Pachyclavulariolides M−R, Six Novel Diterpenoids from a Taiwanese Soft Coral *Pachyclavularia violacea*

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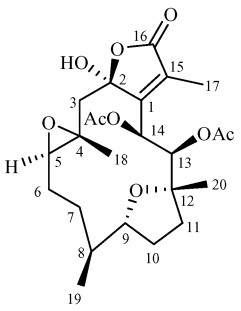
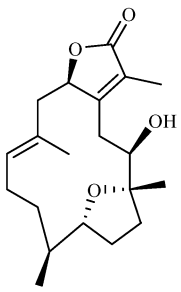
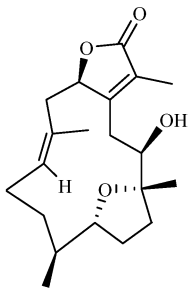
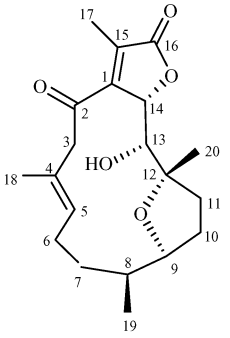
Six novel diterpenoids, pachyclavulariolides M−R (**1**−**6**), have been isolated from a Taiwanese soft coral *Pachyclavularia violacea*. The structures and relative stereochemistry of compounds **1**−**6** were established by spectroscopic analyses. Compound **1** has been shown to exhibit significant cytotoxicity toward P-388 cancer cells. Biosynthesis of **4**−**6** is discussed.

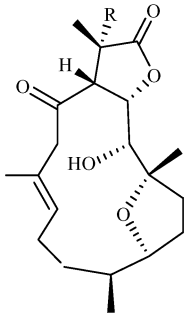
Our research has focused on the search for structurally novel and bioactive metabolites from Formosan marine invertebrates in recent years, and *Briareum excavatum*,[1] *Junceella fragilis*,[2] *Sinularia* sp.,[3] *Suberogorgia suberosa*,[4] and *Isis hippuris*[5] have been investigated. Previous chemical studies on *Pachyclavularia violacea* (Quey & Gaimard, 1833) by other groups and ourselves have revealed that this marine organism produces vasertile metabolites such as pachyclavulariolides,[6,7] secopachyclavulariaenone A,[7] pachyclavulariaenones A−G,[8,9] and secosteroids.[10] In the course of our continuing study on the chemical constituents of *P. violacea*, which was collected along the coast of Kenting, located in the southernmost tip of Taiwan, in September 1995, six new diterpenoids, pachyclavulariolides M−R (**1**−**6**), were isolated. This paper deals with the structure elucidation of these new diterpenoids. The structures and relative stereochemistry of **1**−**6** were established by extensive 1D and 2D NMR experiments (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY). Pachyclavulariolide M has been shown to exhibit significant cytotoxicity against the growth of murine leukemia (P-388) cancer cells. The biosynthetic pathway for the related metabolites **4**−**6** is proposed.

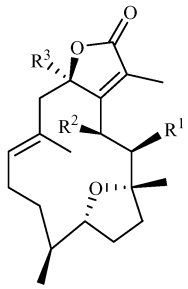












Results and Discussion

Pachyclavulariolide M (**1**) was obtained as a white powder. Its HREIMS established the molecular formula $C_{24}H_{34}O_9$. Thus, eight degrees of unsaturation were determined for **1**. The IR spectrum of **1** showed the presence of a hydroxy group (ν_{max} 3391 cm^{-1}), ester carbonyl groups (ν_{max} 1716 cm^{-1}), and an α,β-unsaturated-γ-lactone unit (ν_{max} 1744 cm^{-1}). The EIMS of **1** showed peaks at *m*/*z* 466 [M]$^+$, 407 [M − OAc]$^+$, and 346 [M − 2 HOAc]$^+$, suggesting the presence of two acetoxy groups in **1**. The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of six methyl groups, including a methyl attached to a methine carbon (δ 0.82, 3H, d, J = 6.6 Hz), two methyls attached to oxygen-bearing carbons (δ 1.53, 3H, s and 1.51, 3H, s), one methyl attached to an α,β-unsaturated-γ-lactone (δ 1.89, 3H, s), and two acetate methyl groups (δ 2.13, 3H, s; 2.18, 3H, s). Three oxygenated methine protons (δ 3.75, 1H, m; 5.03, 1H, dd, J = 5.3, 1.6 Hz; and 5.95, 1H, brs) were assigned to be H-9, H-13, and H-14, respectively, by comparison with the ^{1}H NMR spectral data of pachyclavulariolide E (**7**).[6a] In the ^{13}C NMR spectrum of **1** (Table 1), a set of resonances at δ 173.1 (C-16, s), 155.7 (C-1, s), 128.6 (C-15, s), 105.5 (C-2, s), and 9.5 (C-17, s) could be assigned to the α-methyl-γ-hydroxybutenolide substructure by comparison with the

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Table 1. ^{1}H NMR Chemical Shifts for Diterpenes **1**−**6**

proton	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
1					3.28 dd (9.6, 8.1)	3.32 s
2		4.90 d (10.8)	4.96 d (10.8)			
3α	1.83 d (15.0)[b]	2.79 d (13.6)	2.77 d (13.6)	3.43 d (11.4)	3.43 d (12.6)	3.12 s
3*β*	3.02 d (15.0)	2.19 d (13.6, 10.8)	2.22 dd (13.6, 10.8)	3.15 d (11.4)	2.94 d (12.6)	3.12 s
4						
5	3.45 dd (8.7, 1.8)	5.41 t (7.7)	5.40 m	5.46 t (7.9)	5.29 t (7.2)	5.18 t (7.3)
6	2.04 m	2.05 m	2.10 m	1.20 m	1.98 m	2.08 m
			2.02 m			
					1.52 m	
7	1.37 m	1.88 m	1.85 m	1.84 m	1.77 m	1.83 m
	1.25 m	1.15 m	1.12 m	1.17 m	1.17 m	1.18 m
8	1.67 m	1.01 m	1.11 m	0.86 m	0.93 m	0.98 m
9	3.75 m	3.66 dd (14.4, 8.2)	3.68 m	3.68 ddd (9.7, 9.7, 7.4)	3.67 ddd (11.1, 8.1, 1.5)	3.67 ddd (9.4, 9.4, 7.0)
10α	2.20 m	1.61 m	1.67 m	1.61 m	1.26 m	1.67 m
10*β*		2.11 m	2.08 m			2.11 m
11	1.83 m	2.24 m	1.85 m	2.29 m	2.21 m	2.32 m
		1.68 m	1.66 m	1.67 m	1.26 m	1.67 m
12						
13	5.03 dd (5.3, 1.6)	3.37 d (12.0)	4.80 dd (12.8, 2.5)	3.45 s	3.16 dd (11.1, 8.1)	3.41 s
14α	5.95 brs	2.93 d (12.6)	2.98 d (14.7)			
14*β*		2.63 dd (12.6, 12.0)	2.77 dd (12.8, 12.8)	5.57 br s	5.01 d (8.1)	4.90 s
15					2.98 m	
16						
17	1.89 s	1.84 s	1.80 s	1.92 s	1.31 d (6.9)	1.40 s
18	1.53 s	1.74 s	1.94 s	1.74 s	1.72 s	1.76 s
19	0.82 d (6.6)	0.84 d (6.5)	0.85 d (6.3)	0.80 d (6.4)	0.81 d (6.6)	0.83 d (6.5)
20	1.51 s	1.26 s	1.32 s	1.32 s	1.23 s	1.27 s
acetate CH_3	2.13 s; 2.18 s					

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] *J* values (in Hz) in parentheses. The values are downfield from TMS.

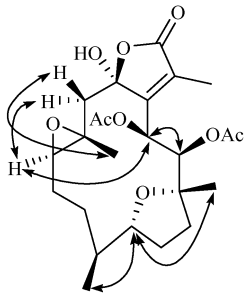


Figure 1. Selective NOE correlations of pachyclavulariolide M (**1**).

^{13}C NMR data of a known metabolite, pachyclavulariolide E, and could be further confirmed by UV absorption at 219 nm. It was found that the spectral data (^{1}H and ^{13}C NMR) of **1** were very similar to those of pachyclavulariolide E, except that the NMR signals for the 4,5-double bond in **7** were missing, and instead the resonances for an epoxide were observed in the ^{1}H NMR (*δ* 3.45, dd, *J* = 8.7, 1.8 Hz) and ^{13}C NMR (*δ* 64.7, d; 58.9, s). On the basis of the above results and by the assistance of ^{1}H−^{1}H COSY and HMBC experiments (Figure 1S, Supporting Information), the molecular framework of **1** could be established. The relative stereochemistry of **1** was further determined on the basis of the results of a NOESY experiment (Figure 1). Key NOE correlations for **1** showed NOE interactions between H-5 and H-14, but not between H-5 and H_3-18. Also H-9 showed NOE responses with H_3-19 and H_3-20, but not with H-13, which exhibited an NOE interaction with H-14. On the basis of these observations, the structure of **1**, including the relative stereochemistry, was established unambiguously.

Pachyclavulariolide N (**2**) was obtained as a white powder. Its HRFABMS established a molecular formula of $C_{20}H_{30}O_4$. Thus, six degrees of unsaturation were determined for **2**. The IR spectrum of **2** showed the presence of hydroxy (ν_{max} 3350 cm^{-1}) and ester carbonyl (ν_{max} 1738 cm^{-1}) groups. The FABMS of **2** showed peaks at *m*/*z* 335 $[M + H]^+$ and 317 $[M + H - H_2O]^+$, suggesting the presence of a hydroxy group. The ^{1}H NMR spectrum of **2** (Table 1) showed signals for three oxymethines (*δ* 3.37, 1H, d, *J* = 12.0 Hz; 3.66, 1H, dd, *J* = 14.4, 8.2 Hz; 4.90, 1H, d, *J* = 10.8 Hz) and four methyls (*δ* 1.26, 3H, s; 1.74, 3H, s; 1.84, 3H, s; 0.84, 3H, d, *J* = 6.5 Hz). The ^{13}C NMR spectrum of **2** (Table 2) showed the presence of 20 carbons, including characteristic signals for an α-methyl-*γ*-butenolide (*δ* 171.0, C-16, s; 163.2, C-1, s; 125.9, C-15, s; 79.2, C-2, d; 8.9, C-17, q), a trisubstituted olefin (*δ* 131.2, d; 130.1, s), two oxymethines (*δ* 84.4, d; 73.8, d), and one quaternary oxygenated carbon (*δ* 84.4, s). The above observations revealed that the structure of **2** should be very similar to that of pachyclavulariolide (**8**),[6b] except that one oxymethine group in **8** had been replaced by a methylene group in **2**. By ^{1}H−^{1}H COSY and HMBC correlations (Figure 2S, Supporting Information), it was found that C-14 of **2** is a methylene carbon. Thus, metabolite **2** should possess the same molecular framework as that of 14-dehydroxypachyclavulariolide. The relative stereochemistry of **2** was determined on the basis of the NOE correlations observed in a NOESY spectrum (Figure 2). Assuming the *β*-orientation of H-9, it was found that H-9 exhibited NOE correlations with H_3-19 (*δ* 0.84, 3H, d, *J* = 6.5 Hz). Thus, H_3-19 should be positioned on the *β*-face, and H-8 should be located on the α-face. H-8 showed an NOE interaction with H-13, which further exhibited an NOE response with H-2 (*δ* 4.90, d, *J* = 10.8 Hz), implying the α-orientation of H-2 and *β*-orientation of the OH-13. The α-oriented H-13 was found to show an NOE interaction with one of the C-14 protons (*δ* 2.93, d, *J* = 12.6 Hz), which was subsequently assigned as the α-oriented protons. Thus, the other H-14 (*δ* 2.63, dd, *J* = 12.6, 12.0 Hz) should be positioned on the *β*-face and was found to exhibit NOE responses with both H_3-20 and H_3-17, revealing the *β*-orientation of H_3-20. On the

Table 2. ^{13}C NMR Chemical Shifts for Diterpenes **1**−**6**

carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
1	155.7 s[b]	163.2 s	161.9 s	159.1 s	60.3 d	62.9 d
2	105.5 s	79.2 d	79.1 d	199.2 s	205.2 s	206.3 s
3	46.7 t	44.5 t	44.3 t	53.4 t	49.7 t	54.0 t
4	58.9 s	130.1 s	130.6 s	126.6 s	127.7 s	127.3 s
5	64.7 d	131.2 d	130.9 d	132.7 d	131.1 d	131.6 d
6	25.0 t	25.2 t	25.0 t	29.3 t	29.8 t	25.0 t
7	29.5 t	32.9 t	32.6 t	33.0 t	33.6 t	33.7 t
8	40.7 d	40.2 d	40.1 d	39.6 d	39.0 d	40.7 d
9	85.3 d	84.4 d	85.2 d	84.4 d	85.0 d	85.0 d
10	30.6 t	30.8 t	30.3 t	30.9 t	30.1 t	30.6 t
11	39.3 t	36.9 t	36.8 t	37.2 t	37.4 t	36.1 t
12	82.3 s	84.4 s	82.9 s	84.2 s	83.7 s	83.8 s
13	74.5 d	73.8 d	72.7 d	72.2 d	76.1 d	76.3 d
14	70.0 d	29.1 t	26.5 t	81.4 d	76.8 d	77.1 d
15	128.6 s	125.9 s	125.5 s	126.5 s	38.8 d	74.9 d
16	173.1 s	171.0 s	170.1 s	172.9 s	177.5 s	176.4 s
17	9.5 q	8.9 q	8.5 q	9.3 q	16.8 q	19.0 q
18	20.0 q	16.7 q	20.7 q	17.4 q	17.0 q	18.2 q
19	15.6 q	16.0 q	15.8 q	15.7 q	15.1 q	16.0 q
20	22.8 q	19.8 q	20.5 q	21.5 q	21.6 q	20.6 q
acetate						
CH_3	20.9 q					
CO	169.6 s					
acetate						
CH_3	21.1 q					
CO	169.0 s					

[a] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [b] Multiplicity deduced by DEPT and indicated by usual symbols. The values are downfield from TMS.

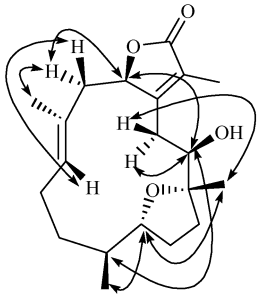


Figure 2. Selective NOE correlations of pachyclavulariolide N(**2**).

basis of the above observations, the relative configuration of **2** was unambiguously established.

Pachyclavulariolide O (**3**) was obtained as a white gum. Its HRFABMS established the molecular formula $C_{20}H_{30}O_4$. Thus, six degrees of unsaturation were determined for **3**. The IR spectrum of **3** showed the presence of a hydroxy group (ν_{max} 3350 cm^{-1}) and an α,β-unsaturated-γ-lactone (ν_{max} 1740 cm^{-1}). The EIMS of **3** showed peaks at *m*/*z* 334 $[M]^+$ and 316 $[M - H_2O]^+$, suggesting the presence of a hydroxy group in **3**. The ^1H NMR spectrum of **3** (Table 1) showed the signals of three oxymethine protons (δ 3.68, 1H, m; 4.80, 1H, dd, J = 12.8, 2.5 Hz; 4.96, 1H, d, J = 10.8 Hz), one olefinic proton (δ 5.40, 1H, m), and four methyls (δ 0.85, 3H, d, J = 6.3 Hz; 1.32, 3H, s; 1.80, 3H, s; 1.94, 3H, s). The ^{13}C NMR spectrum of **3** (Table 2) showed the presence of 20 carbons, including signals for a set of α-methyl-γ-butenolide carbons (δ 170.1, C-16, s; 161.9, C-1, s; 125.5, C-15, s; 79.1, C-2, d; and 8.5, C-17, q), a trisubstituted olefin (δ 130.6, s; 130.9, d), two oxymethines (δ 72.7, d; 85.2, d), and one quaternary oxygenated carbon (δ 82.9, s). The above observations revealed that the structure of **3** should be similar to that of pachyclavulariolide **8**; however, one oxymethine group of **8** had to be converted into a methylene in **3**. Analyses of the $^1H-^1H$ COSY and HMBC correlations (Figure 3S, Supporting Information) revealed that C-14 is a methylene carbon in **3**, as in **2**.

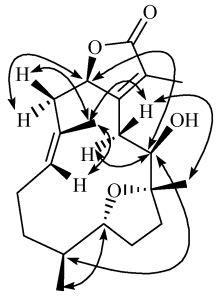


Figure 3. Selective NOE correlations of pachyclavulariolide O (**3**).

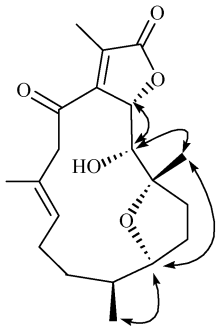
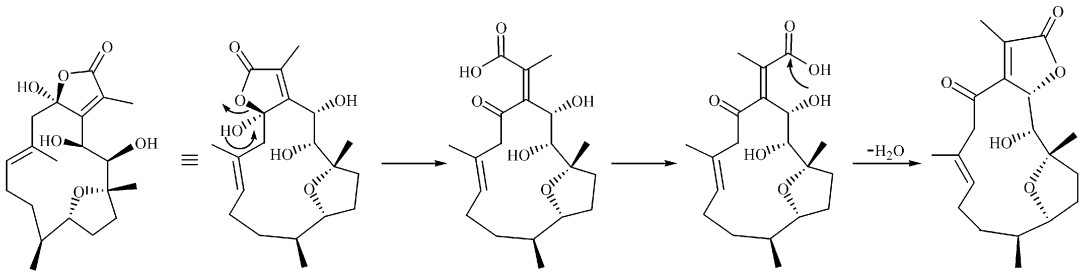


Figure 4. Selective NOE correlations of pachyclavulariolide P (**4**).

However, a NOESY spectrum of **3** showed interactions of H-5 with H_3-18, revealing the *cis* orientation of the 4,5-double bond. Assuming the α-orientation of H-2 (δ 4.96, d, J = 10.8 Hz), it was found that H-2 interacted with H-13 (δ 4.80, dd, J = 12.8, 1.5 Hz). Thus, OH-13 should be positioned on the β-face. On the basis of the above results and other key NOE interactions observed (Figure 3), the structure of **3**, including the relative configuration, was fully determined.

Pachyclavulariolide P (**4**) was isolated as a white solid and had the molecular formula $C_{20}H_{28}O_5$ as determined by HREIMS. Its IR spectrum exhibited stretches for a hydroxy at 3447 cm^{-1}, a carbonyl of an α,β-unsaturated-γ-lactone at 1761 cm^{-1}, and a carbonyl at 1697 cm^{-1}. The EIMS spectrum of **4** showed peaks at *m*/*z* 348 $[M]^+$ and 330 $[M - H_2O]^+$, suggesting the presence of one hydroxy group in **4**. From ^1H and ^{13}C NMR spectral data (Tables 1 and 2), a trisubstituted olefin was deduced from the signals of two carbons at δ 126.6 (s) and 132.7 (d). The ^{13}C NMR spectrum confirmed the presence of an α-methyl-γ-butenolide moiety, which showed signals at δ 172.9 (C-16, s); 159.1 (C-1, s); 126.5 (C-15, s); 81.4 (C-14, d); and 9.3 (C-17, q). An additional carbonyl carbon resonating at δ 199.2 pointed to the presence of a conjugated ketone moiety. Carbons resonating at δ 72.2 (C-13, d), 84.2 (C-12, s), and 84.4 (C-9, d) indicated the presence of two oxymethines and one oxygenated quaternary carbon. According to the above results, together with the correlations observed in $^1H-^1H$ COSY and HMBC spectra (Figure 4S, Supporting Information), the gross structure of **4** was established and found to be highly related with those of **1**−**3**. The relative stereochemistries of the chiral centers were determined by a NOESY experiment (Figure 4). NOE correlations between H-13 and H-14/H_3-20 were observed, suggesting the β-orientations for H-13, H-14, and H_3-20 in compound **4**. Therefore, the structure of pachyclavulariolide P was suggested to be as that illustrated in Figure 4. One reasonable biosynthetic pathway (Figure 5) suggested that the possible precursor, pachyclavulariolide L (**9**), could be hydrolyzed to ketocaboxylic acid **10** and further isomerized at the 1,15-double bond to afford intermediate **11**. Lacton-



9 **10** **11** **4**

Figure 5. Proposed biogenetic relationships between pachyclavulariolides L (**9**) and P (**4**).

ization of **11** should yield metabolite **4**. This proposal predicts the C-8 (*S**), C-9 (*R**), C-12 (*S**), C-13 (*R**), and C-14 (*S**) configurations in **4**.

Pachyclavulariolide Q (**5**) was isolated as a white solid. The molecular formula $C_{20}H_{30}O_5$ was established by HREIMS, indicating six degrees of unsaturation. Its IR spectrum exhibited a hydroxy stretch at 3439 cm^{-1}, a γ-lactone carbonyl at 1743 cm^{-1}, and a carbonyl at 1699 cm^{-1}. The EIMS showed peaks at *m*/*z* 350 $[M]^+$ and 332 $[M - H_2O]^+$, suggesting the presence of one hydroxy group in **5**. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) were nearly identical with those of **4**, except that the signals for carbons of the 1,15-double bond disappeared and were replaced by signals of two sp^3 methine carbons. Thus, the 1,15-double bond in **4** is reduced to a single bond in **5**. Key NOE correlations observed in the NOESY spectrum of **5** revealed that it possesses the same relative configurations at C-8, C-9, C-12, C-13, and C-14 as in **4**. Furthermore, H-1 showed NOE interactions with H-14 and H_3-17. Thus, the structure of **5** was established as shown.

Pachyclavulariolide R (**6**) was isolated as a white solid, and its molecular formula $C_{20}H_{30}O_6$ was assigned from HREIMS. The EIMS showed peaks at *m*/*z* 366 $[M]^+$, 348 $[M - H_2O]^+$, and 330 $[M - 2\ H_2O]^+$, suggesting the presence of two hydroxy groups in **6**. The ^1H and ^{13}C NMR spectra were very similar to those of **5**, while the signal for an additional quaternary oxygenated carbon appeared at δ 74.9 (s). Furthermore, a hydroxy group attached at the C-15 position was confirmed by HMBC correlations observed between H_3-17 (δ 1.40, 3H, s) and C-15, C-16, and C-1; and H-14 (δ 4.90, 1H, s) and C-1, C-2, C-12, C-13, and C-16. The relative configurations at all chiral centers, except C-15, were found to be the same for both **5** and **6**, by comparison of the NOE data. Furthermore H_3-17 showed NOE interactions with H-1, revealing the α-orientation of the hydroxy group at C-15.

Similar to metabolite **4**, compounds **5** and **6** could be biosynthesized via a pathway related to Figure 5. The cytotoxicity of metabolites **1**, **2**, **4**, and **5** against the growth of P-388, KB, A549, and HT-29 was studied. It was found that only **1** showed significant activity against P-388 with an $ED_{50} = 3.2\ \mu g/mL$.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. Melting points were determined using a Fisher-Johns melting point apparatus and were uncorrected. IR spectra were measured on a Hitachi I-2001 or a Jasco FT/IR-5300 infrared spectrophotometer. Ultraviolet spectra were recorded on a Hitachi U-3210 UV spectrophotometer. The NMR spectra were recorded on a Bruker AMX 400 FT NMR at 400 MHz for ^1H and 100 MHz for ^{13}C, in $CDCl_3$ using TMS as an internal standard, unless otherwise indicated. MS spectra were obtained with a VG QUATTRO GC/MS spectrometer. HRMS spectra were recorded on a JEOL JMX-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for CC. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytic TLC.

Collection, Extraction, and Separation. Frozen specimens were freeze-dried and then subjected to extraction, and the crude extracts were evaluated for cytotoxicity as described previously.[7] The extract was subjected to column chromatography on silica gel. Elution was performed with EtOAc−*n*-hexane (stepwise, 0−100% EtOAc) to afford 47 fractions. Fraction 25 eluted with EtOAc−*n*-hexane (1:2) was further separated by silica gel column chromatography eluting with CH_2Cl_2−*n*-hexane (2:1) to obtain compound **2**. Fraction 26 eluted with EtOAc−*n*-hexane (3:2) was further separated by silica gel column chromatography eluting with EtOAc−*n*-hexane (stepwise, 0−100% EtOAc) to obtain a mixture of **3**, **4**, and **5**. Further chromatography of this mixture using pure CH_2Cl_2 as eluent yielded pure **3**, **4**, and **5**. Similarly, fraction 27 eluted with EtOAc−*n*-hexane (1:1) was further purified by silica gel column chromatography eluting with EtOAc−*n*-hexane (2:1) to obtain compound **6**. Finally, fraction 31 eluted with EtOAc−*n*-hexane (2:1) was separated by silica gel chromatography using EtOAc−CH_2Cl_2 (1:3), to obtain compound **1**.

Pachyclavulariolide M (1): white solid (2.9 mg); mp 84−86°C; $[\alpha]^{29}_D$ −14° (*c* 0.15, $CHCl_3$); UV (95% EtOH) λ_{max} 219 nm (ϵ 5680); IR (neat) ν_{max} 3391, 1744, 1716 and 1222 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* 466 [0.2, $(M)^+$], 449 [1, $(M - OH)^+$], 407 [6, $(M - OAc)^+$], and 346 [2, $(M - 2\ HOAc)^+$]; HREIMS *m*/*z* 446.2203 (calcd for $C_{24}H_{34}O_9$, 446.2203).

Pachyclavulariolide N (2): white solid (8.8 mg); mp 108−110 °C; $[\alpha]^{31}_D$ −26° (*c* 0.23, $CHCl_3$); UV (95% EtOH) λ_{max} 215 nm (ϵ 9585); IR (neat) ν_{max} 3350, 2930, 1738, 1668, and 1099 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; FABMS *m*/*z* 335 [5, $(M + H)^+$], 317 [5]; HRFABMS *m*/*z* 335.2222 (calcd for $C_{20}H_{31}O_4$, 335.2223).

Pachyclavulariolide O (3): white gum (1.7 mg); $[\alpha]^{26}_D$ −2° (*c* 0.09, $CHCl_3$); UV (95% EtOH) λ_{max} 210 nm (ϵ 8650); IR (neat) ν_{max} 3350, 2926, 1740, 1440, and 1140 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* 334 [0.6, $(M)^+$], 316 [6, $(M - H_2O)^+$]; HREIMS *m*/*z* 334.2144 (calcd for $C_{20}H_{30}O_4$, 334.2145).

Pachyclavulariolide P (4): white solid (28.5 mg); mp 133−135°C; $[\alpha]^{30}_D$ +131° (*c* 0.68, $CHCl_3$); UV (95% EtOH) λ_{max} 219 nm (ϵ 8920); IR (neat) ν_{max} 3447, 1761, 1697, and 1458 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* 348 [11, $(M)^+$], 331 [2, $(M - OH)^+$], and 330 [0.6, $(M - H_2O)^+$]; HREIMS *m*/*z* 348.1937 (calcd for $C_{20}H_{28}O_5$, 348.1937).

Pachyclavulariolide Q (5): white solid (11.6 mg); mp 186−189 °C; $[\alpha]^{30}_D$ +28° (*c* 0.05, $CHCl_3$); IR (neat) ν_{max} 3439, 1743, 1699, and 1458 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* 350 [7, $(M)^+$], 332 [0.5, $(M - H_2O)^+$]; HREIMS *m*/*z* 350.2093 (calcd for $C_{20}H_{30}O_5$, 350.2094).

Pachyclavulariolide R (6): white solid (4.0 mg); mp 204−205 °C; $[\alpha]^{31}_D$ −103° (*c* 0.20, $CHCl_3$); IR (neat) ν_{max} 3395, 1760, 1703, and 1454 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS *m*/*z* 366 [7, $(M)^+$], 349 [1, $(M - OH)^+$], 348 [0.8, $(M - H_2O)^+$], 331 [0.8, $(M - OH - H_2O)^+$], and 330 [0.2, $(M - 2\ H_2O)^+$]; HREIMS *m*/*z* 366.2039 (calcd for $C_{20}H_{30}O_6$, 366.2043).

Cytotoxicity Testing. KB and P-388 cells were kindly provided by J. M. Pezzuto, University of Illinois at Chicago; A549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) were purchased from the American Type Culture Collection. The cytotoxic activities of tested compounds **1**, **2**, **4**, and **5** were assayed by a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[11]

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Supporting Information Available: Figures of selective $^1H-^1H$ COSY and HMBC correlations of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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