESIMS data (m/z 352.1555 $[M + H]^+$, calcd 352.1549). A comparative analysis of the NMR data (Tables 1 and 2) for compounds **6** and **9** indicated many similarities between these substances. The main difference was the presence of aromatic protons at δ_H 7.74 (d, J = 5.6 Hz, H-4)

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Table 1. ^{1}H NMR Data for Compounds 1−9 and 13 (400 MHz, δ in ppm and J Values in (Hz) in Parentheses)

no.	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[b]	8[b]	9[a]	13[b]
1										
1a										
2										
3										
3a										
3b										
4	2.61−2.57 m	2.63−2.60 m	2.62−2.58 m	2.60−2.56 m	2.65−2.62 m	2.65−2.61 m	2.63−2.60 m	2.62−2.58 m	7.74 d (5.6)	2.60−2.57 m
5	3.62−3.58 m	3.63−3.59 m	3.61−3.58 m	3.61−3.57 m	3.66−3.62 m	3.65−3.62 m	3.57−3.54 m	3.56−3.52 m	8.49 d (5.6)	3.55−3.51 m
6a										
7										
7a										
8	7.54−7.50 m	7.53−7.49 m	7.06 d (2.7)	7.07 d (2.6)	7.51−7.49 m	7.06 d (2.7)	7.00 d (2.6)	6.99 d (2.6)	7.21 d (2.4)	7.01 d (2.5)
9	7.31−7.26 m	7.31−7.27 m			7.31−7.24 m					
10	7.31−7.26 m	7.31−7.27 m	6.82 dd (8.8; 2.7)	6.82 dd (8.6; 2.6)	7.31−7.24 m	6.82 dd (8.8; 2.7)	6.77 dd (8.7; 2.6)	6.75 dd (8.6; 2.6)	6.90 dd (9.0; 2.4)	6.76 dd (8.5; 2.5)
11	8.20−8.16 m	8.48−8.44 m	8.43 d (8.8)	8.13 d (8.6)	8.43−8.39 m	8.37 d (8.8)	8.33 d (8.7)	8.27 d (8.6)	8.81 d (9.0)	8.04 d (8.5)
11a										
(OCH_2O)1-2	6.10 s			6.09 s						6.09 s
OCH_3-1		3.81 s	3.79 s		3.79 s	3.77 s	3.78 s	3.77 s	3.91 s	
OCH_3-2		3.91 s	4.02 s		4.06 s	4.05 s	4.02 s	4.00 s	4.09 s	
OCH_3-3 (OH)	4.04 s	4.02 s	3.89 s	4.01 s			3.89 s			4.01 s
OCH_3-9 (OH)			3.86 s	3.85 s		3.86 s			3.90 s	
CH_3-7 (2×)	1.50 s	1.49 s	1.47 s	1.48 s	1.50 s	1.49 s	1.45 s	1.43 s	1.74 s	1.45 s

[a]The experiments were obtained at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$. [b]In $CDCl_3$ + drops of CD_3OD.

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Table 2. ^{13}C NMR Data for Compounds 1−9 and 13 (100 MHz, δ in ppm)

no.	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[b]	8[b]	9[a]	13[b]
1	143.6, C	150.0, C	149.3, C	142.8, C	148.6, C	148.0, C	149.2, C	148.4, C	149.3, C	142.8, C
1a	111.6, C	122.8, C	122.9, C	111.8, C	119.5, C	119.5, C	123.7, C	119.81, C	116.3, C	112.5, C
2	138.9, C	149.3, C	149.2, C	139.1, C	142.7, C	142.7, C	149.7, C	143.6, C	140.5, C	139.6, C
3	139.2, C	149.3, C	148.5, C	138.5, C	145.1, C	144.4, C	148.3, C	144.9, C	141.4, C	138.3, C
3a	124.4, C	127.0, C	127.0, C	124.4, C	120.2, C	119.69, C	127.0, C	120.2, C	124.5, C	124.5, C
3b	118.0, C	120.1, C	119.6, C	117.4, C	119.6, C	119.65, C	119.4, C	119.84, C	119.2, C	117.4, C
4	19.5, CH_2	19.7, CH_2	19.7, CH_2	19.5, CH_2	19.3, CH_2	19.3, CH_2	19.8, CH_2	19.5, CH_2	112.4, CH	19.6, CH_2
5	46.8, CH_2	46.4, CH_2	46.6, CH_2	46.8, CH_2	46.2, CH_2	46.3, CH_2	46.1, CH_2	46.3, CH_2	142.0, CH	46.5, CH_2
6a	170.5, C	171.3, C	171.0, C	170.4, C	171.7, C	171.5, C	172.8, C	173.1, C	163.3, C	172.2, C
7	42.8, C	42.5, C	42.7, C	43.0, C	42.6, C	42.8, C	42.8, C	42.9, C	42.3, C	43.1, C
7a	143.6, C	143.7, C	146.0, C	145.8, C	143.1, C	145.3, C	145.5, C	145.0, C	146.8, C	145.4, C
8	124.8, CH	124.4, CH	111.0, CH	111.4, CH	124.4, CH	111.0, CH	111.6, CH	111.6, CH	112.1, CH	112.0, CH
9	127.8, CH	127.9, CH	159.1, C	159.2, C	127.5, CH	158.8, C	156.8, C	156.3, C	158.9, C	156.9, C
10	126.5, CH	126.6, CH	110.9, CH	111.0, CH	126.7, CH	111.0, CH	113.5, CH	113.5, CH	111.7, CH	113.5, CH
11	126.8, CH	127.9, CH	129.3, CH	128.2, CH	127.4, CH	128.8, CH	129.6, CH	129.1, CH	128.5, CH	128.5, CH
11a	128.4, C	129.8, C	122.6, C	121.4, C	130.0, C	122.8, C	121.4, C	121.9, C	122.0, C	120.2, C
(OCH_2O)1-2	101.1			101.1						101.3
OCH_3-1		60.90	60.6		60.5	60.3	60.7	60.4	60.3	
OCH_3-2		60.97	61.1		61.2	61.2	61.2	61.1	61.5	
OCH_3-3 (OH)	59.8	61.1	60.9	59.9			61.0			60.0
OCH_3-9 (OH)			55.2	55.2		55.2			55.2	
CH_3-7 (2×)	27.5	27.1	27.1	27.6	27.1	27.2	27.1	27.1	32.2	27.5

[a]The experiments were obtained at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$. [b]In $CDCl_3$ + drops of CD_3OD.

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Table 3. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data for Compounds 10−12 (δ in ppm and J Values in (Hz) in Parentheses)

	10[a]		11[b]		12[a]	
no.	δ_C, type	δ_H mult. (J in Hz)	δ_C, type	δ_H mult. (J in Hz)	δ_C, type	δ_H mult. (J in Hz)
1	147.6, C		148.8, C		149.2, C	
1a	118.4, C		122.7, C		119.3, C	
2	138.5, C		145.2, C		143.6, C	
3	145.6, C		149.6, C		145.5, C	
3a	129.7, C		124.2, C		119.2, C	
3b	117.4, C		129.5, C		118.3, C	
4ax				2.72 dddd (16.9; 11.9; 5.6; 1.7)		2.44 td (16.7; 6.1)
4eq	23.4, CH_2	2.77−2.74 m	23.7, CH_2	2.79 ddt (16.9; 4.2; 1.6)	19.5, CH_2	3.01 ddd (16.6; 5.3; 1.0)
5ax		2.93 ddd (11.9; 10.4; 5.7)		2.88 td (11.9; 4.2)		3.23 ddd (16.7; 15.0; 5.3)
5eq	42.6, CH_2	3.47 dt (11.9; 3.4)	42.7, CH_2	3.40 ddd (11.9; 5.6; 1.6)	45.4, CH_2	4.11 ddd (15.0; 6.1; 1.0)
6a	61.1, CH	3.67 s	61.5, CH	3.62 t (1.6)	170.8, C	
7	38.3, C		38.1, C		72.6, C	
7a	146.4, C		147.0, C		141.8, C	
8	109.9, CH	6.97 d (2.7)	110.5, CH	6.90 d (2.6)	124.5, CH	7.85−7.81 m
9	158.4, C		156.2, C		127.9, CH	7.35−7.28 m
10	110.6, CH	6.81 dd (8.7; 2.7)	113.2, CH	6.75 dd (8.6; 2.6)	127.5, CH	7.35−7.28 m
11	129.0, CH	8.17 d (8.7)	129.6, CH	8.10 d (8.6)	126.9, CH	8.40−8.36 m
11a	123.4, C		122.3, C		128.4, C	
OCH_3-1	60.1	3.67 s	60.5	3.67 s	60.6	3.82 s
OCH_3-2	61.3	3.99 s	61.1	3.96 s	61.2	4.06 s
OCH_3-3 (OH)			60.5	3.89 s		
OCH_3-9 (OH)	55.1	3.85 s				
CH_3-7 (OH)	20.8	0.88 s	20.8	0.85 s		
CH_3-7	23.3	1.49 s	23.3	1.44 s	33.3	1.51 s

[a]The experiments were obtained at 293 K with TMS as internal reference (0.00 ppm) in $CDCl_3$. [b]In $CDCl_3$ + drops of CD_3OD.

and δ_H 8.49 (d, J = 5.6 Hz, H-5), representative of a pyridine ring in **9**. This was supported by the typical coupling constants observed for H-4 and H-5, and by HMBC correlations between H-4 and H-5 with their respective vicinal carbons. On the basis of the spectroscopic data obtained, compound **9** (4,5-dehydro-9-methoxyguatterfriesine) was determined as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **10** was obtained as a yellow amorphous powder. Its molecular formula of $C_{21}H_{25}NO_4$ was determined from its HRESIMS (m/z 356.1865 $[M + H]^+$, calcd 356.1862) and ^{13}C NMR data. A comparative analysis of the NMR data (Tables 1−3) for compounds **6** and **10** confirmed that these two compounds possess similar structures. The main difference observed was the presence of the proton signals at δ_H 2.77−2.74 (m, H-4), δ_H 2.93 (ddd, J = 11.9, 10.4, and 5.7 Hz, H-5_{ax}), and δ_H 3.47 (dt, J = 11.9 and 3.4 Hz, H-5_{eq}), comprising a tetrahydroisoquinoline system. Additionally, a hydrogen atom at C-6a was observed for **10** (δ_H 3.67, s, H-6a) (Table 3). These differences were supported by the absence of characteristic imine resonances (δ_C 170−173) in the ^{13}C NMR spectrum of **10**. The absolute configuration for this compound was established as *R* according to the circular dichroism curve (ECD), which showed a negative Cotton effect at 235 nm.[15] This was also supported by the specific rotation of **10** ($[\alpha]^{25}_D$ −46.8).[8,15] Accordingly, compound **10** ((*R*)-6,6a-dihydro-9-methoxyguatterfriesine) was assigned as a new 7,7-dimethylaporphine alkaloid, as shown.

Compound **11** was isolated as a yellow amorphous powder. Its molecular formula of $C_{20}H_{25}NO_4$ was determined from its HRESIMS (m/z 340.1866 $[M + H]^+$, calcd 356.1862) and ^{13}C NMR data. Its IR, UV, and NMR spectra were similar to those of **7**. The main difference observed was the presence of methylene protons at C-4 (δ_H 2.72, dddd, J = 16.9, 11.9, 5.6, and 1.7 Hz, H-4_{ax} and δ_H 2.79, ddt, J = 16.9, 4.2, and 1.6 Hz, H-4_{eq}) and at C-5 (δ_H 2.88, td, J = 11.9 and 4.2 Hz, H-5_{ax} and δ_H 3.40, ddd, J = 11.9, 5.6, and 1.6 Hz, H-5_{eq}), constituting a tetrahydroisoquinoline system. The relative configuration of **11** was established by its specific rotation, which appeared to be levorotatory ($[\alpha]^{25}_D$ −4.0), indicating the β-orientation of the C-6a hydrogen.[8,15] Thus, compound **11** [(*R*)-4,5,6,6a-tetrahydromelosmidine] was identified as a known 7,7-dimethylaporphine alkaloid of synthetic origin,[14] with this being the first report of **11** as a natural compound.

Compound **12** was isolated as a green amorphous powder. Its molecular formula of $C_{19}H_{19}NO_4$ was determined from its HRESIMS (m/z 326.1392 $[M + H]^+$, calcd 326.1392) and ^{13}C NMR data. A comparative analysis of NMR data (Tables 1−3) for compounds **5** and **12** confirmed that these two compounds possess similar structures. The main difference was the replacement of a methyl group in **5** by a C-7-hydroxy (δ_C 72.6) substituent in **12**. This was supported by the absence of the proton signals in the range δ_H 0.88−0.85 (m) attributed to one of the methyl groups and by HMBC correlations of the protons at δ_H 1.51 (s, CH_3-7) with C-6a, C-7, and C-8. The relative configuration of **12** was deduced by comparison of the ^{13}C NMR resonances for the substituents at C-7. Based on the twisted biphenyl-like core of the aporphines, an anisotropic effect is known for C-7 substituents, allowing the assignment of the substituents of this carbon atom. Substituents with an α-orientation display unshielded chemical shifts when compared with those with a β-orientation.[3] A comparison of the ^{13}C NMR resonances of the CH_3-7 and C-7 carbons (Table 3) for **12** with those reported for dehydroguatteriopsiscine,[3] guattouregidine,[16] and guattouregin[16] suggested the β-orientation of the C-7-hydroxy group. Therefore, compound **12** (9-demethox-

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yisoguattouregine) was assigned as a new 7-hydroxy-7-methylaporphine alkaloid, as shown.

Additionally, the complete and unambiguous NMR assignments of 9-methoxyguadiscine (**13**)[17] (Tables 1 and 2), as well as the IR, UV, and NMR data of guadiscine (**15**) and guadiscidine (**16**), are reported herein. All other known alkaloids, demethoxyguadiscine (**14**),[8] 6,6a-dihydrodemethoxyguadisine (**17**),[8] guatteriopsiscine (**18**),[8] lysicamine (**19**),[18] liriodenine (**20**),[19] and isomoschatoline (**21**),[4] were assigned by spectroscopic data comparison (NMR and MS) with literature values.

Cytotoxic activities of compounds **1**–**4**, **6**–**10**, **12**–**15**, **17**, and **19**–**21** were evaluated against both tumor and normal cells using an Alamar blue assay (Table S1, Supporting Information). Compounds **5**, **11**, and **16** were not tested for their cytotoxic activity due to the small amount of each sample available. Among the compounds tested, liriodenine (**20**) was the most potent, with IC_{50} values of >10, 8.3, 5.5, and 5.0 μM for the B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), HL-60 (human promyelocytic leukemia), and K562 (human chronic myelocytic leukemia) tumor cell lines, respectively. Compound **20** exhibited an IC_{50} value of 34.8 μM for human peripheral blood mononuclear cells. Compound **20** has previously been reported as a cytotoxic agent that binds to DNA and inhibits the action of topoisomerase II.[20−24]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter. UV−vis spectra were recorded on an Agilent HP 8453 spectrophotometer, and IR spectra were measured on a Shimadzu IR Prestige-21 spectrometer. ECD spectra were obtained on a JASCO J-720 spectrometer, and measurements were performed in space, by combining 16 scans at a scanning speed of 50 nm min^{-1} from 100 to 500 nm, with a bandwidth of 1 nm.[25] 1D and 2D NMR experiments were acquired in $CDCl_3$ or in $CDCl_3$ + drops of CD_3OD or CD_3OD at 293 K on a Bruker AVANCE 400 NMR spectrometer operating at 9.4 T (^1H and ^{13}C at 400 and 100 MHz, respectively). The NMR spectrometer was equipped with a 5 mm multinuclear direct detection probe with *z*-gradient. One-bond (HSQC) and long-range (HMBC) $^1H-^{13}C$ NMR correlation experiments were optimized for the average coupling constants, $^1J_{(C,H)}$ and $^{LR}J_{(C,H)}$, of 140 and 8 Hz, respectively. All ^1H and ^{13}C NMR chemical shifts (δ) are presented in ppm relative to the TMS signal at 0.00 ppm as internal reference, and the coupling constants (*J*) are given in Hz. HRESIMS were obtained on a Bruker UltrOTOF-Q mass spectrometer. Column chromatography was performed on silica gel (Merck, 70−230 mesh). TLC analysis (analytical and preparative) was performed using precoated silica gel 60 F254 (0.25 mm, Merck) plates, and spots were visualized by exposure under UV254/365 light, by spraying with *p*-anisaldehyde reagent followed by heating on a plate, or by spraying with Dragendorff's reagent.

Plant Material. The stem bark from a flowering plant of *Guatteria friesiana* was collected in March 2013 on the experimental farm of the Federal University of Amazonas (UFAM), Manaus, Amazonas State, Brazil, and identified by the plant taxonomist Prof. A. C. Webber at the Department of Biology, UFAM. A voucher (number 9282) was deposited in the Herbarium of UFAM. The Institute Chico Mendes for Biodiversity Conservation provided authorization (#25637-1) from the Brazilian Ministry of Environment for the plant collection. This work was performed according to the special authorization for access to genetic resources in Brazil #010240/2013-6, issued by CNPq/MCTI.

Extraction and Isolation. The dried and powdered bark (1900 g) of *G. friesiana* was extracted with *n*-hexane followed by MeOH, to yield a lipid-free MeOH extract (260.6 g). An aliquot of the MeOH extract (250 g) was subjected to an acid−base extraction procedure,[26] giving an alkaloid-containing fraction (5.07 g). This was subjected to 10% $NaHCO_3$-treated silica gel column chromatography (CC),[26] eluted with gradient systems of petroleum ether−CH_2Cl_2 (100:0 to 10:90), CH_2Cl_2−EtOAc (100:0 to 10:90), and EtOAc−MeOH (100:0 to 50:50). The eluted fractions were evaluated and pooled according to TLC analysis, resulting in 21 major fractions. Fraction 3 (51.0 mg), eluted with petroleum ether−CH_2Cl_2 (50:50), was purified by preparative TLC, eluted with petroleum ether−acetone (80:20, 3×), to give **1** (6.0 mg) and **14** (14.4 mg). Fraction 4 (210.2 mg), eluted with petroleum ether−CH_2Cl_2 (40:60), was purified by silica gel CC (treated with 10% $NaHCO_3$ solution), using the same methodology as described above for the initial column chromatography of the alkaloid fraction. Subsequent preparative TLC, eluted with petroleum ether−acetone (80:20, 3×), gave **1** (6.7 mg), **2** (4.8 mg), **3** (6.6 mg), **4** (4.3 mg), **14** (6.7 mg), **15** (6.6 mg), and **17** (18.0 mg). Fraction 5 (343.1 mg), eluted with petroleum ether−CH_2Cl_2 (30:70), was also purified by silica gel CC (treated with 10% $NaHCO_3$ solution), using the same methodology as described above, and subsequent preparative TLC, eluted with petroleum ether−acetone (75:25, 3×), yielded **6** (23.9 mg) and **18** (34.2 mg). Fraction 6 (683.6 mg), eluted with CH_2Cl_2 (100%), was purified by CC (treated with 10% $NaHCO_3$ solution) using this same methodology, and subsequent preparative TLC, eluted with petroleum ether−acetone (70:30, 4×), yielded **5** (8.8 mg), **6** (62.9 mg), **10** (14 mg), **12** (6.2 mg), and **18** (15.3 mg). Fraction 8 (329.4 mg), eluted with CH_2Cl_2−EtOAc (70:30), was also purified by silica gel CC (treated with 10% $NaHCO_3$ solution) using this methodology described above and then by preparative TLC, eluted with petroleum ether−acetone (75:25, 3×), giving **12** (10.0 mg) and **19** (13.6 mg). Additionally, for fraction 8, preparative TLC with petroleum ether−acetone (70:30, 3×, and 75:25, 3×) resulted in the purification of **7** (20 mg), **11** (5.4 mg), **13** (10.5 mg), **16** (3.4 mg), and **20** (9.2 mg). Fraction 10 (320.1 mg), eluted using CH_2Cl_2−EtOAc (50:50, 40:60, 30:70, and 20:80), was purified by preparative TLC, eluted with CH_2Cl_2−MeOH (95:05, 2×), to give **8** (15.0 mg), **9** (8.3 mg), and **20** (69.0 mg). Fraction 15 (367.6 mg), eluted with EtOAc−MeOH (85:15), was purified by silica gel CC (treated with 10% $NaHCO_3$ solution) using this same methodology as described above, followed by preparative TLC, eluted with CH_2Cl_2−MeOH (90:10, 2×), to yield **21** (57 mg).

*3-Methoxy-demethoxyguadiscine (**1**):* yellow oil; UV (MeOH) λ_{max} (log ε) 204 (4.67), 262 (4.50); 296 (3.98), 336 (3.59) nm; IR ν_{max} (film, $CHCl_3$) 2938, 2897, 2844, 1636, 1603, 1571, 1482, 1456, 1418, 1386, 1337, 1293, 1206, 1144, 1094, 1057, 985, 944, 831, 757, 664 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 322.1443 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_3$, 322.1443).

*Guatteriscine (**2**):* yellow oil; UV (MeOH) λ_{max} (log ε) 204 (4.40), 232sh (4.14), 252sh (4.41), 260 (4.49), 291 (3.98), 336 (3.54) nm; IR ν_{max} (film, $CHCl_3$) 2960, 2938, 2844, 1629, 1572, 1469, 1414, 1379, 1351, 1338, 1286, 1199, 1169, 1138, 1118, 1094, 1081, 1073, 1012, 965, 948, 932, 836, 757, 649 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 338.1749 $[M + H]^+$ (calcd for $C_{21}H_{24}NO_3$, 338.1756).

*9-Methoxyguatteriscine (**3**):* light yellow oil; UV (MeOH) λ_{max} (log ε) 204 (4.25), 214sh (4.19), 232sh (4.02), 256sh (4.24), 264 (4.36), 294 (3.91), 344 (3.51) nm; IR ν_{max} (film, $CHCl_3$) 2937, 2837, 1630, 1606, 1575, 1461, 1414, 1382, 1352, 1336, 1294, 1215, 1139, 1092, 1046, 1013, 966, 948, 823, 756, 595 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 368.1862 $[M + H]^+$ (calcd for $C_{22}H_{26}NO_4$, 368.1862).

*3-Methoxyguadiscine (**4**):* light yellow oil; UV (MeOH) λ_{max} (log ε) 204 (4.19), 236sh (3.90), 266 (4.23), 272sh (4.20), 292 (3.80), 302 (3.84), 318sh (3.69), 347 (3.61) nm; IR ν_{max} (film, $CHCl_3$) 2958, 2938, 2840, 1692, 1637, 1603, 1573, 1507, 1479, 1430, 1409, 1384, 1327, 1292, 1213, 1182, 1126, 1057, 987, 948, 823, 756, 663, 596 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 352.1549 $[M + H]^+$ (calcd for $C_{21}H_{22}NO_4$, 352.1548).

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*Guatterfriesine (**5**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 211 (4.07), 236sh (3.95), 256sh (3.87), 289sh (4.11), 298 (4.17), 342 (3.71), 418 (3.35) nm; IR ν_{max} (KBr) 3392, 2972, 2954, 2930, 2854, 1624, 1576, 1460, 1445, 1430, 1413, 1379, 1353, 1304, 1242, 1192, 1147, 1095, 1074, 1032, 982, 944, 902, 842, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 324.1609 $[M + H]^+$ (calcd for $C_{20}H_{21}NO_3 + H^+$, 324.1599).

*9-Methoxyguatterfriesine (**6**):* yellow, needles; UV (MeOH) λ_{max} (log ε) 206 (4.58), 216 (4.56), 233sh (4.38), 258sh (4.65), 265 (4.74), 294 (4.30), 304sh (4.25), 352 (3.78) nm; IR ν_{max} (KBr) 3416, 3005, 2948, 2835, 1626, 1608, 1578, 1463, 1430, 1406, 1355, 1294, 1213, 1143, 1092, 1076, 1045, 983, 858, 829, 756, 733, 594 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 354.1706 $[M + H]^+$ (calcd for $C_{21}H_{24}NO_4$, 354.1705).

*9-Hydroxyguattescine (**7**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 214sh (4.12), 232 (4.16), 289sh (4.06), 301 (4.15), 346 (3.79), 419 (3.45) nm; IR ν_{max} (KBr) 3436, 2988, 2966, 2947, 2931, 2850, 1630, 1611, 1576, 1463, 1416, 1404, 1355, 1339, 1297, 1245, 1213, 1195, 1141, 1095, 1040, 1012, 951, 922, 864, 825, 811, 591 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + drops of CD_3OD) and ^{13}C NMR (100 MHz, $CDCl_3$ + drops of CD_3OD) data, see Tables 1 and 2; HRESIMS *m/z* 354.1707 $[M + H]^+$ (calcd for $C_{20}H_{22}NO_3$, 354.1705).

*9-Hydroxyguatterfriesine (**8**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 206 (4.27), 232sh (4.04), 258sh (4.31), 266 (4.40), 294 (3.96), 304sh (3.91), 354 (3.54) nm; IR ν_{max} (KBr) 3539, 3422, 2967, 2945, 2852, 1630, 1611, 1579, 1462, 1430, 1414, 1355, 1297, 1258, 1215, 1191, 1140, 1085, 1073, 1036, 978, 935, 860, 823, 593, 466 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + drops of CD_3OD) and ^{13}C NMR (100 MHz, $CDCl_3$ + drops of CD_3OD) data, see Tables 1 and 2; HRESIMS *m/z* 340.1544 $[M + H]^+$ (calcd for $C_{20}H_{22}NO_4$, 340.1549).

*4,5-Dehydro-9-methoxyguatterfriesine (**9**):* brown, amorphous powder; UV (MeOH) λ_{max} (log ε) 206 (4.74), 232 (4.60), 258 (4.59), 318sh (4.09), 333 (4.19), 362 (4.20), 366 (4.22), 370sh (4.21) nm; IR ν_{max} (KBr) 3439, 2955, 2936, 2848, 2834, 1610, 1595,1570, 1489, 1453, 1376, 1348, 1293, 1279, 1257, 1199, 1187, 1093, 1044, 983, 956, 917, 884, 834, 795, 729, 688, 605, 554 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 352.1555 $[M + H]^+$ (calcd for $C_{21}H_{22}NO_4$, 352.1549).

*(R)-6,6a-Dihydro-9-methoxyguatterfriesine (**10**):* yellow, amorphous powder; $[\alpha]^{25}_D$ −46.8 (*c* 0.66, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.15), 222sh (4.04), 268sh (3.84), 286 (3.90), 302sh (3.75), 351 (2.65) nm; ECD (*c* 0.66 g/mL, MeOH) λ_{max} ($\Delta\varepsilon$) −22.2 (235); IR ν_{max} (KBr) 3433, 2934; 2836, 1608, 1460, 1430, 1410, 1375, 1353, 1297, 1278, 1212, 1196, 1087, 1070, 1044, 1030, 980, 817, 595 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 3; HRESIMS *m/z* 356.1865 $[M + H]^+$ (calcd for $C_{21}H_{26}NO_4$, 356.1862).

*(R)-4,5,6,6a-Tetrahydromelosmidine (**11**):* yellow, amorphous powder; $[\alpha]^{25}_D$ −4.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.59), 222sh (4.52), 234sh (4.30), 284 (4.35), 298sh (4.24) nm; IR ν_{max} (KBr) 3436, 2976, 2934, 1640, 1610, 1560, 1461, 1418, 1371, 1341, 1297, 1237, 1222, 1085, 1016, 962, 923, 857, 659 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + drops of CD_3OD) and ^{13}C NMR (100 MHz, $CDCl_3$ + drops of CD_3OD) data, see Table 3; HRESIMS *m/z* 356.1866 $[M + H]^+$ (calcd for $C_{21}H_{26}NO_4$, 356.1862).

*9-Demethoxyisoguattouregine (**12**):* green, amorphous powder; $[\alpha]^{25}_D$ −9.1 (*c* 0.7, MeOH) UV (MeOH) λ_{max} (log ε) 204 (3.96), 222sh (3.78), 236sh (3.71), 256sh (3.93), 2.64 (3.99), 292 (3.50), 344 (3.09) nm; IR ν_{max} (KBr) 3411, 2926, 2850, 1640, 1578, 1458, 1417, 1351, 1253, 1217, 1194, 1171, 1138, 1117, 1077, 1034, 983, 953, 921, 848, 760, 664, 638, 570 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 3; HRESIMS *m/z* 326.1392 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_4$, 326.1392).

*3-Methoxyguadiscidine (**13**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 204 (4.37), 234sh (4.12), 266 (4.46), 294 (4.02), 302 (4.02), 352 (3.73) nm; IR ν_{max} (KBr) 3432, 2996, 2946, 2854, 1637, 1603, 1508, 1457, 1423, 1387, 1332, 1300, 1253, 1209, 1062, 987, 954, 820, 654, 602, 576, 530 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + drops of CD_3OD) and ^{13}C NMR (100 MHz, $CDCl_3$ + drops of CD_3OD) data, see Tables 1 and 2; HRESIMS *m/z* 338.1391 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_4$, 338.1392).

*Guadiscine (**15**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 204 (4.08), 230sh (3.91), 264 (4.14), 302 (3.78), 318sh (3.70), 339 (3.58), 352sh (3.54) nm; IR ν_{max} (film, $CHCl_3$) 2925, 2849, 1635, 1608, 1573, 1511, 1459, 1442, 1414, 1380, 1312, 1282, 1250, 1229, 1215, 1186, 1075, 1049, 943, 846, 815, 756, 663, 603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.20 (1H, d 8.6 Hz, H-11), 7.09 (1H, d, 2.6 Hz, H-8), 6.84 (1H, dd, 8.6 and 2.6 Hz, H-10), 6.62 (1H, s, H-3), 6.09 (2H, s, OCH_2O), 3.86 (3H, s, OCH_3-9), 3.64−3.60 (2H, m, H-5), 2.59−2.56 (2H, m, H-4), 1.49 (6H, s, 2× CH_3-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.4 (C-6a), 159.7 (C-9), 149.5 (C-2), 146.6 (C-7a), 141.5 (C-1), 133.0 (C-3a), 128.9 (C-11), 121.3 (C-11a), 117.1 (C-3b), 116.7 (C-1a), 111.4 (C-8), 111.0 (C-10), 106.1 (C-3), 100.9 (OCH_2O), 55.2 (OCH_3-9), 47.1 (C-5), 43.1 (C-7), 27.6 (CH_3-7, 2×), 26.4 (C-4); ESIMS *m/z* 322 $[M + H]^+$.

*Guadiscidine (**16**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 206 (4.25), 230sh (4.06), 266 (4.30), 302 (3.92), 318sh (3.83), 342 (3.73), 352sh (3.71) nm; IR ν_{max} (KBR) 3422, 2960, 2922, 2849, 1636, 1599, 1513, 1460, 1420, 1399, 1383, 1336, 1286, 1249, 1226, 1214, 1073, 1049, 948, 828, 754, 665, 594 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + drops of CD_3OD) δ_H 8.12 (1H, d, 8.5 Hz, H-11), 7.03 (1H, d, 2.5 Hz, H-8), 6.78 (1H, dd, 8.5 and 2.5 Hz, H-10), 6.62 (1H, s, H-3), 6.10 (2H, s, OCH_2O), 3.57−3.54 (2H, m, H-5), 2.61−2.57 (2H, m, H-4), 1.46 (6H, s, 2x CH_3-7); ^{13}C NMR (100 MHz, $CDCl_3$ + drops of CD_3OD) δ_C 172.3 (C-6a), 157.6 (C-9), 149.9 (C-2), 146.2 (C-7a), 141.7 (C-1), 133.0 (C-3a), 128.6 (C-11), 120.0 (C-11a), 117.0 (C-3b), 117.3 (C-1a), 111.7 (C-8), 113.2 (C-10), 105.5 (C-3), 100.8 (OCH_2O), 46.3 (C-5), 43.1 (C-7), 27.4 (CH_3-7, 2×), 26.1 (C-4); ESIMS *m/z* 308 $[M + H]^+$.

Cytotoxicity Assay. The B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), K562 (human chronic myelocytic leukemia), and HL-60 (human promyelocytic leukemia) tumor cell lines were kindly donated by Hospital A.C. Camargo, São Paulo, SP, Brazil. All cell lines were tested for mycoplasma with a mycoplasma stain kit (Sigma-Aldrich) and found to be free from contamination. To obtain normal cells, heparinized blood (from healthy, 20−35-year-old, nonsmoker donors who had not taken any drug at least 15 days prior to sampling) was collected, and peripheral blood mononuclear cells were isolated by a standard protocol using Ficoll (GE Ficoll-Paque Plus) density gradient centrifugation. ConA (10 μg/mL) was added at the beginning of culture, and, after 24 h, cells were treated with the test compound. The Research Ethics Committee of the Oswaldo Cruz Foundation (Salvador, Bahia, Brazil) approved the experimental protocol (number 031019/2013).

Cell viability was quantified by the Alamar blue method, as previously described[27] with minor modifications.[28] The positive control was doxorubicin (IC_{50} 2.3 μM against B16-F10, 0.2 μM against HepG2, 0.7 μM against K562, and 0.8 against HL-60 cells).

Statistical Analyses. Data are presented as half maximal inhibitory concentration (IC_{50}) values obtained by nonlinear regression. All statistical analyses were performed using the GraphPad program (Intuitive Software for Science, San Diego, CA, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b01037.

ECD, 1D NMR, 2D NMR, and HRESIMS of compounds **1**−**13** (PDF)

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Notes

The authors declare no competing financial interest.

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