New Prostanoids with Cytotoxic Activity from Taiwanese Octocoral *Clavularia viridis*

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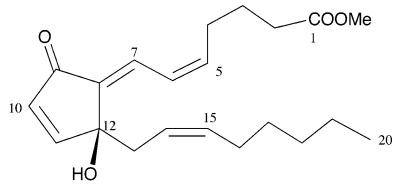
Bioassay-directed fractionation of the CH_2Cl_2−MeOH extract of *Clavularia viridis* collected in Taiwan has afforded seven new prostanoids, designated as 4-deacetoxyl-12-*O*-deacetylclavulone I (**1**), 4-deacetoxyl-12-*O*-deacetylclavulone II (**2**), bromovulone II (**4**), iodovulone II (**5**), 4-deacetoxyl-12-*O*-deacetylclavulone III (**6**), bromovulone III (**7**), and iodovulone III (**8**), in addition to seven known prostanoids (clavulones I, II, III, 7-acetoxy-7,8-dihydroiodovulone, chlorovulones II, III, and 4-deacetoxylclavulone II (**3**, claviridenone E)). The structures of compounds **1**−**8** were determined on the basis of 1D and 2D NMR techniques including COSY, HSQC, and HMBC experiments. Pharmacological study revealed that bromovulone III (**7**) and chlorovulone II exhibited the most promising cytotoxicity against human prostate (PC-3) and colon (HT29) cancer cells.

Marine eicosanoids such as clavulones and halogenated clavulones have attracted much interest because of their novel structures and significant antitumor activities.[1−5] These prostanoids have been found in the different species of marine invertebrates and red alga such as *Clavularia viridis*,[6−8] *Telesto riisei*,[9,10] and *Gracilaria* sp.[11] In addition, the octocoral *C. viridis* is rich in bioactive prostanoids with different structural types such as preclavulone lactones,[12] clavirins,[13] tricycloclavulone, and clavubicyclone.[14] During the course of searching for antitumor natural products from marine creatures, we have surveyed a series of crude extracts of marine soft corals by applying cytotoxicity tests. Among them, an EtOAc extract of the stolonifer *C. viridis* Quoy and Gaimard (class Anthozoa, subclass Octocorallia, order Stolonifera) collected in Green Island was found to possess significant cytotoxicity against human NUGC and HONE-1 tumor cells. Bioassay-directed fractionation of the EtOAc-soluble fraction by normal-phase chromatography has resulted in the isolation of seven new prostanoids, 4-deacetoxyl-12-*O*-deacetylclavulone I (**1**), 4-deacetoxyl-12-*O*-deacetylclavulone II (**2**), bromovulone II (**4**), iodovulone II (**5**), 4-deacetoxyl-12-*O*-deacetylclavulone III (**6**), bromovulone III (**7**), and iodovulone III (**8**), from *C. viridis* in addition to seven known prostanoids (clavulones I, II, III, 7-acetoxy-7,8-dihydroiodovulone, chlorovulones II, III, and 4-deacetoxylclavulone II[15]). Herein we wish to report the isolation, structural elucidation, and biological activity of these marine prostanoids.



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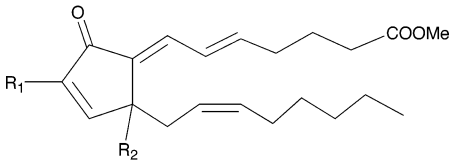
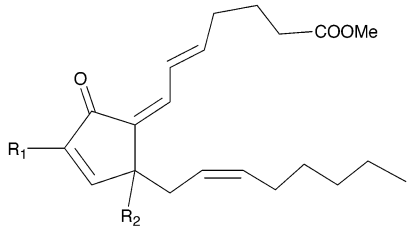


2 R_1 = H, R_2 = β-OH
3 R_1 = H, R_2 = β-OAc
4 R_1 = Br, R_2 = α-OH
5 R_1 = I, R_2 = α-OH



6 R_1 = H, R_2 = β-OH
7 R_1 = Br, R_2 = α-OH
8 R_1 = I, R_2 = α-OH

Results and Discussion

The CH_2Cl_2−MeOH extract of *C. viridis* was partitioned between EtOAc and H_2O. Extensive Si gel column chromatography and HPLC chromatography using gradient solvent combinations yielded seven new compounds. They are 4-deacetoxyl-12-*O*-deacetylclavulone I (**1**, 1.1 mg), 4-deacetoxyl-12-*O*-deacetylclavulone II (**2**, 2.6 mg), bromovulone II (**4**, 3.1 mg), iodovulone II (**5**, 9.1 mg), 4-deacetoxyl-12-*O*-deacetylclavulone III (**6**, 2.2 mg), bromovulone III (**7**, 25 mg), and iodovulone III (**8**, 4.4 mg), in addition to seven known prostanoids from the EtOAc-soluble fraction. The known compounds were identified as clavulones I (15 mg), II (6 mg), and III (8 mg), 7-acetoxy-7,8-dihydroiodovulone (6 mg), chlorovulones II (17 mg) and III (0.9 mg), and 4-deacetoxylclavulone II (**3**, 11.5 mg) by comparison of their spectral data (^{1}H, ^{13}C NMR, MS, and optical rotation) with literature values. The structures of **1**−**8** are described below.

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Table 1. ^{1}H NMR Data ($CDCl_3$, 300 MHz) of Compounds **1**−**4**

position	1	2	3	4
2	2.39 t (7.5)	2.35 t (7.3)	2.35 t (7.3)	2.33 t (7.2)
3	1.82 m	1.82 m	1.81 m	1.82 m
4	2.39 m	2.30 m	2.30 m	2.30 m
5	6.05 dt (8.0, 10.5)	6.21 dt (7.0, 13.2)	6.22 dt (7.8, 14.8)	6.28 dt (7.2, 14.5)
6	6.79 dd (10.5, 12.3)	6.78 dd (12.0, 13.2)	6.54 dd (12.3, 14.7)	6.77 dd (13.2, 14.5)
7	7.25 d (11.0)	6.92 d (11.9)	6.92 d (11.9)	7.04 d (12.0)
10	6.36 d (5.9)	6.33 d (6.0)	6.40 d (6.1)	
11	7.32 d (6.0)	7.30 d (6.0)	7.48 d (6.1)	7.42 s
13a	2.70 dd (8.2, 14.5)	2.70 dd (7.7, 14.2)	2.71 dd (7.9, 14.3)	2.69 dd (8.1, 13.9)
13b	2.78 dd (7.3, 14.5)	2.77 dd (7.8, 14.1)	2.95 dd (7.5, 14.2)	2.78 dd (7.8, 14.0)
14	5.23 dt (10.8, 7.6)	5.22 dt (11.1, 7.5)	5.19 dt (10.8, 8.2)	5.23 dt (11.2, 7.6)
15	5.54 dt (10.8, 7.5)	5.52 dt (11.1, 7.5)	5.51 dt (10.8, 7.2)	5.54 dt (11.0, 7.6)
16	1.98 m	1.97 m	1.95 m	1.98 m
17	1.27 m	1.27 m	1.26 m	1.27 m
18	1.27 m	1.27 m	1.26 m	1.27 m
19	1.27 m	1.27 m	1.26 m	1.27 m
20	0.88 t (6.3)	0.88 t (6.5)	0.88 t (6.0)	0.88 t (6.1)
OMe	3.69 s	3.67 s	3.68 s	3.68 s
OAc			2.04 s	2.06 s

[a] Chemical shifts are in ppm; *J* values in Hz are in parentheses.

Table 2. ^{1}H NMR Data ($CDCl_3$, 300 MHz) of Compounds **5**−**8**

position	5	6	7	8
2	2.32 t (7.2)	2.35 t (6.5)	2.35 t (7.5)	2.34 t (7.2)
3	1.79 m	1.80 m	1.81 m	1.80 m
4	2.32 m	2.31 m	2.35 m	2.33 m
5	6.26 dt (15.0, 7.0)	6.12 dt (15.2, 7.0)	6.21 dt (14.7, 7.7)	6.20 dt (15.6, 7.2)
6	6.77 dd (12.9, 13.8)	7.57 dd (11.5, 15.1)	7.56 dd (11.5, 14.7)	7.58 dd (11.4, 15.2)
7	7.02 d (12.0)	6.60 d (11.3)	6.72 d (11.4)	6.62 d (11.6)
10		6.26 d (6.0)		
11	7.68 s	7.25 d (6.0)	7.37 s	7.64 s
13a	2.67 dd (7.9, 14.3)	2.55 dd (6.7, 14.2)	2.57 dd (7.9, 14.0)	2.53 dd (7.4, 14.1)
13b	2.75 dd (7.5, 14.2)	2.62 dd (8.0, 14.1)	2.63 dd (7.3, 13.7)	2.63 dd (7.4, 13.7)
14	5.22 dt (9.6, 9.0)	5.30 dt (10.7, 7.5)	5.30 dt (7.8, 10.8)	5.30 dt (7.8, 9.3)
15	5.54 dt (12.5, 9.6)	5.56 dt (10.7, 7.4)	5.56 dt (10.7, 7.5)	5.58 dt (10.8, 7.0)
16	1.98 m	2.00 m	1.98 m	2.00 m
17	1.27 m	1.25 m	1.28 m	1.28 m
18	1.27 m	1.25 m	1.28 m	1.28 m
19	1.27 m	1.25 m	1.28 m	1.28 m
20	0.88 t (6.0)	0.88 t (6.0)	0.88 t (6.3)	0.88 t (6.4)
OMe	3.68 s	3.68 s	3.68 s	3.68 s

[a] Chemical shifts are in ppm; *J* values in Hz are in parentheses.

4-Deacetoxyl-12-*O*-deacetylclavulone I (**1**), $[\alpha]_D$ +24° (*c* 0.4, CH_2Cl_2), had a molecular formula of $C_{21}H_{30}O_4$ as derived from a molecular ion at *m*/*z* 347 $[M + H]^+$ in the HRFABMS spectrum. Its IR bands indicated the presence of a hydroxyl (3450 cm^{-1}), an α,*β*-unsaturated cyclopentenone (1706 cm^{-1}), and an ester (1734 cm^{-1}) group. The ^{1}H NMR spectrum of **1** (Table 1) exhibited a methoxyl singlet (*δ* 3.69), a terminal methyl triplet (*δ* 0.88), and seven olefinic protons (*δ* 6.05, dt, *J* = 8.0, 10.2 Hz, H-5; *δ* 6.79, dd, *J* = 11.1, 12.3 Hz, H-6; *δ* 7.25, d, *J* = 11.0 Hz, H-7; *δ* 6.36, d, *J* = 5.9 Hz, H-10; *δ* 7.32, d, *J* = 6.0 Hz, H-11; *δ* 5.23, dt, *J* = 10.8, 7.6 Hz, H-14; *δ* 5.54, dt, *J* = 10.8, 7.5 Hz, H-15). To determine the proton sequence of **1**, a COSY spectrum revealed the connectivities of H-7/H-6/H-5/H-4, H-11/H-12, and H-13/H14/H-15/H-16. Thus, it located the signals of H-4, H-13a, H-13b, and H-16 at *δ* 2.39, 2.70, 2.78, and 1.98, respectively. The ^{13}C NMR spectrum and HMQC of **1** showed 21 carbon signals for one methyl (*δ* 14.0 q), one methoxyl (*δ* 51.6 s), eight methylenes (*δ* 22.5 t, 24.5 t, 27.1 t, 27.4 t, 29.1 t, 31.5 t, 33.3 t, 36.5 t), seven olefinic methines (*δ* 122.2 d, 123.8 d, 126.3 d, 134.6 d, 134.8 d, 142.6 d, 161.1 d), and five quaternary carbons (Table 3) including the ester carbonyl (*δ* 173.7 s), the conjugated ketone carbonyl (*δ* 195.3 s), and the hydroxylated carbon (*δ* 79.5 s). Detailed analysis of the ^{1}H and ^{13}C NMR spectra revealed that **1** is a prostanoid having an α,*β*-unsaturated cyclopentenone connected with a conjugated diene system.[6,7] This finding was further confirmed from the observation of long-range correlations of C-1/H-2, C-8/H-7, C-9/H-7; C-10/H-11, C-12/H-11, C-12/H-13, C-12/H-7 in the HMBC spectrum of **1**. The correlation of the methoxyl group with the ester carbonyl was also observed. The (5*Z*, 14*Z*) configurations of two disubstituted double bonds were determined on the basis of coupling constants between the olefinic protons H-5 and H-6 (10.5 Hz), and H-14 and H-15 (10.8 Hz). The deshielding effect of H-7 (*δ* 7.25) due to the anisotropy of the C-9 carbonyl group indicated that the geometry of C-7 and C-8 is an *E* form. In addition, the assignment of **1** was concluded from the NOESY experiment. The above spectroscopic evidence clearly indicated that the structure of **1** is similar to that of claviridenone F, which has an acetoxyl group at C-12.[15] The absolute stereochemistry of **1** was determined by comparison of its CD spectral data with that of clavulone I, whose structure was established previously.[16] The CD spectral data of **1** showed a positive Cotton effect (λ_{ex} 209 nm, +3.80 mdeg; λ_{ex} 263 nm, −0.46; λ_{ex} 363.5 nm, +0.38) and thus confirmed the 12*R* configuration at the *ω* side chain.

4-Deacetoxyl-12-*O*-deacetylclavulone II (**2**), $[\alpha]_D$ +54° (CH_2Cl_2), had the molecular formula $C_{21}H_{30}O_4$ as deduced from HRFABMS and NMR data. The IR and UV spectra of **2** showed similar absorbances as in **1**, suggesting that **2** was an analogue of **1**. A comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** revealed that they were very similar. The difference between them is the proton chemical shift of H-5 (*δ* 6.21 in **2**; *δ* 6.05 in **1**) and coupling constant between the two olefinic protons H-5 and H-6 (13.2 Hz in **2**; 10.5 Hz in **1**) as well as the ^{13}C chemical shifts at C-5, C-6, and C-7 (*δ* 146.1, 126.0, 132.2 in **2**; *δ* 142.6, 123.8, 126.3 in **1**). Compound **2** was thus determined as a 5*E* isomer of **1**. The COSY, HMQC, and HMBC spectra of **1** confirmed its structural assignment. Because the spectral data of the *ω* side chain in **2** and its CD data were similar to those of **1**, the stereochemistry of **2** was therefore assigned as 12*R*, identical with **1**.

Bromovulone II (**4**), $[\alpha]_D$ +23° (CH_2Cl_2), had the molecular formula $C_{21}H_{29}O_4Br$ as derived from FABMS and NMR data. The ^{1}H and ^{13}C NMR spectra of **4** were very similar to those of **2**, suggesting a close analogue of **2**. Detailed comparison of the ^{1}H NMR spectrum of **4** with that of **2** revealed that the H-10 in **2** was missing in **4**. Instead, a bromine atom appeared at C-10, and thus the multiplicity of H-11 changed from a doublet to a singlet. Additionally, the ^{1}H chemical shift of H-11 was shifted downfield from *δ* 7.30 in **2** to *δ* 7.42 in **4**. Moreover, the ^{13}C chemical shifts of C-8, C-9, C-10, and C-11 shifted upfield from *δ* 136.8, 195.2, 134.8, and 160.9 in **2** to *δ* 133.9, 193.2, 126.8, and 158.3 in **4**, respectively. Supported by the HMBC, **4** showed correlations of H-7/C-8, C-9, C-10 and H-11/C-10, C-12. Thus the *E* configuration at C-5 and *Z* configuration at C-14 agreed with the coupling constants of $J_{5,6}$ = 14.5 Hz and $J_{14,15}$ = 11.0 Hz. The *R* configuration at C-12 in **4** was elucidated by comparison of the CD

Table 3. ^{13}C NMR Data ($CDCl_3$, 75 MHz) of Compounds **1**−**8**

carbon	**1**[b]	**2**[b]	**3**	**4**	**5**	**6**	**7**	**8**[c]
1	173.7 s	173.6 s	173.6 s	173.9 s	173.6 s	173.6 s	173.7 s	173.6 s
2	27.1 t	33.3 t	33.2 t	33.6 t	32.8 t	33.5 t	33.3 t	33.3 t
3	24.5 t	23.9 t	23.8 t	24.0 t	23.8 t	24.2 t	23.8 t	23.8 t
4	33.3 t	32.7 t	32.7 t	33.0 t	33.3 t	32.4 t	32.7 t	32.8 t
5	142.6 d	146.1 d	146.4 d	148.0 d	147.4 d	145.4 d	147.5 d	147.5 d
6	123.8 d	126.0 d	125.3 d	126.1 d	125.9 d	126.7 d	126.0 d	125.9 d
7	126.3 d	132.2 d	131.4 d	134.3 d	133.7 d	135.5 d	133.9 d	135.1 d
8	138.3 s	136.8 s	133.9 s	133.9 s	132.9 s	136.6 s	134.3 s	136.3 s
9	195.3 s	195.2 s	193.8 s	193.2 s	189.9 s	195.4 s	188.2 s	191.5 s
10	134.8 d	134.8 s	135.5 d	126.8 s	106.1 s	136.2 d	126.5 s	105.2 s
11	161.1 d	160.9 d	157.5 d	158.3 d	166.2 d	159.2 d	158.4 d	159.0 d
12	79.5 s	79.6 s	85.5 s	79.6 s	81.4 s	79.1 s	79.2 s	79.5 s
13	36.5 t	36.5 t	35.6 t	36.8 t	36.5 t	37.3 t	36.2 t	36.4 t
14	122.2 d	122.3 d	121.3 d	121.9 d	121.7 d	122.5 d	121.7 d	121.8 d
15	134.6 d	134.5 d	134.8 d	137.3 d	135.0 d	134.6 d	137.2 d	135.1 d
16	27.4 t	27.4 t	27.4 t	27.7 t	27.4 t	27.4 t	27.3 t	27.4 t
17	29.1 t	29.1 t	29.1 t	29.3 t	29.1 t	29.2 t	29.0 t	29.7 t
18	31.5 t	31.5 t	31.5 t	31.8 t	31.6 t	31.6 t	31.5 t	31.5 t
19	22.5 t	22.5 t	22.5 t	22.7 t	22.5 t	22.6 t	22.5 t	22.5 t
20	14.0 q	14.0 q	14.0 q	14.3 q	14.0 q	14.1 q	14.0 q	14.0 q
OMe	51.6 s	51.6 s	51.6 s	51.9 s	51.6 s	51.6 s	51.6 s	51.6 s
OAc			169.3 s					
			21.3 q					

[a] Assignments were made using HMQC and HMBC techniques. [b] Measured at 100 MHz. [c] Measured at 125 MHz.

spectrum of **4** with that of bromovulone I, in which the chiral center has already been determined as *R*.[5,17] The positive Cotton effect of **4** (λ_{ex} 209 nm, +2.54 mdeg; λ_{ex} 265 nm, −0.60; λ_{ex} 354 nm, +0.46) and the positive Cotton effect of bromovulone I established the stereochemistry of the tertiary hydroxyl in **4**.

The ^1H and ^{13}C NMR spectra of iodovulone II (**5**), $[\alpha]^{25}_D$ +44.6° (*c* 0.9, CH_2Cl_2), were very similar in both chemical shifts and coupling constants to those of bromovulone II (**4**), except for the signals of H-11, C-9, C-10, and C-11 (δ_H 7.68, δ_C 189.9, 106.1, 166.2 in **5**; δ 7.42, 193.2, 126.8, 158.3 in **4**). The deshielding effect of H-11 (+0.26 ppm) and the heavy atom effect of C-11 clearly indicated that the bromine atom at C-11 in **4** was replaced by the iodine atom in **5**. Indeed, the EIMS of **5** exhibited a quasi-molecular ion at *m/z* 473 ($[M + H]^+$). Furthermore, the assignments of ^1H and ^{13}C NMR data of **5** were accomplished by COSY, HMQC, and HMBC techniques as shown in Tables 2 and 3, respectively. The *R* configuration at C-12 in **5** was determined by comparison of the spectral data including the CD of **5** with those of **4**.

4-Deacetoxyl-12-*O*-deacetylclavulone III (**6**), $[\alpha]_D$ +15° (CH_2Cl_2), had the molecular formula $C_{21}H_{30}O_4$ as deduced from HRFABMS (*m/z* 347 $[M + H]^+$) and NMR data. A comparison of the ^1H and ^{13}C NMR spectra of **6** with those of **2** revealed that they were very similar. The difference is the proton chemical shifts of H-6 and H-7 (δ 7.57, 6.60 in **6**, δ 6.78, 6.92 in **2**) and the ^{13}C chemical shift at C-7 (δ 135.5 in **6**, δ 132.2 in **2**) due to the anisotropic effect of the C-9 carbonyl. Compound **6** was thus determined as a 7*Z* isomer of **2**. The ^1H and ^{13}C NMR assignment of **6** was completed by COSY, HMQC, and HMBC spectra. The stereochemistry of **6** was determined as 12*R* on the basis of the comparison of CD spectra data with that of **2**.

Bromovulone III (**7**), $[\alpha]_D$ +39° (CH_2Cl_2), had the molecular formula $C_{21}H_{29}O_4Br$ as established from HRFABMS. It is an isomer of **4**. They had very similar ^1H and ^{13}C NMR spectral data. A comparison of the ^1H and ^{13}C NMR spectra of **7** with those of **4** revealed the difference between them were the chemical shifts of H-6 and H-7 (δ 7.56, 6.72 in **7**, δ 6.77, 7.04 in **4**). Furthermore, the coupling constants of $J_{5,6} = 14.7$ Hz and $J_{14,15} = 10.7$ Hz in **7** agreed with the 5*E* and 14*Z* configuration. The CD spectrum of **7** showed the same positive Cotton effect (λ_{ex} 216 nm, +1.96 mdeg; λ_{ex} 265 nm, −0.97; λ_{ex} 363 nm, +0.47) as those of **4**, suggesting that C-12 had an *R* configuration. The above spectral data established the structure of **7**.

Iodovulone III (**8**), $[\alpha]^{25}_D$ +8.6° (CH_2Cl_2), is an isomer of **5**. Its ^1H and ^{13}C NMR spectral data (Tables 2 and 3) were very similar to those of iodovulone II (**5**) and bromovulone III (**7**). The chemical shifts of the α-side chain (from C-1 to C-7) in **8** were superimposable to those of **7**. On the other hand, a close comparison of the resonances from H-11 to the end of the *ω*-side chain in **8** with those of **5** established the structure of **8**. The *R* configuration at C-12 was determined by comparison of the spectral data of **8** with those of **5** and **7**. Finally, the assignments of ^1H and ^{13}C NMR data of **8** were accomplished by COSY, HMQC, and HMBC experiments.

The cytotoxicities of the isolated marine prostanoids were evaluated in vitro against human prostate carcinoma (PC-3) and colon adenocarcinoma (HT29) cells. As illustrated in Table 3, compound **7** showed the most promising antiumor activity against human PC-3 cells and HT29 cancer cells at an IC_{50} of 0.5 μM. Clorovulones II and III were used as standard compounds, which also exhibited potent cytotoxicity againt human PC-3 cells and HT29 cancer cells but slightly weaker than **7**.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. Circular dichroisms were measured with a Jasco J-810 spectrophotometer. The IR spectra were taken with a HORIBA FT-720 spectrophotometer. ^1H, ^{13}C NMR, DEPT, COSY, HSQC, HMBC, and NOESY spectra were recorded using a Bruker FT-300 (AVANCE) or a Varian FT-500 (INOVA) NMR instrument. EIMS and FABMS were measured with VG Quattro 5022 and JEOL JMS-SX 102 mass spectrometers.

Animal Material. *Clavularia viridis* Quoy and Gaimard was collected in Green Island, Taiwan, in April 2001. This soft coral was identified by one of the authors. A voucher specimen (GSC-4) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. The whole animal of *C. viridis* (wet, 10.5 kg) was ground and extracted with CH_2Cl_2−MeOH (1:1, 20 L) to afford a crude extract, which was partitioned

between H_2O (2 L) and EtOAc (2 L) to yield an EtOAc-soluble fraction (65 g). This residue was chromatographed on a Si gel column (600 g) and eluted with solvent mixture of *n*-hexane−EtOAc (200:1 to 20:20:1, each 1 L) to afford 17 fractions. Fraction 8 (1.17 g) was applied to a preparative HPLC Si gel column and eluted with a mixture of *n*-hexane−EtOAc (3:1) to yield six fractions, Fa (17 mg), Fb (76 mg), Fc (25 mg), Fd (102 mg), Fe (153 mg), and Ff (57 mg). Fraction Fa was further purified with HPLC (Si gel column, *n*-hexane−EtOAc, 5:1) to give compound **8** (1.2 mg). Fraction Fb was applied to a HPLC column (Si gel) and developed with *n*-hexane−EtOAc (5:1) to yield compound **5** (9.1 mg). Fraction Fc was applied to a HPLC column (Si gel) and developed with *n*-hexane−EtOAc (5:1) to yield compounds **7** (25 mg), **4** (3.1 mg), and chlorovulone II (6.4 mg). Fraction Fe was chromatographed on a HPLC column (Si gel) eluted with *n*-hexane−EtOAc (5:1) to afford chlovulone III (0.9 mg), chlorovulone II (10.5 mg), and compound **3** (11.5 mg). Fraction 9 (2.2 g) was applied to a flash column (Si gel) and developed with *n*-hexane−CH_2Cl_2−MeOH (100:100:1−40:40:1, each 200 mL) to yield two fractions, F9a (0.4 g) and F9b (0.3 g). Fraction 9a was separated with a HPLC column (Si gel, *n*-hexane−EtOAc, 1:2) to give clavulones II (6 mg) and III (8 mg). Fraction 9b was purified with a HPLC column (Si gel) and eluted with the same solvent system to give 7-acetoxy-7,8-dihydroiodovulone (6 mg). Fraction 10 (2.1 g) was chromatographed on a flash column (Si gel) and developed with *n*-hexane−CH_2Cl_2−MeOH (80:80:1−40:40:1, each 200 mL) to yield two fractions, F10a (0.9 g) and F10b (0.2 g). Fraction 10a was purified with a HPLC column (Si gel, *n*-hexane−EtOAc, 1:2) to give clavulone I (15 mg). Fraction 10b was separated on a HPLC column (Si gel) and eluted with the same solvent system to give compounds **6** (2.2 mg), **1** (1.1 mg), and **2** (2.6 mg).

4-Deacetoxyl-12-*O*-deacetylclavulone I (1): pale yellowish oil; $[\alpha]^{25}_D$ +24° (*c* 0.4, CH_2Cl_2); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 209 (+3.80), 263 (−0.46), 363.5 (+0.38); IR (neat) ν_{max} 3450, 2915, 2848, 1734, 1706, 1697, 1539, 1454 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 3, respectively; FABMS *m*/*z* 347 ($[M + H]^+$), 369 ($[M + Na]^+$); HRFABMS *m*/*z* 347.2220 ($[M + H]^+$, calcd for $C_{21}H_{31}O_4$, 347.2223).

4-Deacetoxyl-12-*O*-deacetylclavulone II (2): pale yellowish oil; $[\alpha]^{25}_D$ +54° (*c* 0.8, CH_2Cl_2); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 264 (−0.43), 366 (+0.30); IR (neat) ν_{max} 2952, 2922, 2848, 2361, 1734, 1712, 1701, 1649, 1557, 1539, 1454, 1432 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 3, respectively; FABMS *m*/*z* 346 $[M]^+$, 369 ($[M + Na]^+$); EIMS *m*/*z* (rel int) 267 (1), 251 (2), 235 (2), 219 (5), 203 (5), 173 (5), 161 (5), 133 (9), 129 (22), 99 (31), 81 (27), 69 (44), 55 (100); HRFABMS *m*/*z* 347.2227 ($[M + H]^+$, calcd for $C_{21}H_{31}O_4$, 347.2223).

Bromovulone II (4): pale yellowish oil; $[\alpha]^{25}_D$ +23° (*c* 0.25, CH_2Cl_2); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 209 (+2.54), 265 (−0.59), 355 (+0.46); IR (neat) ν_{max} 3749, 2915, 2848, 2354, 1734, 1715, 1646, 1635, 1540, 1456, 1432, 1369, 1059, 790 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 3, respectively; FABMS *m*/*z* 407, 409 $[M - OH + H]^+$.

Iodovulone II (5): pale yellowish oil; $[\alpha]^{25}_D$ +44.6° (*c* 0.9, CH_2Cl_2); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 209 (+3.80), 283 (−0.25), 365 (+0.15); IR (neat) ν_{max} 3431, 2925, 2848, 2354, 1715, 1649, 1541, 1456, 1258, 1166, 1059 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 2 and 3, respectively; FABMS *m*/*z* 473 $[M + H]^+$.

4-Deacetoxyl-12-*O*-deacetylclavulone III (6): pale yellowish oil; $[\alpha]^{25}_D$ +15° (*c* 0.8, CH_2Cl_2); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 219 (+0.4), 279 (−0.1); IR (neat) ν_{max} 3445, 2952, 2926, 2848, 2361, 1734, 1716, 1649, 1539, 1456, 1435, 1369, 1236, 1166, 1059 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 2 and 3, respectively; FABMS *m*/*z* 347 $[M + H]^+$, 369 ($[M + Na]^+$); EIMS *m*/*z* (rel int) 275 (0.3), 255 (0.4), 235 (21), 203 (58), 185 (10), 175 (11), 147 (12), 133 (21), 123 (15), 109 (55), 97 (13), 91 (32), 81 (35), 69 (59), 41 (100); HRFABMS *m*/*z* 347.2221 ($[M + H]^+$, calcd for $C_{21}H_{31}O_4$, 347.2223).

Bromoclavulone III (7): pale yellowish oil; $[\alpha]^{25}_D$ +39° (*c* 0.38, CH_2Cl_2); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 216 (+1.96), 265 (−0.97), 364 (+0.47); IR (neat) ν_{max} 3445, 2927, 2855, 2359, 1734, 1713, 1633, 1454, 1436, 1373, 1268, 1196, 1170, 1059, 984, 876, 794, 761, 725 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 2 and 3, respectively; FABMS *m*/*z* 425, 427 $[M + H]^+$; EIMS *m*/*z* (rel int) 424 (1), 426 (1), 409 (4), 407 (4), 363 (5), 345 (2), 315 (17), 313 (21), 283 (6), 281 (5), 237 (58), 219 (9), 201 (13), 187 (9), 160 (9), 174 (10), 145 (12), 129 (16), 115 (13), 107 (23), 91 (22), 79 (14), 77 (16), 69 (44), 55 (100); HRFABMS *m*/*z* 425.1315 ($[M + H]^+$, calcd for $C_{21}H_{30}O_4Br$, 425.1318).

Table 4. Results of Cytotoxicities (IC_{50}, μM) of Isolated Prostanoids[a]

compd/tumor cells	PC-3	HT29
1	7.2	6.0
2	5.4	4.1
3	3.5	>10
4	5.6	5.4
5	3.9	6.5
6	3.9	7.9
7	0.5	0.5
8	6.7	>10
chlorovulone II	0.8	NT[b]
chlorovulone III	1.9	2.7

[a] The concentration of compound inhibiting 50% (IC_{50}) of the growth of human tumor cell lines, PC-3 (prostate) and HT-29 (colon adenocarcinoma), after 48 h drug exposure. [b] Not tested.

Iodovulone III (8): pale yellowish oil; $[\alpha]^{25}_D$ +8.6° (*c* 0.27, CH_2Cl_2); ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 2 and 3, respectively.

Cytotoxicity Assay. A bioassay against PC-3 (human androgen-independent prostate carcinoma) and HT-29 (human colon adenocarcinoma) tumor cells is based on the sulforhodamine B (SRB) assay method.[18] Cells are inoculated into 96-well microtiter plates in RPMI 1640 medium containing 5% fetal bovine serum and incubated at 37 °C, 5% CO_2, 95% air. After 24 h, two plates of each cell line are fixed in situ with trichloracetic acid (TCA). Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. The assay is terminated by the addition of cold TCA. The supernatant is discarded, and the plates are washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% w/v in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing three times with 1% acetic acid and the plates are air-dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. The IC_{50} value was defined, by a comparison with the untreated cells, as the concentration of test sample resulting in a 50% reduction of absorbance.

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