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Pochonins K–P: new radicicol analogues from *Pochonia chlamydosporia* var. *chlamydosporia* and their WNT-5A expression inhibitory activities

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ABSTRACT

WNT-5A, a secretory glycoprotein, is related to the proliferation of dermal papilla cells. To develop a hair-growth stimulant, we have been searching for an inhibitor of WNT-5A expression, and have identified an active compound, radicicol (**1**), and isolated six new radicicol analogues, pochonins K–P (**2**–**7**), together with ten known radicicol analogues, including monorden analogue-1 (**8**), pochonin E (**9**), and pochonin F (**10**), from a culture broth of the fungus *Pochonia chlamydosporia* TF-0480. This report describes the structural elucidation of **2**–**7**, determination of the stereochemistries of **8**–**10**, and their biological activities against WNT-5A.

1. Introduction

Hair loss or hair thinning is a common complaint of people living in today's stressful world.[1] Some drugs to counteract such hair loss, such as minoxidil[2,3] and finasteride,[4] have been developed, but their therapeutic effects have been limited. Consequently, there is a need for a new drug that induces hair-growth through a new mechanism.

Human hair follicles are made up of epithelial and mesenchymal dermal cells such as keratinocytes, dermal papilla cells, fibroblasts, and sebaceous cells, and the hair-growth cycle (hair cycle) is controlled via interactions among these cells. The hair shaft is formed by the proliferation and differentiation (keratinization) of follicular keratinocytes. Dermal papillae regulate the proliferation, differentiation and apoptosis of these follicular keratinocytes, which play a key role in the control of the hair cycle.[5] WNT-5A (wingless-type mouse mammary tumor virus integration site family, member 5A) is a secretory glycoprotein that belongs to the WNT family. WNTs are important intercellular signaling molecules that regulate axis formation and organ formation during the fetal stage.[6,7] To develop a hair-growth stimulant, we have been studying molecules that regulate the proliferation of dermal papilla cells. We recently found that WNT-5A was highly expressed in the dermal papillae of depilated skin. WNT-5A expression inhibitor promotes the proliferation of dermal papilla cells.[8] By using inhibitory activity against WNT-5A expression as a guide for bioassay, we obtained radicicol (**1**)[9–12] as a potently active compound and isolated new unique analogues[13] from a culture broth of the fungus *Pochonia chlamydosporia* var. *chlamydosporia*. As a continuation of this work, we isolated six new compounds **2**–**7** (Fig. 1) along with ten known radicicol analogues including monorden analogue-1 (**8**),[14] pochonin E (**9**), and pochonin F (**10**).[15] This paper describes the structures of the new compounds, the determination of the stereochemistries of the three known compounds **8**–**10** (Fig. 2), and their WNT-5A expression inhibitory activities.

2. Results and discussion

The inhibition of WNT-5A expression was measured using the QuantiGene assay (a signal amplification nucleic acid probe assay for the direct quantification of cellular mRNA).[16] Cytotoxicity against dermal papilla cells was measured using the Alamar Blue™ assay. Strain TF-0480 was isolated from a soil sample collected at Fujioka City, Tochigi Prefecture, Japan (1994). Based on its morphological characteristics, the strain was identified as *P. chlamydosporia* var. *chlamydosporia*.[8] The cultured broth was centrifuged and the supernatant was treated with Diaion HP-20. The resin was washed with water and methanol, and the methanol eluent was extracted with ethyl acetate. The ethyl acetate extract was separated chromatographically to give radicicol (**1**) {$[\alpha]_D^{20}$ +194.6

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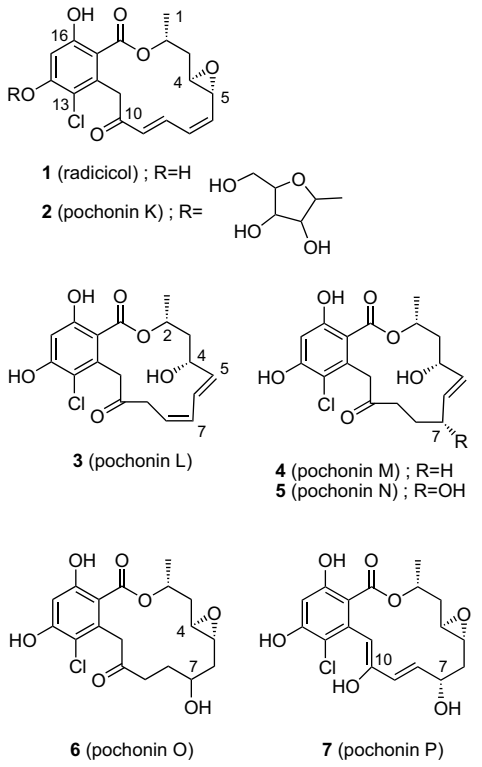

Figure 1. Structures of pochonins K–P (**2**–**7**).

(*c* 1.00, chloroform), lit.;[10] $[\alpha]_D$ +216 (*c* 1.00, chloroform)} and six new compounds **2**–**7**, which were designated pochonins K–P.[17]

The molecular formula of pochonin K (**2**), $[\alpha]_D^{20}$ +23.1 (*c* 0.24, methanol), was established as $C_{23}H_{25}ClO_{10}$ by HRESI-MS (found *m*/*z* 495.1053; calcd for $C_{23}H_{24}^{35}Cl_1O_{10}$ $[M-H]^-$, 495.1058, Δ −0.5 mmu). The UV spectrum of **2** showed maximal absorptions (λ_{max}) at 213 and 262 nm, similar to the findings for **1**. The ^{13}C NMR spectra of **2** exhibited 23 carbon signals (Table 1); 18 carbon signals were almost identical to those of **1**, and the remaining five were attributed to aldofuranose [δ_C 103.3 (d, C-1′), 73.5 (d, C-2′), 71.0 (d, C-3′), 88.4 (d, C-4′), and 63.1 (t, C-5′)]. The location of the aldofuranose was confirmed using long-range H/C coupling from the anomeric proton (δ_H 5.64, H-1′) to the phenolic carbon (δ_C 156.6, C-14) of the radicicol skeleton observed in the HMBC spectrum, and a sharp singlet signal (δ_H 10.2; assigned to 16-OH) was observed in the ^1H NMR spectrum (DMSO-d_6). These findings suggest that an intramolecular hydrogen bond is formed between the hydroxyl group at C-16 and the ester carbonyl group at C-18, and the aldofuranose is linked to position 14, not to position 16.

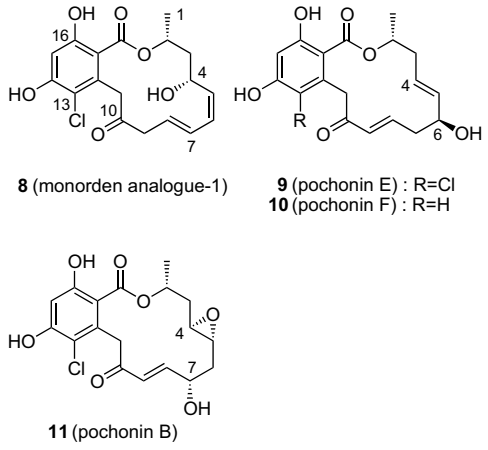

Figure 2. Structures of known radicicol analogues, pochonins B (**11**), E (**9**), and F (**10**), and monorden analogue-1 (**8**).

The molecular formula of pochonin L (**3**), $[\alpha]_D^{20}$ −88.1 (*c* 1.00, acetone), was established as $C_{18}H_{19}ClO_6$ by HRESI-MS, which has two more hydrogens than that of **1** ($C_{18}H_{17}ClO_6$). In the ^1H and ^{13}C NMR (methanol-d_4) spectra of **3** (Tables 1 and 2), signals of the moieties in common with those of **1** were observed: (1) 2-alkyl-3-chloro-4,6-dihydroxybenzoate (C11–C18); (2) a secondary methyl (C-1); and (3) an acyloxy methine (C-2). The NMR signals of the dienone group and epoxide of **1** were absent in the spectrum of **3**; instead, compound **3** exhibited signals assignable to a non-conjugated carbonyl (δ_C 208.3, C-10), a diene [δ_H 5.64 (1H, m, H-5), 6.10 (1H, dd, *J*=14.9, 10.7 Hz, H-6), 6.18 (1H, t, *J*=10.7 Hz, H-7), 5.65 (1H, m, H-8); δ_C 136.9 (d, C-5), 128.2 (d, C-6), 132.1 (d, C-7), 124.9 (d, C-8)], and one additional oxymethine [δ_H 4.14 (1H, td, *J*=9.4, 5.5 Hz, H-4); δ_C 71.8 (s, C-4)]. The proton network from H_3-1 to H-6 and from H-7 to H_2-9 was deduced from the ^1H–^1H COSY spectrum. The long-range H/C couplings between H-4/C-3 (δ_C 42.7), H-4/C-6, H-6/C-4, H-4/C-8, H-8/C-6, and H-8/C-10 suggested a diene moiety located at the C5–C8 position. The coupling constants ($J_{H\text{-}5/H\text{-}6}$=14.9 Hz, $J_{H\text{-}7/H\text{-}8}$=10.7 Hz) of these olefinic protons indicated that the two olefins (C5–C6 and C7–C8) had *E*- and *Z*-configurations, respectively. Thus, the structure of pochonin L was elucidated as **3**, i.e., a C5–C6 and C7–C8 diastereomer of monorden analogue-1 (**8**).[14]

The relative stereochemistry of **3** was assigned according to the results of a NOESY experiment (Fig. 3). The NOE correlations between H-2/H-3b, H-3a/H-4, H-4/H-6, and H-6/H-9a indicated the relative configuration of **3** at C-2, together with the conformation of **1**, and the hydroxyl group at C-4 was determined to have an α-orientation. This conformation was verified using an MM2 calculation (CambridgeSoft, Chem 3D Pro software).

The molecular formula of pochonin M (**4**), $[\alpha]_D^{20}$ −57.0 (*c* 0.61, acetone), was established as $C_{18}H_{21}ClO_6$ by HRESI-MS, which has two more hydrogens than that of **3**. The ^1H and ^{13}C NMR (methanol-d_4) spectra of **4** were similar to those of **3**, except for the absence of one olefin (Tables 1 and 2). The proton network from H_3-1 to H_2-9 was deduced from the COSY spectrum, and two new methylene signals were assigned to the C-7 [δ_H 2.18 (1H, m, H-7a), 1.91 (1H, m, H-7b); δ_C 32.4 (t, C-7)] and C-8 [δ_H 1.75 (1H, m, H-8a), 1.65 (1H, m, H-8b); δ_C 21.0 (t, C-8)] positions. The coupling constant ($J_{H\text{-}5/H\text{-}6}$= 15.3 Hz) of olefinic protons indicated that the geometry of the olefin at the C5–C6 position had an *E*-configuration.

The relative stereochemistry of **4** was assigned based on a ^1H–^1H coupling constants (Table 2) and NOESY data (Fig. 4). The NOE correlations observed for H_3-1/H-4, H-4/H-6, and H-6/H-9a in the NOESY spectrum of **4**, suggesting that the orientation of H_3-1, H-4, H-6, and H-9a in **4** was on the same side of the macrocyclic ring. The NOE correlations were observed for H-5/H-7b and H-7b/H-9b in the NOESY spectrum of **4** and the large $J_{H\text{-}4/H\text{-}5}$ value (7.9 Hz) indicates that the relationship for H-4/H-5 was *anti*. The relative configurations of **4** were determined to be α-methyl (C-2) and α-hydroxyl (C-4).

The molecular formula of pochonin N (**5**), $[\alpha]_D^{20}$ −77.3 (*c* 1.00, methanol), was established as $C_{18}H_{21}ClO_7$ by HRESI-MS, which has one more oxygen than that of **4**. The ^1H and ^{13}C NMR (methanol-d_4) spectra of **5** were similar to those of **4** except for one additional oxymethine [δ_H 3.92 (1H, dt, *J*=8.5, 4.9 Hz, H-7); δ_C 73.7 (d, C-7)] (Tables 1 and 2). The long-range H/C couplings between H-7/C-5 (δ_C 134.6), H-7/C-8 (δ_C 29.3), H-9b (δ_H 2.29)/C-7, H-5 (δ_H 5.33)/C-6 (δ_C 135.1), and H-5/C-7 suggested a new hydroxyl group located at the C-7 position. The geometry of the olefin at C5-C6 was determined to have an *E*-configuration based on the coupling constant ($J_{H\text{-}5/H\text{-}6}$= 15.3 Hz).

Table 1
^{13}C NMR data (500 MHz) for radicicol (**1**) and pochonins K–P (**2**–**7**)

Position	**1**[a]		**2**[b]		**3**[b]		**4**[b]		**5**[b]		**6**[b]		**7**[b]	
	δ_C	Mult.	δ_C	Mult.	δ_C	Mult.	δ_C	Mult.	δ_C	Mult.	δ_C	Mult.	δ_C	Mult.
1	18.5	q	18.7	q	20.6	q	20.5	q	20.6	q	19.2	q	23.4	q
2	71.9	d	72.3	d	71.3	d	71.8	d	71.7	d	72.6	d	66.5	d
3	37.0	t	37.9	t	42.7	t	43.1	t	43.2	t	37.8	t	42.1	t
4	56.1	d	56.8	d	71.8	d	71.2	d	70.9	d	55.7	d	57.3	d
5	55.8	d	56.5	d	136.9	d	134.4	d	134.6	d	55.2	d	56.7	d
6	135.9	d	137.2	d	128.2	d	133.0	d	135.1	d	39.6	t	40.5	t
7	130.4	d	130.9	d	132.1	d	32.4	t	73.7	d	68.2	d	70.2	d
8	139.0	d	140.7	d	124.9	d	21.0	t	29.3	t	31.5	t	139.3	d
9	131.2	d	131.7	d	42.5	t	40.2	t	38.0	t	38.3	t	122.4	d
10	199.1	s	199.1	s	208.3	s	208.5	s	207.7	s	209.2	s	153.8	s
11	46.5	t	46.3	t	45.0	t	47.3	t	47.4	t	47.5	t	103.6	d
12	136.4	s	134.4	s	135.8	s	137.2	s	137.3	s	136.5	s	137.5	s
13	115.6	s	117.2	s	116.0	s	116.6	s	116.6	s	116.1	s	108.2	s
14	157.9	s	156.6	s	157.9	s	159.8	s	159.9	s	159.2	s	163.1	s
15	103.8	d	105.3	d	104.0	d	104.0	d	104.1	d	104.0	d	103.5	d
16	160.6	s	157.8	s	158.5	s	163.4	s	163.9	s	162.4	s	163.2	s
17	111.0	s	117.2	s	113.4	s	109.0	s	108.1	s	110.0	s	100.3	s
18	168.8	s	168.4	s	168.9	s	171.5	s	171.6	s	170.8	s	166.4	s
1′			103.3	d										
2′			73.5	d										
3′			71.0	d										
4′			88.4	d										
5′			63.1	t										

[a] Measured in acetone-d_6.
[b] Measured in methanol-d_4.

The relative stereochemistry of **5** was assigned based on ^{1}H–^{1}H coupling constants ($J_{H\text{-}4/H\text{-}5}$=8.5 Hz, $J_{H\text{-}6/H\text{-}7}$=8.5 Hz) (Table 2) and NOESY data (Fig. 4) in the same manner described for **4**.

Pochonins L (**3**), M (**4**), and N (**5**) are the first examples of radicicol analogues in which the double bond has an *E*-configuration at C5–C6.

The molecular formula of pochonin O (**6**), $[\alpha]_D^{20}$ −46.7 (*c* 1.00, acetone), was established as $C_{18}H_{21}ClO_7$ by HRESI-MS. This formula is the same as that of **5**. The NMR signals of the olefin moiety of **5** were absent in the spectrum of **6** and, instead, compound **6** had signals assignable to an epoxide [δ_H 2.83 (1H, m, H-4), 2.69 (1H, m, H-5); δ_C 55.7 (d, C-4), 55.2 (d, C-5)]. The locations of the epoxide at C4–C5 and the hydroxyl group at C-7 were deduced from the COSY and HMBC spectra. Compound **6** is too flexible to enable determination of the relative configuration of the secondary alcohol function at C-7 by NOESY correlation.

The molecular formula of pochonin P (**7**), $[\alpha]_D^{20}$ −18.6 (*c* 0.36, acetone), was established as $C_{18}H_{19}ClO_7$ by HRESI-MS. This formula is the same as that of a known radicicol analogue, pochonin B (**11**) (Fig. 2). The ^{13}C NMR (methanol-d_4) spectrum of **7** was similar to those of **11**, except for the carbonyl moiety (Table 1 and Experimental section). The signal caused by the carbonyl group at C-10 of **11** was absent in the ^{13}C NMR spectrum of **7** and, instead, compound **7** had signals assignable to one olefinic proton signal [δ_H 6.80 (1H, s, H-11); δ_C 103.6 (d, C-11)] and one sp^2 quaternary carbon [δ_C 153.8 (s, C-10)]. The proton network from H_3-1 to H-9 was deduced from the COSY and HMBC spectra. The locations of the epoxide, hydroxyl group, and one olefin moiety were easily assigned to C4–C5, C-7, and C8–C9, respectively. The long-range H/C couplings between H-11/C-9 (δ_C 122.4), H-11/C-10, H-11/C-12 (δ_C 137.5), H-11/C-13 (δ_C 108.2), and H-11/C-17 (δ_C 100.3) suggested the presence of a tri-substituted olefin at C10–C11. The geometry of olefin at C8–C9 was determined to have an *E*-configuration based on the coupling constant ($J_{H\text{-}8/H\text{-}9}$=15.9 Hz). Thus, the structure of **7** was determined to be the enol-form tautomer of **11**.

The relative stereochemistry of **7** was assigned based on the NOESY experiment. The NOE correlations between H-4/H-8, H-7/H-9, H-8/H-11, and H-5/H-7 made it possible to elucidate the relative configuration of **7** at C-2, C-4, C-5, and C-7 together with the conformation of **11**.

It should be noted that compound **7** is an enol-form tautomer of pochonin B (**11**). Compound **7** is substantially stable and shows a single spot on silica gel TLC (R_f value: **7**; 0.35, **11**; 0.50, developing solvent: chloroform/methanol=90:10, v/v) and the presence of **7** in the culture broth was recognized by TLC and HPLC analyses. Compound **7** maintained high purity (about 95%) in the solid state for 7 days. After 30 days, the ratio of **7** and **11** shifted from 95:5 to 50:50 in the solid state by HPLC analysis. Compound **11** was stable under the same conditions.

Pochonins A–F were reported by Hellwig et al. (2003).[15] The relative stereochemistry of pochonin B (**11**) was assigned by the NOESY experiment and the 7-hydroxyl group was determined to have an α-configuration (Fig. 2). Both pochonins E (**9**) and F (**10**) have a 6-hydroxyl group, but lack an epoxide ring (Fig. 2) and are too flexible to determine their relative configuration at C-6.[15] We also isolated compounds **9** and **10** and attempted to assign a relative configuration at C-6 based on the results of a NOESY experiment. The geometries of the two olefins at C4–C5 and C8–C9 in **9** and **10** were determined to be *E*-configurations based on the coupling constant ($J_{H\text{-}4/H\text{-}5}$=15.3 Hz, $J_{H\text{-}8/H\text{-}9}$=15.9 Hz, respectively) (Table 3). NOE correlations were observed for H_3-1/H-4, H-4/H-6, H-6/H-8, and H-8/H-11a in the NOESY spectra of **9** and **10**, suggesting that the orientations of H_3-1, H-4, H-6, H-8, and H-11a in **9** and **10** were on the same side of the macrocyclic ring. The relative configurations of **9** and **10** were both determined to be α-methyl (C-2) and β-hydroxyl (C-6) (Fig. 5).

We also isolated known compound, monorden analogue-1 (**8**),[14] that have only been reported to have a planar structure. The relative stereochemistry of **8** was elucidated based on ^{1}H–^{1}H coupling constants (Table 3) and NOESY data (Fig. 5). The coupling constants ($J_{H\text{-}5/H\text{-}6}$=11.0 Hz, $J_{H\text{-}7/H\text{-}8}$=15.2 Hz) of these olefinic protons indicated that the geometries of the two olefins (C5–C6 and C7–C8) in **8** were the *Z*- and *E*-configurations, respectively (Table 3). NOE correlations were observed for H-4/H-7, H-3b/H-5, H-5/H-6, H-6/H-8, and H-7/H-11a in the NOESY spectrum of **8**, and the $J_{H\text{-}4/H\text{-}5}$ value was relatively large (11.0 Hz), indicating that the relationship for

Table 2
^{1}H NMR data (500 MHz) for radicicol (**1**) and pochonins K–P (**2**–**7**) (*J* in Hz)

Position	**1**[a]		**2**[b]		**3**[b]		**4**[b]	
	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)
1	1.55	d (6.6)	1.52	d (6.1)	1.34	d (6.1)	1.37	d (6.7)
2	5.43	ddq (3.8, 3.3, 6.6)	5.38	m	5.03	m	5.45	m
3	2.43	ddd (15.1, 3.3, 2.7)	2.43	dt (14.6, 2.4)	2.20	ddd (14.0, 11.6, 5.5)	2.10	ddd (15.3, 7.9, 3.0)
	1.78	ddd (15.1, 8.5, 3.8)	1.65	ddd (14.6, 8.5, 3.7)	1.73	ddd (14.0, 9.4, 3.4)	2.00	m
4	3.04	dt (8.5, 2.7)	3.06	dt (8.5, 2.4)	4.14	td (9.4, 5.5)	4.23	td (7.9, 3.0)
5	3.19	br s	3.35	m	5.64	m	5.24	dd (15.3, 7.9)
6	5.75	dd (10.7, 3.6)	5.75	dd (10.4, 4.3)	6.10	dd (14.9, 10.7)	5.50	ddd (15.3, 10.4, 4.3)
7	6.24	dd (10.7, 9.6)	6.20	t (10.4)	6.18	t (10.7)	2.18	m
							1.91	m
8	7.50	dd (15.9, 9.6)	7.57	dd (15.9, 10.4)	5.65	m	1.75	m
							1.65	m
9	6.12	d (15.9)	6.06	d (15.9)	3.29	dd (15.5, 6.7)	2.58	ddd (19.5, 10.4, 3.0)
					3.06	dd (15.5, 8.2)	2.30	ddd (19.5, 6.7, 3.0)
11	4.44	d (16.2)	4.01	d (16.5)	4.48	d (18.3)	4.42	d (18.3)
	3.90	d (16.2)	3.92	d (16.5)	3.91	d (18.3)	4.12	d (18.3)
15	6.57	s	6.85	s	6.47	s	6.45	s
1′			5.64	d (4.9)				
2′			4.22	dd (6.7, 4.9)				
3′			4.08	dd (6.7, 3.0)				
4′			4.17	dd (3.7, 3.0)				
5′			3.68	dd (12.2, 3.7)				
			3.64	dd (12.2, 3.7)				

Position	**5**[b]		**6**[b]		**7**[b]	
	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)
1	1.36	d (6.7)	1.40	d (6.7)	1.21	d (6.7)
2	5.49	m	5.25	m	3.94	m
3	2.10	ddd (15.3, 7.3, 3.0)	1.99	m	1.72	dt (14.0, 6.1)
	2.03	ddd (15.3, 8.5, 1.8)	1.80	m	1.60	dt (14.0, 5.5)
4	4.27	td (8.5, 3.0)	2.83	m	2.90	m
5	5.33	dd (15.3, 8.5)	2.69	m	2.88	m
6	5.43	dd (15.3, 8.5)	2.10	m	1.84	m
			1.43	m		
7	3.92	dt (8.5, 4.9)	3.75	m	4.48	dd (5.5, 4.9)
8	1.75–1.84	m	1.89	m	6.63	dd (15.9, 5.5)
			1.60	m		
9	2.54	ddd (19.5, 9.8, 3.0)	2.43	m	6.44	d (15.9)
	2.29	ddd (19.5, 6.7, 3.0)	1.77	m		
11	4.37	d (17.7)	4.25	d (18.3)	6.80	s
	4.08	d (17.7)	4.17	d (18.3)		
15	6.47	s	6.42	s	6.46	s

[a] Measured in acetone-d_6.
[b] Measured in methanol-d_4.

H-4/H-5 was *anti*. The relative configurations of **8** were determined to be α-methyl (C-2) and α-hydroxyl (C-4).

The WNT-5A expression inhibitory activities and cytotoxicities against dermal papilla cells of pochonins K–P (**2**–**7**), radicicol (**1**) and known isolated radicicol analogues (monocillins I–IV,[18,19] pochonins B and D–F, monorden E,[20,21] and monorden analogue-1) (Figs. 2 and 6) are summarized in Table 4.

Radicicol (**1**) showed the strongest WNT-5A expression inhibitory activity of the tested samples, although it also showed high cytotoxicity. The inhibitory activities of pochonins K (**2**) and O (**6**) were 10-fold weaker than that of **1**. Interestingly, compound **6** showed no cytotoxicity at concentrations above 100 μM. The data in Table 4 imply that the 4,5-epoxide or 4,5-*E*-olefin moieties present in **2** and **6**, or **9** and **10**, respectively, may be necessary for radicicol-type compounds that are designed to inhibit WNT-5A expression. The chlorine atom at C-13 may decrease the toxicity against dermal papilla cells.

3. Experimental section

3.1. General experimental procedures

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz using a JEOL Alpha 500, Lambda 500, or ECA 500 spectrometer. The coupling constants (*J*) are given in hertz (Hz), and the abbreviations s, d, t, q, br, and m refer to singlet, doublet, triplet, quartet, broad, and multiplet peaks, respectively. All assignments were based on ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single- or multiple-quantum coherence (HSQC or HMQC, respectively), heteronuclear multiple-bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY) methods. Chemical shifts are reported in parts per million (ppm) with the solvent peaks used as an internal standard. Electrospray ionization (ESI) mass spectra were obtained using a Micromass Platform LC mass spectrometer. Melting points were

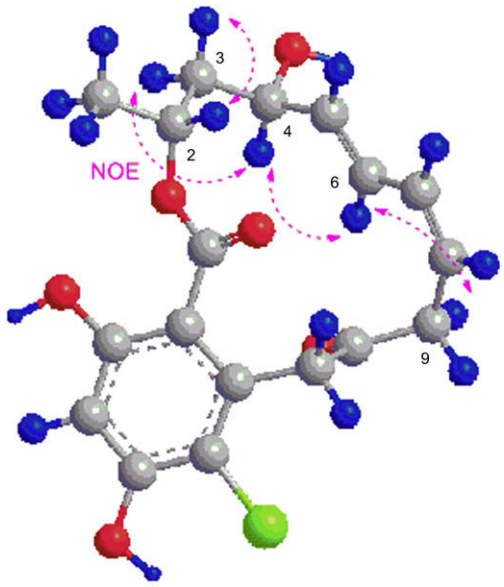

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Figure 3. NOEs observed for pochonin L (**3**) in the NOESY spectrum. NOEs are expressed as dotted-line arrows. The conformation was energy-minimized based on MM2 calculations.

Table 3
^{1}H NMR data (500 MHz) for radicicol analogues **8**–**10** (*J* in Hz)

Position	**8**[b]		**9**[b]		**10**[a]	
	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)	δ_H	Mult. (*J* in Hz)
1	1.33	d (6.1)	1.19	d (6.4)	1.28	d (6.7)
2	4.78	ddq (11.6, 1.8, 6.1)	5.20	m	5.38	m
3	2.15	ddd (13.4, 11.6, 3.7)	2.47	ddd (14.3, 7.6, 3.7)	2.65	ddd (14.0, 7.9, 4.3)
	1.56	ddd (13.4, 11.0, 1.8)	2.16	ddd (14.3, 7.6, 5.5)	2.24	ddd (14.0, 7.9, 4.3)
4	4.65	td (11.0, 3.7)	5.38	dt (15.3, 7.6)	5.52	dt (15.3, 7.9)
5	5.25	t (11.0)	5.23	dd (15.3, 6.1)	5.37	m
6	6.15	t (11.0)	4.24	m	4.41	m
7	6.04	dd (15.2, 11.0)	2.36	ddd (12.8, 7.3, 3.4)	2.48	ddd (14.0, 7.3, 4.9)
			2.25	dt (12.8, 7.3)	2.34	dt (14.0, 8.5)
8	5.78	dt (15.2, 6.1)	6.69	dt (15.9, 7.3)	6.73	ddd (15.9, 8.5, 7.3)
9	3.26	dd (13.4, 6.1)	5.80	d (15.9)	5.89	d (15.9)
	2.96	dd (13.4, 6.1)				
11	4.68	d (18.3)	4.14	d (17.7)	4.04	d (16.5)
	3.82	d (18.3)	4.10	d (17.7)	3.98	d (16.5)
15	6.47	s	6.35	s	6.31	d (2.4)

[a] Measured in acetone-d_6.
[b] Measured in methanol-d_4.

determined using a MP-500D micro-melting point apparatus (Yanaco). Optical rotations were measured using an AUTOPOL V digital polarimeter (Rudolph Research Analytical). UV spectra were recorded using a V-520 UV/VIS spectrophotometer (JASCO). IR spectra were measured using a Spectrum One FT-IR spectrometer (PerkinElmer). Preparative HPLC was performed using a Waters M600 system as follows: preparative HPLC system, Waters M600; column, YMC-Pack Pro C_{18}, AS-343, 5 μm, 20×250 mm; flow rate, 10 mL/min; UV detection, 254 nm; temperature, 40 °C; eluent, MeCN/H_2O (+0.25% acetic acid). To separate the compounds, the following materials were used: silica gel column chromatography, Merck silica gel 60; preparative TLC, Merck silica gel 60 F_{254} thin-layer plate; gel filtration, Sephadex LH-20 (Pharmacia Co.).

3.2. Biological assays

Inhibition of WNT-5A expression (quantification of mRNA using the QuantiGene method): human dermal papilla cells, which were a gift from Dr. S. Arase (University of Tokushima Faculty of Medicine), were cultured in MEM (Invitrogen) containing 12% FBS. The dermal papilla cells in the fifth subculture generation were sowed in a 96-well plate to give a density of 1×10^4 cells/well and cultured overnight. The medium was then replaced with a medium to which no compounds had been added or a medium that contained the test compounds and the culture was continued for an additional 24 h. After the culture was complete, the amount of WNT-5A mRNA was measured using a QuantiGene High Volume Kit (Bayer Medical) by the branched DNA (bDNA) signal amplification method.[16] In accordance with the manufacturer's protocol, the cells were lysed with a lysis mixture and the lysis solution was added to the capture plate.

Next, a set of probes specific to WNT-5A[8] was added and the reaction was allowed to proceed at 53 °C for 20 h. After the plate was washed using 0.1×SSC (3.0 M NaCl and 0.3 M sodium citrate) containing 0.03% lauryl sulfate, an amplification probe that contained bDNA was added and allowed to react at 46 °C for 1 h. After the plate was washed, a labeling probe labeled with alkaline phosphatase was added and allowed to react at 46 °C for 1 h. After the plate was washed, the substrate Lumi-Phos Plus was added and the reaction was allowed to proceed at 46 °C for 30 min. Luminescence was then measured using WALLAC 1420 ARVOsx.

Cytotoxicity against dermal papilla cells: dermal papilla cells were sowed in a 96-well plate to yield a density of 5×10^3 cells/well and cultured 16 h in MEM containing 12% FBS. The medium was then replaced with a medium to which no compounds had been added or a medium that contained the test compounds and the culture was continued for an additional 24 h. The medium was then replaced with a medium containing 10% Alamar Blue™ (Wako Pure Chemical Industries) and the culture was continued for an additional 4 h. Finally, the fluorescence intensity (Ex 544 nm, Em 590 nm) was measured using a WALLAC 1420 ARVOsx.

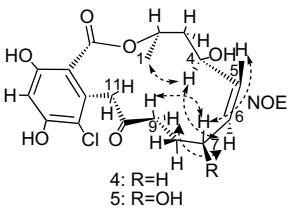

Figure 4. Relative stereochemistry of pochonin M (**4**) and N (**5**). NOEs are expressed as dotted-line arrows.

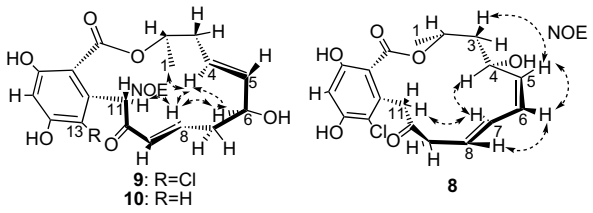

Figure 5. Relative stereochemistry of pochonin E (**9**) and F (**10**), and monorden analogue-1 (**8**). NOEs are expressed as dotted-line arrows.

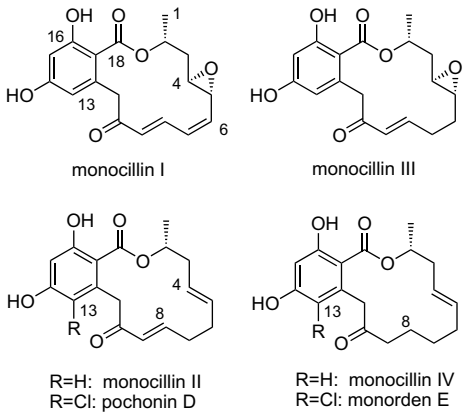

Figure 6. Structures of known isolated radicicol analogues from *P. chlamydosporia* var. *chlamydosporia*, TF-0480.

3.3. Microorganism

Strain TF-0480 was deposited at the National Institute of Advanced Industrial Science and Technology, Japan, and was labeled as FERM BP-8332.

3.4. Fermentation and extraction

A slant culture of strain TF-0480 was inoculated into a 500-mL Erlenmeyer flask containing 100 mL of seed medium consisting of 2.0% glucose, 4.0% mannitol, 2.0% oatmeal, 0.4% yeast extract, 0.014% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.001% $ZnSO_4 \cdot 7H_2O$, 0.001% $MnSO_4 \cdot 4\text{–}5H_2O$, 0.0005% $CuSO_4 \cdot 5H_2O$, adjusted to pH 6.0 before sterilization. The flask was shaken at 26 °C for 3 days on a rotary shaker at 200 rpm. Next, 300 mL of the seed culture was transferred to a 50-liter jar fermentor containing 30 L of production medium with the same composition as the seed medium. Fermentation was carried out at 26 °C for 6 days with an aeration rate of 1.0 v/v/min and agitation at 300 rpm. The culture broth was adjusted to pH 8.5–9.0 using 1 M sodium hydroxide and stirred for 1 h. The broth was then separated into mycelial cake and the supernatant using a Sharpless-type centrifuge. The supernatant was then stirred with Diaion HP-20 (1.5 L, Mitsubishi Chemical Co.) for adsorption. The resin was washed with water (3 L) and eluted with methanol (3 L). The eluates were combined and evaporated under reduced pressure to yield an aqueous residue. The residue was extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated in vacuo to give a brownish syrup (60 g) (lot 1 extract). TF-0480 was subjected to fermentation two more times using the same production method. These fermentations were performed in 50-liter and 1-ton jar fermentors containing 30 L and 600 L of production medium to give two crude extracts [47 g (lot 2) and 550 g (lot 3), respectively].

Table 4
WNT-5A expression inhibitory activities of radicicol (**1**), pochonins K–P (**2**–**7**), and known isolated radicicol analogues

Compound	IC_{50} (μM)	TC_{50} (μM)[a]
	WNT-5A	
Radicicol (**1**)	0.19	15.35
Pochonin K (**2**)	8.57	75.02
Pochonin L (**3**)	11.53	15.14
Pochonin M (**4**)	>50	>100
Pochonin N (**5**)	>50	>100
Pochonin O (**6**)	9.39	>100
Pochonin P (7)	>100	>100
Monorden Analogue-1 (**8**)	>50	>100
Pochonin E (**9**)	8.28	77.20
Pochonin F (**10**)	8.17	25.94
Pochonin B (**11**)	12.16	69.63
Monocillin I	1.93	2.90
Monocillin II	7.36	17.62
Monocillin III	9.43	28.87
Monocillin IV	>50	N.T.
Pochonin D	17.74	92.77
Monorden E	>50	N.T.

N.T.: not tested.
[a] TC_{50}: half maximal toxic concentration against dermal papilla cells.

3.5. Purification

Half of the crude extract (lot 1, 30 g) was purified by silica gel column chromatography eluted with *n*-hexane/ethyl acetate by a stepwise increase in the ethyl acetate contents to give four fractions (eluent: *n*-hexane/ethyl acetate=4:1, 2:1, 1:1, 1:2). Fraction 1 (130 mg) was purified by preparative HPLC (MeCN/H_2O=55:45) to yield monocillin IV (23.5 mg) and monorden E (4.8 mg). Fraction 2 (1.88 g) was recrystallized from acetone to yield monocillin II (277 mg). Fraction 3 (780 mg) was purified by preparative TLC (developing solvent: *n*-hexane/acetone=3:2) to yield monocillin I (80.3 mg). Fraction 4 (3.56 g) was purified by silica gel column chromatography eluted stepwise with chloroform/methanol. The fractions eluted with chloroform/methanol (98:2) were concentrated and recrystallized from acetone/methanol to yield monocillin III (339 mg). The fractions eluted with chloroform/methanol (97:3) were concentrated and purified by Sephadex LH-20 eluted with methanol, and further purified by preparative HPLC (eluent: MeCN/H_2O=30:70) to yield pochonin F (**10**) (4.5 mg). The other half of the crude extract (lot 1, 30 g) was purified by silica gel column chromatography eluted with chloroform/methanol by a stepwise increase in the methanol contents to give two fractions (eluent: chloroform/methanol=98:2, 93:7). Fraction 1 was recrystallized from methanol to yield radicicol (**1**) (2.0 g). Fraction 2 (156 mg) was purified by preparative HPLC (eluent: MeCN/H_2O=30:70) to yield pochonin K (**2**) (4.5 mg), pochonin L (**3**) (13.7 mg), pochonin P (**7**) (5.1 mg), monorden analogue-1 (**8**) (23.5 mg), pochonin E (**9**) (4.1 mg), and pochonin B (**11**) (18.7 mg).

The crude extract (lot 2, 40 g) was washed with *n*-hexane (300 mL×2). The residue was purified by silica gel column chromatography eluted with chloroform/methanol by a stepwise increase in the methanol contents to give two fractions (eluent: chloroform/methanol=100:0, 88:12). Fraction 1 (1.5 g) was purified by silica gel column chromatography eluted stepwise with *n*-hexane/ethyl acetate. The fractions eluted with *n*-hexane/ethyl acetate=2:1 were concentrated and further purified by preparative HPLC (MeCN/H_2O=45:55 to 70:30, gradient) to yield pochonin D (14.4 mg). Fraction 2 (318 mg) was purified by preparative HPLC (MeCN/H_2O=30:70) to yield pochonin M (**4**) (8.0 mg) and pochonin N (**5**) (15.3 mg).

The crude extract (lot 3, 500 g) was purified by silica gel column chromatography eluted stepwise with chloroform/methanol. The fractions eluted with chloroform/methanol=95:5 were concentrated and further purified by silica gel column chromatography eluted with *n*-hexane/ethyl acetate. The fractions eluted with *n*-hexane/ethyl acetate=2:1 were concentrated and further purified by preparative HPLC (MeCN/H_2O=45:55) to yield pochonin O (**6**) (38.1 mg).

All or partial NMR data of the known compounds (radicicol, monocillin I, II, III, IV, pochonin D, and monorden E) have been described previously,[11,15,19,21] and were identified.

3.6. Characteristics of radicicol analogues

*3.6.1. Radicicol (**1**)*

Colorless crystal; mp 181–183 °C, lit.:[10] 195 °C; $[\alpha]_D^{20}$ +194.6 (*c* 1.00, chloroform), lit.:[10] $[\alpha]_D$ +216 (*c* 1.00, chloroform); UV (methanol) λ_{max} (log ε) 215 (4.58), 266 (4.34), and 308 sh (3.92) nm; IR (KBr) ν_{max} 3418, 2988, 1706, 1655, 1607, 1438, 1360, 1306, 1244, 1110, 1043, 982, 925, 848, 732, 712, 670, 602 cm^{-1}; ESI-MS (neg.) *m*/*z* (%) 363.0 (100, $[M-H]^-$), 365.0 (40); HRESI-MS *m*/*z* 363.0647 (calcd for $C_{18}H_{16}Cl_1O_6$ $[M-H]^-$, 363.0635, Δ +1.2 mmu).

*3.6.2. Pochonin K (**2**)*

Colorless oil; $[\alpha]_D^{20}$ +23.1 (*c* 0.24, methanol); UV (methanol) λ_{max} (log ε) 213 (4.08), 262 (4.03), and 308 sh (3.70) nm; IR (KBr) ν_{max} 3431, 2969, 2929, 1711, 1672, 1646, 1620, 1569, 1462, 1432, 1384, 1362, 1311, 1246, 1132, 1086, 1037, 951, 853, 794, 716, 666, 624, 530, 506 cm^{-1}; NMR data, see Tables 1 and 2; ESI-MS (neg.) *m*/*z* (%) 495.2 (100, $[M-H]^-$), 497.2 (40); HRESI-MS *m*/*z* 495.1053 (calcd for $C_{23}H_{24}^{35}Cl_1O_{10}$ $[M-H]^-$, 495.1058, Δ −0.5 mmu).

*3.6.3. Pochonin L (**3**)*

Colorless oil; $[\alpha]_D^{20}$ −88.1 (*c* 1.00, acetone); UV (methanol) λ_{max} (log ε) 221 (4.27) and 287 sh (3.75) nm; IR (KBr) ν_{max} 3396, 2981, 2933, 1707, 1655, 1608, 1444, 1361, 1311, 1242, 1186, 1160, 1114, 1086, 1026, 914, 846, 794, 670, 628, 532 cm^{-1}; NMR data, see Tables 1 and 2; ESI-MS (neg.) *m*/*z* (%) 365.1 (100, $[M-H]^-$), 367.1 (40); HRESI-MS *m*/*z* 365.0797 (calcd for $C_{18}H_{18}Cl_1O_6$ $[M-H]^-$, 365.0792, Δ +0.5 mmu).

*3.6.4. Pochonin M (**4**)*

Colorless oil; $[\alpha]_D^{20}$ −57.0 (*c* 0.61, acetone); UV (methanol) λ_{max} (log ε) 222 (4.26), 262 (3.91), and 314 (3.78) nm; IR (KBr) ν_{max} 3421, 2991, 2931, 1711, 1650, 1607, 1580, 1496, 1440, 1420, 1381, 1368, 1313, 1245, 1184, 1105, 1088, 1049, 1024, 995, 971, 910, 875, 846, 798, 690, 670, 628, 553 cm^{-1}; NMR data, see Tables 1 and 2; ESI-MS (neg.) *m*/*z* (%) 367.1 (100, $[M-H]^-$), 369.1 (40); HRESI-MS *m*/*z* 367.0961 (calcd for $C_{18}H_{20}Cl_1O_6$ $[M-H]^-$, 367.0948, Δ +1.3 mmu).

*3.6.5. Pochonin N (**5**)*

Colorless oil; $[\alpha]_D^{20}$ −77.3 (*c* 1.00, methanol); UV (methanol) λ_{max} (log ε) 221 (4.29), 263 (3.92), and 315 (3.80) nm; IR (KBr) ν_{max} 3419, 2991, 2935, 1714, 1651, 1607, 1580, 1493, 1420, 1379, 1365, 1314, 1247, 1191, 1155, 1107, 1050, 1023, 973, 909, 846, 802, 696, 668, 634, 601, 565 cm^{-1}; NMR data, see Tables 1 and 2; ESI-MS (neg.) *m*/*z* (%) 383.1 (100, $[M-H]^-$), 385.1 (40); HRESI-MS *m*/*z* 383.0910 (calcd for $C_{18}H_{20}Cl_1O_7$ $[M-H]^-$, 383.0898, Δ +1.2 mmu).

*3.6.6. Pochonin O (**6**)*

Colorless oil; $[\alpha]_D^{20}$ −46.7 (*c* 1.00, acetone); UV (methanol) λ_{max} (log ε) 220 (4.21), 260 (3.81), and 298 (3.58) nm; IR (KBr) ν_{max} 3426, 2974, 2936, 1706, 1651, 1607, 1579, 1496, 1442, 1387, 1360, 1311, 1244, 1193, 1118, 1083, 1034, 976, 947, 846, 796, 702, 670, 622 cm^{-1}; NMR data, see Tables 1 and 2; ESI-MS (neg.) *m*/*z* (%) 383.1 (100, $[M-H]^-$), 385.1 (40); HRESI-MS *m*/*z* 383.0904 (calcd for $C_{18}H_{20}Cl_1O_7$ $[M-H]^-$, 383.0898, Δ +0.6 mmu).

*3.6.7. Pochonin P (**7**)*

Colorless oil; $[\alpha]_D^{20}$ −18.6 (*c* 0.36, acetone); UV (methanol) λ_{max} (log ε) 262 (4.56), 300 sh (3.98), 320 (3.92), 351 (3.94), and 366 (3.85) nm; IR (KBr) ν_{max} 3402, 2970, 2929, 1680, 1653, 1621, 1563, 1463, 1393, 1363, 1298, 1238, 1184, 1118, 1078, 966, 845, 793, 770, 712, 666, 625 cm^{-1}; NMR data, see Tables 1 and 2; ESI-MS (neg.) *m*/*z* (%) 381.2 (100, $[M-H]^-$), 383.2 (40); HRESI-MS *m*/*z* 381.0753 (calcd for $C_{18}H_{18}Cl_1O_7$ $[M-H]^-$, 381.0741, Δ +1.2 mmu).

*3.6.8. Monorden analogue-1 (**8**)*

Colorless oil; $[\alpha]_D^{20}$ −96.3 (*c* 0.37, methanol), lit.:[14] $[\alpha]_D^{24}$ −137 (*c* 0.05, chloroform); UV (methanol) λ_{max} (log ε) 226 (4.30) and 296 (3.71) nm; IR (KBr) ν_{max} 3425, 2985, 2928, 1708, 1653, 1608, 1443, 1415, 1362, 1300, 1238, 1112, 1090, 1032, 954, 841, 811, 788, 735, 670, 601, 517 cm^{-1}; NMR data, ^1H NMR: see Table 3, ^{13}C NMR (methanol-d_4, 125 MHz) δ 206.4 (s, C-10), 168.7 (s, C-18), 157.7 (s, C-16), 157.5 (s, C-14), 135.2 (s, C-12), 133.9 (d, C-5), 130.5 (d, C-6), 129.0 (d, C-7), 128.5 (d, C-8), 115.9 (s, C-13), 114.3 (s, C-17), 104.0 (d, C-15), 71.6 (d, C-2), 65.4 (d, C-4), 45.2 (t, C-9), 44.6 (t, C-11), 44.0 (t, C-3), 20.6 (q, C-1); ESI-MS (neg.) *m*/*z* (%) 365.1 (100, $[M-H]^-$), 367.1 (40); HRESI-MS *m*/*z* 365.0797 (calcd for $C_{18}H_{18}Cl_1O_6$ $[M-H]^-$, 365.0792, Δ +0.5 mmu).

*3.6.9. Pochonin E (**9**)*

Colorless oil; $[\alpha]_D^{20}$ −16.6 (*c* 0.87, acetone); UV (methanol) λ_{max} (log ε) 223 (4.37), 259 sh (3.95), and 310 (3.72) nm; IR (KBr) ν_{max} 3387, 2991, 2929, 1709, 1651, 1609, 1580, 1488, 1440, 1415, 1384, 1356, 1313, 1244, 1166, 1116, 1073, 1045, 979, 948, 909, 848, 806, 750, 708, 670, 632, 606, 534 cm^{-1}; NMR data, ^1H NMR: see Table 3, ^{13}C NMR (methanol-d_4, 125 MHz) δ 198.6 (s, C-10), 170.6 (s, C-18), 162.1 (s, C-16), 159.1 (s, C-14), 146.2 (d, C-8), 137.2 (d, C-5), 136.8 (s, C-12), 132.2 (d, C-9), 126.3 (d, C-4), 116.3 (s, C-13), 110.2 (s, C-17), 103.9 (d, C-15), 73.6 (d, C-2), 72.6 (d, C-6), 45.7 (t, C-11), 40.8 (t, C-7), 38.0 (t, C-3), 18.9 (q, C-1); ESI-MS (neg.) *m*/*z* (%) 365.1 (100, $[M-H]^-$), 367.1 (40); HRESI-MS *m*/*z* 365.0791 (calcd for $C_{18}H_{18}Cl_1O_6$ $[M-H]^-$, 365.0792, Δ −0.1 mmu).

*3.6.10. Pochonin F (**10**)*

Colorless oil; $[\alpha]_D^{20}$ +2.8 (*c* 0.89, acetone); UV (methanol) λ_{max} (log ε) 216 (4.45), 262 (4.06), and 302 (3.78) nm; IR (KBr) ν_{max} 3383, 2985, 2933, 1706, 1647, 1621, 1591, 1496, 1453, 1418, 1385, 1351, 1316, 1263, 1208, 1172, 1138, 1108, 1090, 1074, 1036, 977, 925, 901, 850, 807, 745, 718, 690, 626, 601, 573, 551, 535 cm^{-1}; NMR data, ^1H NMR: see Table 3, ^{13}C NMR (acetone-d_6, 125 MHz) δ 196.8 (s, C-10), 171.0 (s, C-18), 165.8 (s, C-16), 163.0 (s, C-14), 144.9 (d, C-8), 141.2 (s, C-12), 137.6 (d, C-5), 132.5 (d, C-9), 125.2 (d, C-4), 113.2 (d, C-13), 106.6 (s, C-17), 102.8 (d, C-15), 72.8 (d, C-2), 72.1 (d, C-6), 48.3 (t, C-11), 40.7 (t, C-7), 37.2 (t, C-3), 18.7 (q, C-1); ESI-MS (neg.) *m*/*z* (%) 331.2 (100, $[M-H]^-$), 332.1 (20); HRESI-MS *m*/*z* 331.1189 (calcd for $C_{18}H_{19}O_6$ $[M-H]^-$, 331.1160, Δ +2.9 mmu).

*3.6.11. Pochonin B (**11**)*

Colorless oil; $[\alpha]_D^{20}$ +20.8 (*c* 1.00, acetone); UV (methanol) λ_{max} (log ε) 224 (4.44), 260 (4.23), and 313 (3.86) nm; IR (KBr) ν_{max} 3386, 2997, 2928, 1678, 1651, 1619, 1580, 1495, 1462, 1420, 1390, 1359, 1312, 1247, 1200, 1183, 1114, 1077, 1045, 1012, 984, 953, 917, 892, 850, 802, 774, 750, 709, 674, 637, 614, 590, 562, 514 cm^{-1}; NMR data, ^1H NMR (methanol-d_4, 500 MHz) δ 6.83 (1H, dd, *J*=16.5, 9.8 Hz, H-8), 6.48 (1H, s, H-15), 6.14 (1H, d, *J*=16.5 Hz, H-9), 5.22 (1H, m, H-2), 4.55 (1H, d, *J*=18.3 Hz, H-11a), 4.38 (1H, d, *J*=18.3 Hz, H-11b), 4.35 (1H, td, *J*=9.8, 4.3 Hz, H-7), 2.88 (1H, td, *J*=4.3, 2.4 Hz, H-4), 2.62 (1H, dt, *J*=9.8, 4.3 Hz, H-5), 2.54 (1H, ddd, *J*=12.8, 4.3, 2.4 Hz, H-6a), 2.00 (1H, dt, *J*=15.9, 2.4 Hz, H-3a), 1.77 (1H, dt, *J*=15.9, 4.3 Hz, H-3b), 1.45 (3H, d, *J*=6.7 Hz, H_3-1), 1.25 (1H, dt, *J*=12.8, 9.8 Hz, H-6b); ^{13}C NMR (methanol-d_4, 125 MHz) δ 198.5 (s, C-10), 171.3 (s, C-18), 164.2 (s, C-16), 160.0 (s, C-14), 150.7 (d, C-8), 137.2 (s, C-12), 129.4 (d, C-9), 116.8 (s, C-13), 107.9 (s, C-17), 104.0 (d, C-15), 73.4 (d, C-2), 70.5 (d, C-7), 56.8 (d, C-4), 55.4 (d, C-5), 45.7 (t, C-11), 41.2 (t, C-6), 37.4 (t, C-3), 18.0 (q, C-1); ESI-MS (neg.) *m*/*z* (%) 381.2 (100, $[M-H]^-$), 383.2 (40); HRESI-MS *m*/*z* 381.0754 (calcd for $C_{18}H_{18}Cl_1O_7$ $[M-H]^-$, 381.0741, Δ +1.3 mmu).

3.6.12. Monocillin I

Colorless oil; $[\alpha]_D^{20}$ +6.9 (*c* 1.00, acetone); UV (methanol) λ_{max} (log ε) 217 (4.13), 262 (3.98), and 294 sh (3.67) nm; IR (KBr) ν_{max}

3388, 2980, 2941, 1708, 1651, 1625, 1588, 1504, 1451, 1384, 1346, 1311, 1262, 1209, 1169, 1121, 1099, 1044, 1027, 993, 924, 850, 802, 754, 718, 693, 667, 618, 520 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 10.94 (1H, br s, OH-16), 9.16 (1H, br s, OH-14), 7.83 (1H, dd, J=16.1, 11.3 Hz, H-8), 6.30 (1H, dd, J=11.3, 10.7 Hz, H-7), 6.29 (1H, d, J=2.7 Hz, H-15), 6.28 (1H, d, J=2.7 Hz, H-13), 5.97 (1H, d, J=16.1 Hz, H-9), 5.83 (1H, dd, J=10.7, 2.7 Hz, H-6), 5.49 (1H, ddq, J=3.7, 3.7, 6.7 Hz, H-2), 4.97 (1H, d, J=14.0 Hz, H-11a), 3.56 (1H, d, J=14.0 Hz, H-11b), 3.32 (1H, q, J=2.7 Hz, H-5), 3.12 (1H, dt, J=8.5, 2.7 Hz, H-4), 2.44 (1H, ddt, J=14.9, 3.7, 2.7 Hz, H-3a), 1.85 (1H, ddd, J=14.9, 8.5, 3.7 Hz, H-3b), 1.60 (3H, d, J=6.7 Hz, H_3-1); ^{13}C NMR (acetone-d_6, 125 MHz) δ 198.9 (s, C-10), 170.4 (s, C-18), 165.5 (s, C-16), 162.9 (s, C-14), 141.6 (d, C-8), 140.3 (s, C-12), 137.2 (d, C-6), 131.7 (d, C-9), 130.6 (d, C-7), 110.1 (d, C-13), 106.0 (s, C-17), 102.8 (d, C-15), 72.0 (d, C-2), 56.0 (d, C-4), 55.7 (d, C-5), 43.9 (t, C-11), 36.9 (t, C-3), 18.9 (q, C-1); ESI-MS (neg.) m/z (%) 329.1 (100, [M−H]$^-$), 330.1 (20); HRESI-MS m/z 329.1016 (calcd for $C_{18}H_{17}O_6$ [M−H]$^-$, 329.1025, Δ −0.9 mmu).

3.6.13. Monocillin II

Colorless crystal; mp 188–191 °C, lit.:[19] 198–200 °C; $[\alpha]_D^{20}$ +23.7 (c 0.43, acetone); UV (methanol) λ_{max} (log ε) 224 (4.60), 262 (4.18), and 302 (3.90) nm; IR (KBr) ν_{max} 3313, 2944, 1674, 1646, 1612, 1594, 1498, 1485, 1460, 1442, 1414, 1366, 1332, 1317, 1258, 1216, 1173, 1140, 1108, 1036, 979, 924, 892, 860, 823, 790, 728, 691, 625, 544 cm^{-1}; NMR data were identical to the reported data;[21] ESI-MS (neg.) m/z (%) 315.1 (100, [M−H]$^-$), 316.1 (20); HRESI-MS m/z 315.1228 (calcd for $C_{18}H_{19}O_5$ [M−H]$^-$, 315.1232, Δ −0.4 mmu).

3.6.14. Monocillin III

Colorless crystal; mp 197–200 °C, lit.:[19] 204–205 °C; $[\alpha]_D^{20}$ −0.8 (c 1.00, acetone); UV (methanol) λ_{max} (log ε) 217 (4.49), 264 (4.00), and 304 (3.72) nm; IR (KBr) ν_{max} 3613, 3418, 2985, 1674, 1643, 1621, 1584, 1493, 1464, 1440, 1418, 1389, 1358, 1342, 1312, 1260, 1217, 1196, 1175, 1143, 1100, 1078, 1059, 1036, 1021, 986, 922, 896, 879, 850, 805, 738, 585, 556, 502 cm^{-1}; NMR data were identical to the reported data;[21] ESI-MS (neg.) m/z (%) 331.0 (100, [M−H]$^-$), 332.0 (20); HRESI-MS m/z 331.1188 (calcd for $C_{18}H_{19}O_6$ [M−H]$^-$, 331.1182, Δ +0.6 mmu).

3.6.15. Monocillin IV

Colorless oil; $[\alpha]_D^{20}$ +48.3 (c 1.00, acetone); UV (methanol) λ_{max} (log ε) 218 (4.64), 264 (4.38), and 304 (4.09) nm; IR (KBr) ν_{max} 3307, 3050, 2988, 2943, 2860, 1692, 1646, 1622, 1584, 1529, 1497, 1482, 1464, 1440, 1412, 1393, 1382, 1365, 1345, 1317, 1292, 1261, 1201, 1170, 1134, 1107, 1032, 1010, 988, 920, 879, 840, 808, 779, 726, 682, 614, 547 cm^{-1}; NMR data, ^1H NMR (acetone-d_6, 500 MHz) δ 11.76 (1H, br s, OH-16), 9.19 (1H, br s, OH-14), 6.31 (1H, d, J=2.4 Hz, H-15), 6.24 (1H, d, J=2.4 Hz, H-13), 5.48 (1H, dd, J=15.9, 5.5 Hz, H-5), 5.45 (1H, dd, J=15.9, 5.5 Hz, H-4), 5.28 (1H, dq, J=3.7, 6.7 Hz, H-2), 4.36 (1H, d, J=17.1 Hz, H-11a), 3.56 (1H, d, J=17.1 Hz, H-11b), 2.56 (1H, ddd, J=14.0, 3.7, 3.0 Hz, H-3a), 2.30 (1H, dt, J=14.0, 5.5 Hz, H-3b), 2.49 (2H, m, H_2-9), 2.03 (1H, m, H-6b), 2.10 (1H, m, H-6a), 1.50 (2H, m, H_2-7), 1.59 (2H, m, H_2-8), 1.36 (3H, d, J=6.7 Hz, H_3-1); ^{13}C NMR (acetone-d_6, 125 MHz) δ 207.3 (s, C-10), 171.8 (s, C-18), 166.4 (s, C-16), 163.1 (s, C-14), 140.9 (s, C-12), 135.2 (d, C-5), 125.8 (d, C-4), 113.2 (d, C-13), 106.2 (s, C-17), 102.7 (d, C-15), 73.5 (d, C-2), 50.4 (t, C-11), 41.1 (t, C-9), 38.1 (t, C-3), 33.0 (t, C-6), 26.0 (t, C-7), 22.9 (t, C-8), 19.1 (q, C-1); ESI-MS (neg.) m/z (%) 317.0 (100, [M−H]$^-$), 318.0 (20); HRESI-MS m/z 317.1387 (calcd for $C_{18}H_{21}O_5$ [M−H]$^-$, 317.1389, Δ −0.2 mmu).

3.6.16. Pochonin D (or monorden D)

Colorless oil; $[\alpha]_D^{20}$ +1.70 (c 0.94, acetone), lit.:[21] $[\alpha]_D^{26}$ +21.4 (c 0.1, chloroform); UV (methanol) λ_{max} (log ε) 224 (4.38), 262 (3.86), and 312 (3.71) nm; IR (KBr) ν_{max} 3370, 2985, 2938, 1706, 1655, 1609, 1580, 1496, 1434, 1415, 1384, 1358, 1313, 1246, 1188, 1159, 1117, 1070, 1034, 981, 945, 900, 850, 819, 806, 746, 708, 668, 630, 534 cm^{-1}; NMR data were identical to the reported data;[15,21] ESI-MS (neg.) m/z (%) 349.2 (100, [M−H]$^-$), 351.2 (40); HRESI-MS m/z 349.0851 (calcd for $C_{18}H_{18}Cl_1O_5$ [M−H]$^-$, 349.0843, Δ +0.8 mmu).

3.6.17. Monorden E

Colorless oil; $[\alpha]_D^{20}$ −21.2 (c 1.00, acetone), lit.:[21] $[\alpha]_D^{26}$ −22.7 (c 0.28, methanol); UV (methanol) λ_{max} (log ε) 223 (4.45), 262 (3.90), and 311 (3.70) nm; IR (KBr) ν_{max} 3370, 2985, 2937, 1710, 1652, 1607, 1580, 1493, 1448, 1418, 1381, 1357, 1312, 1245, 1187, 1163, 1119, 1074, 1054, 1032, 980, 925, 846, 803, 760, 693, 668, 630 cm^{-1}; NMR data were identical to the reported data;[21] ESI-MS (neg.) m/z (%) 351.0 (100, [M−H]$^-$), 353.0 (40); HRESI-MS m/z 351.1004 (calcd for $C_{18}H_{20}Cl_1O_5$ [M−H]$^-$, 351.0999, Δ +0.5 mmu).

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