Cytotoxic Dolabellane Diterpenes from the Formosan Soft Coral *Clavularia inflata*

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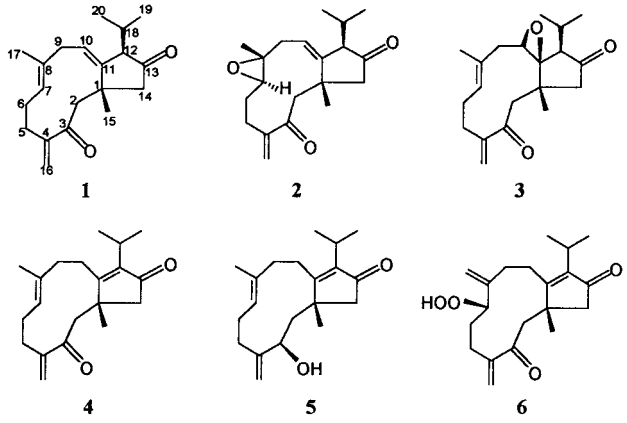
Six new cytotoxic dolabellane diterpenes, (1*R**,12*R**)-dolabella-4(16),7,10-triene-3,13-dione (**1**), (1*R**,7*R**,8*S**,-12*R**)-dolabella-4(16),10-diene-7,8-epoxy- 3,13-dione (**2**), (1*R**,10*R**,11*S**,12*R**)-dolabella-4(16),7-diene-10,11-epoxy-3,13-dione (**3**), (1*R**)-dolabella-4(16),7,11(12)-triene-3,13-dione (**4**), (1*R**,3*R**)-3-hydroxydolabella-4(16),7,11(12)-triene-3,13-dione (**5**), and (1*R**,7*R**)-7-hydroperoxydolabella-4(16),8(17),11(12)-triene-3,13-dione (**6**), have been isolated from the Formosan soft coral *Clavularia inflata*. The structures of compounds **1**−**6** were determined by 1D and 2D spectral analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

Diterpenes with the dolabellane skeleton were originally isolated from the herbivorous sea hare *Dolabella californica*[1] and subsequently from the brown algae *Glossophora galapagensis*,[2] upon which the sea hare feeds, *Dictyota dichotoma*,[3−5] *D. pardarlis*,[6−9] and *Dilophus fasciola*.[10] They have also been isolated from the sea whips *Eunicea calyculata*,[11] *E. laciniata*,[12,13] and *E. tourneforti*,[14] from the mollusc *Aplysia dactylomela*,[15] from the liverworts *Odontoschisma denudatum*,[16] *Barbilophozia floerkei, B. lycopodioides, B. attenuata*,[17] from the higher plant *Chrozophora oblique*,[18] and from the soft corals of the genus *Clavularia*.[19,20]

As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Clavularia inflata* Schenk (Stolonifera) was studied because CH_2Cl_2 extracts showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[21,22] Bioassay-guided fractionation resulted in the isolation of six new cytotoxic dolabellane diterpenes, (1*R**,12*R**)-dolabella-4(16),7,10-triene-3,13-dione (**1**), (1*R**,7*R**,8*S**,12*R**)-dolabella-4(16),10-diene-7,8-epoxy-3,13-dione (**2**), (1*R**,10*R**,11*S**,12*R**)-dolabella-4(16),7-diene-10,11-epoxy-3,13- dione (**3**), (1*R**)-dolabella-4(16),7,11-(12)-triene-3,13-dione (**4**), (1*R**,3*R**)-3-hydroxydolabella-4(16),7,11(12)-triene-3,13-dione (**5**), and (1*R**,7*R**)-7-hydroperoxydolabella-4(16),8(17),11(12)-triene-3,13-dione (**6**).



Results and Discussion

Compound **1** was isolated as a colorless oil. HREIMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **1** as $C_{20}H_{28}O_2$. Thus, seven degrees of unsaturation were determined for **1**. The IR spectrum of **1** indicated the presence of an α,β-unsaturated ketone (ν_{max} 1685 cm^{-1}), a nonconjugated ketone (ν_{max} 1729 cm^{-1}), and an *exo*-methylene at 1617 cm^{-1}. A strong UV absorption at λ_{max} 226 nm suggested the presence of an α,β-unsaturated ketone. The presence of eight sp^2-hybridized carbon atoms in the molecule, as deduced from the ^{13}C and DEPT NMR spectra (Table 1), corresponding to three carbon−carbon double bonds and two carbon−oxygen double bonds, as the only multiple bonds, indicated compound **1** to be bicyclic. The ^{13}C NMR singlet at δ 135.6 and a doublet at δ125.2 that was correlated in the HMBC experiment (Figure 1) with the ^1H NMR signal at δ 4.89 (t, J = 5.1 Hz, 1H) together with the vinylic methyl signals at δ 1.58 (br s, 3H) in the ^1H NMR spectrum and at δ 16.8 (q) in the ^{13}C NMR spectrum were assigned to an *E*-trisubstituted double bond bearing a methyl group.[19] HMQC correlation of δ_H 5.34 (dt, 1H) with δ_C 123.7 (d) and HMBC correlation of δ_H 5.34 (dt, 1H) with δ_C 146.0 (s) indicated the presence of the other trisubstituted double bond. HMQC correlation of δ_H 5.71 (s, 1H), 5.77 (s, 1H) with δ_C 124.2 (t) as well as HMBC correlation of δ_H 5.71 (s, 1H), 5.77 (s, 1H) with δ_C 151.5 (s) and 201.6 (s) indicated that **1** contained a keto function α to an exocyclic methylene. The doublets at δ_H 1.11 (3H) and 0.92 (3H) showed HMBC correlations with δ_C 34.1 (d), indicating the presence of an isopropyl group. A tertiary methyl group (δ_H 1.36 s; δ_C 28.9 q) was located at a bridgehead position based on HMBC correlations between δ_H 1.36 and δ_C 42.1 (s) and 146.0 (s). Measurement of the $^{13}C-^{13}C$ homonuclear shift correlation 2D spectrum (INADEQUATE) (Figure 2) of **1** together with COSY, HMQC, and HMBC experiments established its chemical structure and enabled also the assignment of all resonances in the NMR spectra. The relative stereochemistry of **1** was

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Table 1. ^{1}H NMR Chemical Shifts of Diterpenes **1**−**3**

proton	**1**[a]	**2**[b]	**3**[b]
2	3.26 d (16.4)[c]	3.53 d (15.8)	3.02 d (16.4)
	2.61 d (16.4)	2.58 d (15.8)	2.63 d (16.4)
5α	2.87 m	2.98 m	2.96 m
5*β*	2.03 m	2.19 s	2.06 m
6α	2.11 m	2.12 m	1.69 m
6*β*	2.13 m	1.22 m	1.69 m
7	4.89 t (5.1)	2.40 d (10.8)	5.03 t (6.5)
9α	2.79 d (12.1)	1.71 d (12.0)	1.79 m
9*β*	2.40 dd (12.1, 3.5)	2.57 dd (12.0, 3.4)	2.37 d (12.6)
10	5.34 dt (2.7, 12.1)	5.38 dt (12.6, 3.0)	2.77 d (10.5)
12	2.99 br d (2.4)	2.74 br d (2.3)	2.53 br s
14α	2.70 d (17.4)	2.78 d (17.8)	2.69 d (18.0)
14*β*	2.32 d (17.4)	2.37 d (17.8)	2.32 d (18.0)
15	1.36 s	1.42 s	1.02 s
16	5.71 s	5.88 s	5.75 s
	5.77 s	6.06 s	5.79 s
17	1.58 br s	1.22 s	1.66 br s
18	1.92 m	1.89 m	1.81 m
19	1.11 d (6.9)	1.07 d (6.9)	1.22 d (6.9)
20	0.92 d (6.9)	0.91 d (6.9)	0.92 d (6.9)

[a] Spectrum recorded at 400 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments. [c]*J* values (in Hz) in parentheses.

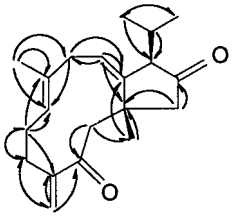

Figure 1. HMBC correlations of **1**.

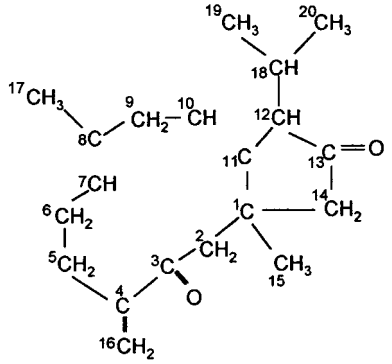

Figure 2. 2D INADEQUATE correlations of **1**.

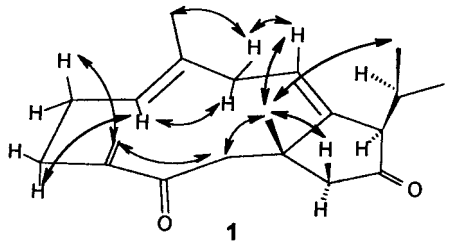

Figure 3. Preferred conformations for **1**. NOE (↔).

deduced from a 2D NOESY experiment (Figure 3), which indicated that the bridgehead methyl at C-1, the methyl at the *E*-trisubstituted olefin, the olefinic proton at C-10, and the *exo*-methylene at C-4 are on one side of the molecule, while H-7, H-12, H-5α, and H-6α are on the opposite side of the molecule. From the aforementioned data, **1** can be formulated as (1*R**,12*R**)-dolabella-4(16),7,-10-triene-3,13-dione.

Compound **2** was isolated as a colorless oil, whose molecular formula, $C_{20}H_{28}O_3$, was revealed by HREIMS and NMR spectra. The IR spectrum of **2** suggested the presence of an α,*β*-unsaturated ketone group (ν_{max} 1686 cm^{-1}), a nonconjugated ketone (ν_{max} 1730 cm^{-1}), and an *exo*-methylene at 1616 cm^{-1}. A strong UV absorption at λ_{max} 233 nm also suggested the presence of an α,*β*-unsaturated ketone functionality. The NMR features of compound **2** were analogous to those of compound **1** with the exception that the resonances for the methyl-bearing *E*-trisubstituted olefin were replaced by those of methyl-bearing *E*-trisubstituted epoxide[19] (δ_H 1.22 s, 2.40 d; δ_C 17.2 q, 61.0 d, 62.1 s). Cross-peaks in the ^{1}H−^{1}H COSY spectrum showed couplings between the epoxide methine proton at δ 2.40 (d, H-7) and methylene proton at δ 1.22 (m, 1H, H-6*β*) and from H-6*β* to H-5*β* (δ 2.19 m). HMBC correlations between H-7 and C-8, C-6; H-9 and C-8, C-17, C-10, C-11; H-5 and C-6, C-7, C-4, C-16; and H-10 and C-8, C-11, C-1, C-12 positioned the methyl-bearing trisubstituted *E* epoxide at C-7, C-8, and C-17. The relative stereochemistry of **1** was deduced from a 2D NOESYexperiment, which indicated that H-10, H_3-15, H-2*β*, H_2-16, H-5*β*, and H_3-17 are oriented to the same side of the molecule, while H-7 and H-9α are oriented to the opposite side of the molecule. Compound **2** is thus (1*R**,7*R**,8*S**,12*R**)-dolabella-4(16),10-diene-7,8-epoxy-3,13- dione.

Compound **3** was an isomer of compound **2**. The molecular formula, $C_{20}H_{28}O_3$, was obtained by HREIMS, ^{13}C NMR, and DEPT spectra. Spectroscopic data of **3** showed close similarity to those of **2**. In contrast to **2**, compound **3**, however, contained no epoxide methyl (δ_H 1.22 s), but an olefinic methyl (δ_H 1.66 br s) at C-8. HMBC correlations between H-10 and C-11, C-12; H-12 and C-10, C-11; H-15 and C-11; H-9 and C-11, C-8, C-7; H_3-17 and C-8, C-7, C-9; and H-6 and C-7, C-8, C-5, C-4 clearly positioned the trisubstituted olefin and epoxide. H-9*β* did not couple to H-10, implying a dihedral angle approaching 90° between these two protons. The relative stereochemistry of **3** was deduced from a NOESY experiment, which indicated H-20, H_3-15, H_3-17, H_2-2, H_2-16, and H-9*β* are on one side of the molecule, while H-7, H-10, H-12, H-5α, and H-9α are on the opposite side. Compound **3** is thus (1*R**,10*R**,11*S**,-12*R**)-dolabella-4(16),7-diene-10,11-epoxy-3,13-dione.

Compound **4** was isolated as an oil of molecular formula $C_{20}H_{28}O_2$ as indicated by HREIMS and ^{13}C NMR spectral methods. The NMR features of **4** were also analogous to those of **1**. Analyses of 2D NMR data revealed that **4** possessed an 11-membered and a five-membered ring with substitution identical to that in **1**. However, there was a significant difference that indicated the presence of a tetrasubstituted olefin (δ_H 172.9 s, 146.1 s) and a ketone α to the tetrasubstituted olefin (δ_C 208.0, C-13) in **4** instead of a trisubstituted olefin (δ_H 5.34 dt; δ_C 127.3 d, 146.0 s) and a nonconjugated ketone as in **1**. HMBC correlations between H-18 and C-11, C-12, C-13, C-19, C-20; H-10 and C-11, C-12, C-1, C-9; H-14 and C-1, C-12, C-13, C-15; H-15 and C-11, C-1, C-2, C-14; and H-9 and C-10, C-11, C-7, C-8, C-17 clearly positioned the tetrasubstituted double bond at C11−C12. The relative stereochemistry of **4** was deduced from a 2D NOESY experiment, which indicated that the bridgehead methyl at C-1, the methyl at the *E*-trisubstituted olefin, the olefin proton at C-10, and the *exo*-methylene at C-4 are oriented to one side of the molecule, while H-7, H-5α, and H-9α are all oriented to the opposite

side of the molecule. Compound **4** is thus (1*R**)-dolabella-4(16),7,11(12)-triene-3,13-dione.

Compound **5** has the molecular formula $C_{20}H_{30}O_2$, as determined by HREIMS and NMR spectral data. The IR spectrum showed the presence of hydroxy (3500 cm^{-1}) and α,*β*-unsaturated ketone (1690 cm^{-1}) moieties. The NMR spectra of **5** were similar to those of **4** except that the resonances for the ketone function (δ_C 200.8 s) α to the exocyclic methylene (δ_H 5.30 s, 5.34 s; δ_C 118.7 t, 152.0 s) in **4** were replaced by a secondary hydroxy (δ_H 3.60 t; δ_C 71.6 d) α to the exocyclic methylene (δ_H 5.05 s, 4.93 s; δ_C 112.1 t, 156.7 s) in **5**. COSY cross-peaks between H-3 (*δ* 3.60, t) and H_2-2 (*δ* 1.73, m), as well as HMBC correlations between H-3 and C-4, C-16, C-2, C-1; H_2-2 and C-3, C-1, C-11, C-15, C-14, C-4; and H_2-16 and C-3, C-4, C-5 clearly positioned the secondary hydroxy at C-3. The secondary hydroxy at C-3 was assigned as *β* on the basis of NOEs between H_3-15 and H-14*β*, H-10*β*, H_2-2; between H-16 and H-6*β*, H_2-2; between H-3 and H-5α; between H-5α and H-7; and between H-7 and H-6α. Compound **5** is thus (1*R**,3*R**)-3-hydroxydolabella-4(16),7,11(12)-triene-3,13-dione.

Compound **6** was analyzed for $C_{20}H_{28}O_4$ by HREIMS and NMR spectral data. The IR spectrum showed the presence of an α,*β*-unsaturated ketone (1688 cm^{-1}) moiety. The ^1H and ^{13}C NMR spectra resembled those of **4**. However, a secondary hydroperoxy (δ_H 4.11 dd; δ_C 89.0 d) α to an exocyclic methylene (δ_H 5.29 s, 5.34 s; δ_C 115.4 t, 144.7 s) in **6** replaced the *E*-trisubstituted double bond bearing a methyl group (δ_H 4.82 dd, 1.45 br s; δ_C 128.9 d, 133.2 s, 14.8 q) in **4**. COSY cross-peaks between H-7 (*δ* 4.11 dd) and H_2-6 and between H_2-6 and H_2-5 as well as HMBC correlations between H-9 and C-8, C-10, C-11; H-7 and C-8, C-17, C-6, C-9; H_2-6 and C-7, C-8; and H-17 and C-8, C-9 positioned the secondary hydroperoxy group at C-7. In the NOESY experiment, NOEs between H-7 and H-5α, H-9α; between H_3-15 and H_2-2, H-14*β*; between H_2-16 and H-6*β*, H_2-2; and between H_2-17 and H-6*β*, H-10*β* allowed the secondary hydroperoxy and exocyclic methylene at C-8 to be assigned to the *β* face of the molecule. Compound **6** is thus (1*R**,7*R**)-7-hydroperoxydolabella-4(16),8(17),11(12)-triene- 3,13-dione.

The cytotoxicity of compounds **1**−**6** is shown in Table 4. Compound **6**, which contains a secondry hydroperoxy group, exhibited cytotoxicity against A-549, HT-29, and P-388 cell lines with ED_{50} values of 0.56, 0.31, and 0.052 *μ*g mL^{-1}, respectively. Compounds **1**−**5** showed moderate cytotoxicity against the P-388 cell line.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26−30 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C or on a Bruker AMX 400 NMR spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMT at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *C. inflata* was collected at Orchid Island, off Taiwan, in September 1998, at a depth of 20 m and was stored for 6 days in a freezer until extraction.

Table 2. ^1H NMR Chemical Shifts of Diterpenes **4**−**6**

proton	**4**[a]	**5**[b]	**6**[a]
2	2.86 d (18.6)[c]	1.73 m	2.95 m
	2.63 d (18.6)	1.73 m	2.95 m
3		3.60 t (3.0)	
5α	2.04 m	2.06 m	1.70 m
5*β*	2.75 m	2.48 m	2.74 m
6α	2.07 m	2.07 m	2.24 m
6*β*	1.97 m	2.29 m	1.76 m
7	4.82 dd (10.5, 4.5)	5.14 dd (12.0, 4.5)	4.11 dd (9.6, 1.8)
9α	2.21 m	2.36 m	2.28 m
9*β*	2.22 m	2.36 m	2.70 m
10α	2.41 dd (12.6, 7.2)	2.46 dd (14.0, 7.0)	2.65 m
10*β*	2.20 m	2.25 m	2.28 m
14α	2.73 d (18.2)	3.35 d (18.5)	2.59 d (17.8)
14*β*	1.92 d (18.2)	1.95 d (18.5)	2.12 d (17.8)
15	0.98 s	1.14 s	1.19 s
16	5.30 s	5.05 s	5.66 s
	5.34 s	4.93 s	5.71 s
17	1.45 br s	1.62 br s	5.29 s, 5.34 s
18	2.41 m	2.78 septet (7.0)	2.70 m
19	1.19 d (6.9)	1.36 d (7.0)	1.33 d (6.9)
20	1.03 d (6.9)	1.19 d (7.0)	1.21 d (6.9)
OOH			8.01 br s

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. [b]Spectrum recorded at 500 MHz in $CDCl_3$ at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments. [c]*J* values (in Hz) in parentheses.

Table 3. ^{13}C NMR Chemical Shifts of Diterpenes **1**−**6**

carbon	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[b]
1	42.1 s	41.6 s	39.9 s	42.4 s	44.2 s	42.3 s
2	53.9 t	51.8 t	50.4 t	43.7 t	42.1 t	44.3 t
3	201.6 s	201.6 s	201.3 s	200.8 s	71.6 d	200.3 s
4	151.5 s	150.4 s	150.7 s	152.0 s	156.7 s	150.6 s
5	28.9 t	24.4 t	28.7 t	32.5 t	36.5 t	33.7 t
6	29.6 t	29.0 t	28.9 t	29.6 t	30.3 t	29.5 t
7	125.2 d	61.0 d	127.8 d	128.9 d	126.4 d	89.0 d
8	135.6 s	62.1 s	131.3 s	133.2 s	134.0 s	144.7 s
9	40.2 t	40.6 t	36.9 t	39.5 t	39.6 t	24.2 t
10	123.7 d	119.9 d	57.3 d	23.1 t	23.8 t	27.8 t
11	146.0 s	151.9 s	70.8 s	172.9 s	176.3 s	176.8 s
12	58.0 d	58.4 d	54.4 d	146.1 s	147.2 s	144.8 s
13	217.8 s	216.8 s	215.4 s	208.0 s	209.3 s	208.1 s
14	53.8 t	53.7 d	54.4 t	48.5 t	47.3 t	49.8 t
15	28.9 q	29.7 q	24.2 q	28.8 q	28.9 q	28.9 q
16	124.2 t	126.2 t	123.8 t	118.7 t	112.1 t	122.2 t
17	16.8 q	17.2 q	17.4 q	14.8 q	15.7 q	115.4 t
18	34.1 d	33.5 d	29.8 d	26.2 d	26.4 d	26.2 q
19	20.9 q	20.9 q	20.9 q	19.9 q	20.4 q	20.2 q
20	16.8 q	19.5 q	18.8 q	19.8 q	19.8 q	19.9 q

[a] Spectrum recorded at 100 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. [c]Spectrum recorded at 125 MHz in $CDCl_3$ at 25 °C. Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments.

Table 4. Cytotoxicity[a] of Diterpenes **1**−**6**

	cell lines ED_{50} (*μ*g/mL)		
compound	A549	HT-29	P-388
1	>50	27.3	2.60
2	8.56	7.84	2.48
3	7.74	6.39	3.83
4	>50	>50	3.89
5	8.10	9.19	3.82
6	0.57	0.31	0.052

[a] For significant activity of pure compounds, an ED_{50} of ≤4.0 *μ*g/mL is required.

A voucher specimen, NSULY-001, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *C. inflata* were freeze-dried to give 1.65 kg of a solid, which was extracted with CH_2Cl_2 (2.0 L × 3). After removal of solvent in vacuo, the residue (80 g) was chromatographed over Si gel 60 using CH_2Cl_2 and CH_2Cl_2−acetone mixtures of increasing polarity. Elution by CH_2Cl_2 afforded fractions containing **1** and **3**. Elution by CH_2Cl_2−acetone (19:1) afforded fractions containing **4** and **6**. Elution by CH_2Cl_2−acetone (9:1) afforded fractions containing **5**. Elution by CH_2Cl_2−acetone (7:3) afforded fractions containing **2**. Compounds **1** and **3** were obtained by Si gel column chromatography, by eluting with *n*-hexanes−EtOAc (9:1) and *n*-hexane−EtOAc (10:1), respectively. Compounds **4** and **6** were obtained by Si gel column chromatography, by eluting with *n*-hexane−acetone (10:1) and acetone, respectively. Compound **5** was obtained by Si gel column chromatography, by eluting with *n*-hexane−CH_2Cl_2 (1:1). Compound **2** was obtained by Si gel column chromatography, by eluting with *n*-hexane−CH_2Cl_2 (2:1).

Compound 1: colorless oil (460 mg); $[\alpha]^{25}_D$ −92.3° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 226 (4.24) nm; IR (KBr) ν_{max} 1729, 1685, 1617 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* 300 $[M]^+$ (76), 285 (8), 271 (15), 257 (18), 232 (15), 203 (19), 189 (24), 173 (26), 159 (32), 149 (68), 135 (100); HREIMS *m/z* 300.2086 (calcd for $C_{20}H_{28}O_2$, 300.2090).

Compound 2: colorless oil (50 mg); $[\alpha]^{25}_D$ −82.3° (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 233 (4.22) nm; IR (KBr) ν_{max} 1730, 1686, 1616 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* 316 $[M]^+$ (12), 299(3), 283 (6), 273 (8), 255 (6), 233 (27), 175 (18), 164 (28), 149 (43), 135 (66), 109 (93), 55 (91), 43 (100); HREIMS *m/z* 316.2042 (calcd for $C_{20}H_{28}O_3$, 316.2039).

Compound 3: colorless oil (6 mg); $[\alpha]^{25}_D$ +56.2° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.28) nm; IR (KBr) ν_{max} 1726, 1688, 1619 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* 316 $[M]^+$ (10), 299(4), 283 (5), 273 (6), 233 (23), 164 (23), 149 (48), 135 (69), 109 (92), 55 (90), 43 (100); HREIMS *m/z* 316.2036 (calcd for $C_{20}H_{28}O_3$, 316.2039).

Compound 4: colorless oil (120 mg); $[\alpha]^{25}_D$ −264.5° (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (4.16) nm; IR (KBr) ν_{max} 1726, 1683, 1617 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS *m/z* 300 $[M]^+$ (7), 285 (4), 271 (3), 257 (12), 242 (25), 208 (49), 189 (16), 149 (35), 107 (63), 91 (100); HREIMS *m/z* 300.2092 (calcd for $C_{20}H_{28}O_2$, 300.2090).

Compound 5: colorless oil (7 mg); $[\alpha]^{25}_D$ +76.1° (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 228 (4.28) nm; IR (KBr) ν_{max} 3500, 1690, 1615 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS *m/z* 302 $[M]^+$ (12), 285 (19), 269 (9), 256 (28), 233 (38), 164 (86), 149 (90), 135 (100), 107 (72), 91 (75), 55 (96); HRFABMS *m/z* 302.2251 (calcd for $C_{20}H_{30}O_2$, 302.2247).

Compound 6: colorless oil (8 mg); $[\alpha]^{25}_D$ +95.6° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.24) nm; IR (KBr) ν_{max} 3316, 1686, 1616 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS *m/z* 332 $[M]^+$ (5), 316 (1), 257 (3), 241 (6), 223 (5), 201 (6), 189 (8), 173 (7), 163 (14), 149 (25), 135 (24), 107 (39), 91 (72), 55 (100); HREIMS *m/z* 332.1986 (calcd for $C_{20}H_{28}O_4$, 332.1988).

Cytotoxicity Testing. KB and P-388 cells were kindly supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[23]

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