Sucutiniranes C−F, Cassane-Type Diterpenes from *Bowdichia nitida*

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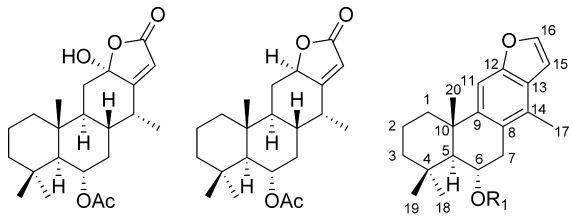
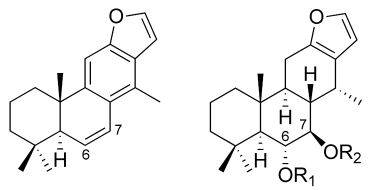
Four new cassane-type diterpenes, sucutiniranes C−F (**3**−**6**), have been isolated from seeds of *Bowdichia nitida*, and their structures were elucidated by using 2D NMR data, chemical correlations, and X-ray analysis. Sucutiniranes E (**5**) and F (**6**) were moderately cytotoxic against human blood premyelocytic leukemia (HL-60), breast adenocarcinoma (MCF-7), and colon cancer (HCT-116) cells.

Bowdichia nitida Spruce ex Benth. (Leguminosae), common name "sucupira", is found in tropical regions of South America.[1] The seeds, which contain alkaloids, triterpenes, isoflavonoids, benzofuranes, and benzopyranes, are used for treatment of rheumatic, antipyretic, and gouty conditions.[2−4] We recently isolated two cassane-type diterpenes, sucutiniranes A (**1**) and B (**2**), and 6α,7β-diacetoxyvouacapane, which showed antiplasmodial activity, from the seeds of *B. nitida*.[5] Our efforts to identify additional diterpenes with biological activity from *B. nitida* led to the isolation of new diterpenes **3**−**6**. This paper describes the structure elucidation of **3**−**6** and their cytotoxic activity against three human cancer cell lines.



1 **2** **3**: R_1=Ac; **7**: R_1=H



4 **5**: R_1=Ac, R_2=H; **6**: R_1=H, R_2=Ac; **8**: R_1=H, R_2=H; **9**: R_1=H, R_2=*p*-Br-Bz

Seeds of *B. nitida* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% aqueous tartaric acid. The EtOAc-soluble materials were subjected to silica gel column chromatography (hexane/EtOAc and $CHCl_3$/MeOH) and ODS column chromatography (MeOH/H_2O), followed by ODS HPLC (MeOH/H_2O), to afford **3** (0.0002%), **4** (0.0001%), **5** (0.01%), and **6** (0.003%), together with sucutiniranes A (**1**) and B (**2**),[5] 6α-acetoxyvouacapane,[6] and 6α,7β-diacetoxyvouacapane.[7]

Compound **3**, $[\alpha]_D$ +36 (*c* 0.2, $CHCl_3$), showed a pseudomolecular ion peak at *m*/*z* 363 $(M + Na)^+$ in the ESIMS, and the molecular formula $C_{22}H_{28}O_3$ was established by HRESITOFMS [*m*/*z* 363.1957 $(M + Na)^+$]. IR absorptions implied the presence of an ester carbonyl (1735 cm^{-1}) functionality. ^1H NMR data (Table 1) showed the presence of an aromatic ring, a furan ring, an acetyl, and four methyl groups. The ^{13}C NMR data (Table 2) revealed 22 carbon signals due to five sp^2 quaternary carbons, three sp^2 methines, one ester carbonyl, two sp^3 quaternary carbons, two sp^3 methines, four sp^3 methylenes, and five methyl groups.

Partial structures C-1 to C-3 (**a**), C-5 to C-7 (**b**), and C-15 to C-16 (**c**) were deduced from analysis of the $^1H-^1H$ COSY spectrum of **3**. The presence of an α,β-disubstituted furan ring was substantiated by the signals of two sp^2 methines (δ_H 6.73 and 7.55; δ_C 105.1 and 144.2) and two sp^2 quaternary carbons (δ_C 125.5 and 153.7). The HMBC cross-peaks of H-15 and H-16 to C-12 and of H-11, H-16, and H_3-17 to C-13 indicated that the furan ring was connected to a benzene ring through C-12 and C-13 with a methyl group at C-14. The HMBC cross-peaks of H-11 and H_3-17 to C-8, H-5 and H_3-20 to C-9, H_3-18 and H_3-20 to C-5, H_3-19 to C-3, and H_3-20 to C-1 indicated the connection among partial structures **a** and **b** and a benzene ring through C-8 and C-9. The placement of an acetoxy group was deduced to be at C-6 by the observation of an HMBC correlation between H-6 and C-21 as well as H_3-22 and C-21. Thus, **3** had a cassane-type skeleton with the methyl group at C-14, an acetoxy group at C-6, and the furan ring at C-12 and C-13.

The relative configuration of **3** was elucidated by NOESY correlations. NOESY correlations of H-6/H_3-19 and H_3-20, H-5/H_3-18, and H-1/H-11 were observed, indicating that H-5 was α-oriented and H-6, H_3-19, and H_3-20 were β-oriented. The ^3J coupling constant (9.5 Hz) supported the antiperiplanar relationship between H-5 and H-6. To determine the absolute configuration at C-6, **3** was converted into its (*S*)- and (*R*)-MTPA esters of deacetyl derivative **7** prepared from **3** by $LiAlH_4$. The values of $\Delta\delta$ obtained from the ^1H NMR spectra indicated that the absolute configuration of **3** at C-6 was *S*.[8] Thus, the structure of **3** was assigned as shown, and it was named sucutinirane C.

Compound **4**, $[\alpha]_D$ +30 (*c* 0.1, $CHCl_3$), showed a pseudomolecular ion peak at *m*/*z* 303 $(M + Na)^+$ in the ESIMS, and the molecular formula $C_{20}H_{24}O$ was established by HRESITOFMS [*m*/*z* 303.1757 $(M + Na)^+$], smaller than **3** by a $C_2H_4O_2$ unit. ^1H and ^{13}C NMR data of **4** (Tables 1 and 2) were analogous to those of **3** with a cassane-type skeleton, although NMR signals due to an acetoxy group were present for **3** and, instead, signals due to a double bond (δ_H 6.02 and 6.86; δ_C 128.8 and 124.3) were observed for **4**. The gross structure of **4** was elucidated by 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC) data, and the relative configuration was assigned as shown. Treatment of the deacetyl derivative **7** with *p*-toluenesulfonic acid afforded a dehydro derivative, whose spectroscopic data and specific rotation were identical with **4**,

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Table 1. ^{1}H NMR (400 MHz) Data (J, Hz) of Compounds **3**−**6** in $CDCl_3$ at 300 K[a]

	3	4	5	6
1a	1.61 (1H, m)	1.67 (1H, m)	1.00 (1H, m)	1.00 (1H, m)
1b	2.27 (1H, m)	2.22 (1H, m)	1.68 (1H, m)	1.70 (1H, m)
2a	1.70 (1H, m)	1.75 (1H, m)	1.43 (1H, m)	1.47 (1H, m)
2b	1.80 (1H, m)	1.80 (1H, m)	1.50 (1H, m)	1.52 (1H, m)
3a	1.26 (1H, m)	1.24 (1H, m)	1.21 (1H, m)	1.24 (1H, m)
3b	1.49 (1H, m)	1.53 (1H, m)	1.36 (1H, m)	1.39 (1H, m)
5	1.53 (1H, d, 9.5)	2.11 (1H, t, 3.0)	1.24 (1H, m)	1.06 (1H, m)
6	5.54 (1H, ddd, 9.5, 6.4, 3.2)	6.02 (1H, dd, 10.0, 3.0)	5.22 (1H, dd, 7.8, 9.5)	3.86 (1H, dd, 9.5, 9.5)
7a	3.01 (1H, dd, 17.1, 3,2)	6.86 (1H, dd, 10.0, 3.0)	3.44 (1H, dd, 8.3, 8.3)	4.90 (1H, dd, 9.5, 9.5)
7b	3.24 (1H, dd, 17.1, 6.4)			
8			1.87 (1H, ddd, 9.8, 9.8, 4.5)	2.07 (1H, m)
9			1.50 (1H, m)	1.58 (1H, m)
11a	7.29 (1H, s)	7.21 (1H, s)	2.31 (1H, dd, 13.8, 8.6)	2.35 (1H, dd, 16.6, 9.4)
11b			2.56 (1H, dd, 13.8,5.5)	2.61 (1H, dd, 16.6, 6.6)
14			3.01 (1H, m)	2.78 (1H, m)
15	6.73 (1H, d, 1.8)	6.74 (1H, d, 2.2)	6.17 (1H, s)	6.18 (1H, s)
16	7.55 (1H, d, 1.8)	7.53 (1H, d, 2.2)	7.20 (1H, s)	7.22 (1H, s)
17	2.39 (3H, s)	2.49 (3H, s)	1.03 (3H, d, 6.0)	1.05 (3H, d, 7.1)
18	0.92 (3H, s)	0.99 (3H, s)	1.02 (3H, s)	1.21 (3H, s)
19	1.10 (3H, s)	1.08 (3H, s)	0.92 (3H, s)	1.09 (3H, s)
20	1.24 (3H, s)	1.08 (3H, s)	1.00 (3H, s)	0.99 (3H, s)
22	2.01 (3H, s)		2.12 (3H, s)	2.14 (3H, s)

[a] δ in ppm.

Table 2. ^{13}C NMR (100 MHz) Data of Compounds **3**−**6** in $CDCl_3$ at 300 K[a]

	3	4	5	6
1	39.7	36.7	39.7	39.9
2	19.0	19.1	18.2	18.3
3	42.4	41.0	43.4	43.7
4	34.1	32.8	32.9	33.5
5	53.5	50.5	54.5	56.4
6	77.8	128.8	76.3	73.5
7	32.3	124.3	75.7	79.5
8	125.8	126.2	43.0	40.5
9	147.1	147.0	43.2	43.2
10	38.5	38.9	38.5	37.9
11	103.3	103.0	22.4	22.5
12	153.7	154.4	148.8	148.7
13	125.5	126.1	121.8	121.5
14	127.8	125.3	27.3	27.5
15	105.1	105.4	109.6	109.3
16	144.2	144.1	140.6	140.7
17	15.9	15.4	16.8	17.0
18	33.6	32.6	36.2	36.6
19	22.7	22.5	22.5	22.2
20	22.4	20.6	15.6	15.6
21	170.9		172.1	172.3
22	21.8		21.9	21.0

[a] δ in ppm.

confirming the absolute configuration. Thus, the structure of **4** was assigned as shown, and it was named sucutinirane D.

Compound **5**, $[\alpha]_D$ +49 (c 0.1, $CHCl_3$), was revealed to have the molecular formula $C_{22}H_{32}O_4$ by HRESITOFMS [m/z 383.2195 $(M + Na)^+$]. IR absorptions implied the presence of OH (3450 cm^{-1}) and ester carbonyl (1720 cm^{-1}) groups. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) and 2D NMR correlations indicated that **5** had the same cassane-type skeleton as that of sucutinirane A,[5] except for the presence of furan (δ_H 6.17 and 7.20, δ_C 109.6, 121.8, 140.6, and 148.8) and an OH (δ_H 3.44, δ_C 75.7) group. The relative configuration of **5** was deduced from the NOESY spectrum and ^3J coupling constants. α-Orientation of H-5, H-9, and H_3-17 and β-orientation of H-6, H-8, H_3-19, and H_3-20 were indicated by NOESY cross-peaks of H-5/H-9, H-7/H_3-17, H-6/H-8 and H_3-19, and H-8/H_3-20. The coupling constants $^3J_{H5/H6} = 9.5$ and $^3J_{H6/H7} = 9.5$ Hz supported an antiperiplanar conformation among H-5, H-6, and H-7. Thus, the structure of **5** was assigned as shown, and it was named sucutinirane E.

The molecular formula, $C_{22}H_{32}O_4$, of **6**, $[\alpha]_D$ +29 (c 1.0, $CHCl_3$), was established by HRESITOFMS [m/z 383.2192 $(M + Na)^+$], which was the same as that of **5**. Analyses of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra of **6** indicated that it was a stereoisomer of **5** with an OH at C-6 and an acetoxy group at C-7, and it was named sucutinirane F. Reduction of **5** and **6** with $LiAlH_4$ gave the same deacetyl derivative (**8**), followed by esterification with *p*-bromobenzoyl chloride to give a 7-*p*-bromobenzoate of **8** (**9**) as colorless needles. The absolute configurations of **5** and **6** were elucidated by the single-crystal X-ray diffraction analysis of **9**.

The effects of sucutiniranes C−F (**3**−**6**) and sucutiniranes A (**1**) and B (**2**) on cancer cell growth were examined. Sucutiniranes E (**5**) and F (**6**) showed moderate cytotoxicity against three cancer cell lines: human blood premyelocytic leukemia (HL-60) with IC_{50} values of 12 and 7.5 μM, respectively, breast adenocarcinoma (MCF-7) with IC_{50} values of 35 and 18 μM, respectively, and colon cancer (HCT-116) cells with IC_{50} values of 36 and 30 μM, respectively. Sucutiniranes C (**3**) and D (**4**) were inactive against three cancer cell lines at 50 μM. Additionally, sucutiniranes A (**1**) and B (**2**) were cytotoxic only against HL-60, with IC_{50} values of 14 and 32 μM, respectively. None of **3**−**6** showed antiplasmodial activity, as in a previous paper.[5]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 polarimeter. UV spectra were recorded on a Shimadzu UV-250 spectrophotometer, and IR spectra on a JASCO FTIR-230 spectrometer. Mass spectra were obtained with a Micromass LCT spectrometer. ^{1}H and 2D NMR spectra were recorded on a 400 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 100 MHz spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AV 400 spectrometer, and chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. X-ray analysis were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite-monochromated Cu Kα radiation.

Material. A voucher specimen of the seeds of *Bowdichia nitida* was identified by Dr. Chieko Hirobe, Seisen University, Tokyo, Japan, and a voucher specimen (no. 261205) was deposited at the herbarium of Hoshi University, Tokyo, Japan.

Extraction and Isolation. The seeds of *B. nitida* (1 kg) were extracted with MeOH to give 300 g of dried extract. A part of the MeOH extract (12 g) was treated with 3% aqueous tartaric acid (pH 2) and then partitioned with EtOAc. The EtOAc fraction (10 g) was

purified by silica gel column chromatography (CC) (hexane/EtOAc 1:0 → 0 → 1 and $CHCl_3$/MeOH 1:0 → 0 → 1) and ODS CC (MeOH/H_2O 0:1 → 1:0), followed by ODS HPLC (MeOH/H_2O, 7:3; UV detection at 254 nm) to afford **3** (2.0 mg, 0.0002%), **4** (1.0 mg, 0.0001%), **5** (140.0 mg, 0.01%), and **6** (33.0 mg, 0.003%), together with sucutiniranes A (**1**) and B (**2**),[5] 6α-acetoxyvouacapane,[6] and 6α,7β-diacetoxyvouacapane.[7]

Sucutinirane C (3): colorless solid; $[\alpha]_D$ +36 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 2930 and 1735 cm^{-1}; ^1H and ^{13}C NMR data (Tables 1 and 2); ESIMS *m/z* 363 $(M + Na)^+$; HRESITOFMS *m/z* 363.1957 $[(M + Na)^+]$ (calcd for $C_{22}H_{28}O_3Na$, 363.1936).

Sucutinirane D (4): colorless solid; $[\alpha]_D$ +30 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 2930 cm^{-1}; ^1H and ^{13}C NMR data (Tables 1 and 2); ESIMS *m/z* 303 $(M + Na)^+$; HRESITOFMS *m/z* 303.1757 $[(M + Na)^+]$ (calcd for $C_{20}H_{24}ONa$, 303.1725).

Sucutinirane E (5): colorless solid; $[\alpha]_D$ +49 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3450, 2930, and 1720 cm^{-1}; ^1H and ^{13}C NMR data (Tables 1 and 2); ESIMS *m/z* 383 $(M + Na)^+$; HRESITOFMS *m/z* 383.2195 $[(M + Na)^+]$ (calcd for $C_{22}H_{32}O_4Na$, 383.2198).

Sucutinirane F (6): colorless solid; $[\alpha]_D$ +29 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3450, 2930, and 1740 cm^{-1}; ^1H and ^{13}C NMR data (Tables 1 and 2); ESIMS *m/z* 383 $(M + Na)^+$; HRESITOFMS *m/z* 383.2192 $[(M + Na)^+]$ (calcd for $C_{22}H_{32}O_4Na$, 383.2198).

Conversion of Sucutinirane C (3) to Deacetyl Derivative (7). $LiAlH_4$ (2.0 mg) was added to a solution of **3** (10 mg) in dry ether (0.4 mL) at 0 °C, and the mixture was warmed to room temperature. After 1 h, ice cold water was added to the reaction mixture and extracted with ethyl acetate. After evaporation, the residue was applied to a silica gel column (hexane/EtOAc, 30:1) to give compound (**7**, 6.0 mg): colorless oil; IR (KBr) ν_{max} 3450 and 2880 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.40 (m, H-6), 3.01 (dd, 4.0, 16.8, H-7), 3.32 (dd, 6.3, 16.5, H-7), 7.28 (s, H-11), 6.74 (d, 2.2, H-15), 7.54 (d, 2.2, H-16), 2.54 (s, H-17), 1.15 (s, H-18), 1.12 (s, H-19), 1.22 (s, H-20); ESIMS *m/z* 299 $(M + H)^+$.

(*R*)- and (*S*)-MTPA Esters of 7. To a solution of **7** (1.0 mg) in CH_2Cl_2 (0.25 mL) was added (*R*)-(−)-MTPA chloride or (*S*)-(+)-MTPA chloride (0.8 mL) and *N*,*N*-dimethylaminopyridine (0.5 mg). The mixture was allowed to stand at 40 °C for 30 min. After addition of ice cold water and evaporation of solvent, the residue was passed through a silica gel column (hexane/EtOAc, 20:1) to afford (*S*)-MTPA or (*R*)-MTPA ester of **7** (each 0.7 mg). (*S*)-MTPA ester: colorless oil; ^1H NMR ($CDCl_3$) δ 5.76 (m, H-6), 2.98 (dd, 2.7, 16.9, H-7), 3.26 (dd, 6.2, 17.4, H-7), 7.24 (s, H-11), 6.69 (d, 2.2, H-15), 7.55 (d, 2.2, H-16), 2.18 (s, H-17), 1.08 (s, H-18), 0.73 (s, H-19), 1.23 (s, H-20); ESIMS *m/z* 537 $(M + Na)^+$. (*R*)-MTPA ester: colorless oil; ^1H NMR ($CDCl_3$) δ 5.65 (m, H-6), 3.25 (d, 3.8, H-7), 7.27 (s, H-11), 6.73 (d, 2.2, H-15), 7.58 (d, 2.2, H-16), 2.28 (s, H-17), 1.01 (s, H-18), 0.58 (s, H-19), 1.23 (s, H-20); ESIMS *m/z* 537 $(M + Na)^+$.

Conversion of 7 to 4. To a solution of **7** (5.0 mg) in benzene (0.4 mL) was added *p*-toluenesulfonic acid (1.0 mg), and the mixture was kept at 60 °C for 1 h. The mixture was poured into a saturated aqueous solution of $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the residue was applied to a silica gel column (hexane) to give a compound (1.0 mg) whose spectroscopic data were identical with those of **4**.

Conversion of 5 and 6 to Deacetyl Derivative 8. $LiAlH_4$ (5 mg) was added to a solution of **5** (10 mg) in dry ether (0.3 mL), and the mixture was kept at room temperature for 1 h. Ice cold water was added to the mixture and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the residue was applied to a silica gel column (hexane/EtOAc, 7:3) to give **8** (7 mg), whose spectroscopic data and $[\alpha]_D$ value were identical with those of **6**: colorless powder; $[\alpha]_D$ +94 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3420 and 2880 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.77 (m, H-6), 3.39 (m, H-7), 2.34 (dd, 10.5, 16.6, H-11), 2.60 (dd, 6.6, 16.6, H-11), 3.07 (m, H-14), 6.21 (s, H-15), 7.23 (s, H-16), 1.08 (d, 7.1, H-17), 1.10 (s, H-18), 1.19 (s, H-19), 0.98 (s, H-20); ESIMS *m/z* 319 $(M + H)^+$.

Conversion of 8 to Its *p*-Bromobenzoate Derivative (9). A solution of **8** (10 mg), *p*-bromobenzoyl chloride (48 mg), and *N*,*N*-dimethylaminopyridine (5 mg) in $CHCl_3$ (1.0 mL) were heated at 60 °C for 5 h. The reaction was stopped by ice cold water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous Na_2CO_3 and brine and dried over Na_2SO_4. After removal of the solvent, the residue was applied to a silica gel column (hexane/EtOAc, 30:1 → 15:1) to give to give 6-*p*-bromobenzoate (1.4 mg), 7-*p*-bromobenzoate (8.5 mg), and 6,7-di-*p*-bromobenzoate (0.5 mg). 7-*p*-Bromobenzoate of **8** (**9**): colorless needles; $[\alpha]_D$ +53 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3440, 2880, and 1710 cm^{-1}; ^1H NMR data ($CDCl_3$) δ 4.03 (dd, 9.8, 9.8, H-6), 5.16 (dd, 9.8, 9.8, H-7), 2.34 (dd, 10.4, 16.5, H-11), 2.60 (dd, 6.5, 16.5, H-11), 2.84 (m, H-14), 6.17 (s, H-15), 7.24 (s, H-16), 1.10 (d, 7.1, H-17), 1.24 (s, H-18), 1.12 (s, H-19), 1.07 (s, H-20), 7.96 (d, 7.4, H-2' and H-6′), 7.61(d, 7.4, H-3′ and H-5′); ESIMS *m/z* 501 $(M + H)^+$; HRESITOFMS *m/z* 501.1640 $[(M + H)^+]$ (calcd for $C_{27}H_{34}O_4Br$, 501.1635). 6-*p*-Bromobenzoate of **8**: $[\alpha]_D$ +45 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3700 and 1720 cm^{-1}; ^1H NMR data ($CDCl_3$) δ 3.62 (dd, 9.8, 9.8, H-6), 5.59 (dd, 10.0, 10.5, H-7), 2.40 (dd, 10.6, 16.5, H-11), 2.65 (dd, 6.2, 16.5, H-11), 3.12 (m, H-14), 6.23 (s, H-15), 7.26 (s, H-16), 1.09−1.11 (d, overlapped, H-17), 1.28 (s, H-18), 0.98 (s, H-19), 1.05 (s, H-20), 7.97 (d, 7.5, H-2′ and H-6′), 7.61(d, 7.5, H-3′ and H-5′). 6,7-Di-*p*-bromobenzoate of **8**: $[\alpha]_D$ −31 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 2370 and 1730 cm^{-1}; ^1H NMR data ($CDCl_3$) δ 5.46 (dd, 9.8, 9.8, H-6), 5.81 (dd, 10.1, 10.1, H-7), 6.15 (s, H-15), 7.24 (s, H-16), 1.17 (d, 6.4, H-17), 1.21 (s, H-18), 1.00 (s, H-19), 1.00 (s, H-20), 7.65−7.72 (d, 7.7, H-2′ and H-6′), 7.45−7.49 (d, 7.7, H-3′ and H-5′).

X-ray Analysis of 9. *p*-Bromobenzoate of **8** (**9**) was crystallized from MeOH/$CHCl_3$ to give colorless needles (mp 164−167 °C). Crystal data: $C_{27}H_{33}BrO_4$, space group $P2_12_12_1$ (#19), a = 10.04377(18) Å, b = 12.1650(2) Å, c = 19.2375(7) Å, V = 2350.48(10) $Å^3$, Z = 4, D_{calc} = 1.417 g/cm^3, Cu Kα radiation (λ = 1.54187 Å), T −180(1) °C. The structure was solved by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on F^2 was based on 4274 observed reflections and converged with unweighted and weighted agreement factors of R1 = 0.0198 [$I > 2.00\sigma(I)$] and wR2 = 0.0502. The absolute configuration was determined based on a Flack parameter of −0.022(9),[9] refined using 1834 Friedel pairs. Complete crystallographic data of **9** have been deposited in the Cambridge Crystallographic Data Centre (CCDC 725108).[10]

Cytotoxic Activity. HL-60 (human blood premyelocytic leukemia), HCT-116 (colon cancer), and MCF-7 (breast adenocarcinoma) cells were used. Each cell line was seeded onto 96-well microtiter plates at 1×10^4 and 5×10^3 cells per well for HL-60, and HCT-116 and MCF-7, respectively. Cells were preincubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. Different concentrations of each compound (10 μL) were added to the cultures, and then the cells were incubated at 37 °C for 48 h. On the third day, 15 μL of MTT solution (5 mg/mL) was added into each well of the cultured medium. After a further 2 h of incubation, 100 μL of 10% SDS/0.01 N HCl solution was added to each well, and the formazan crystals in each well were dissolved by stirring with a pipet. Optical density measurements were made using a micropipet reader (Benchmark Plus microplate spectrometer, BIO-RAD) equipped with a two-wavelength system (550 and 700 nm). In each experiment, three replicate wells were prepared for each sample. The ratio of living cells was determined on the basis of the difference of the absorbance between those of samples and controls. These differences are expressed in percentage, and cytotoxic activity was indicated as an IC_{50} value.

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Supporting Information Available: Selected 2D NMR correlations for **3** and **5** and an ORTEP drawing and CIF file of **9** are available free of charge via the Internet at http://pubs.acs.org.

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(10) CCDC 725108 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/deposit, or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

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