Penicillenols from *Penicillium* sp. GQ-7, an Endophytic Fungus Associated with *Aegiceras corniculatum*

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Six new tetramic acids derivatives, penicillenols A_1, A_2, B_1, B_2, C_1, and C_2 (1—6), together with citrinin, phenol A acid, phenol A, and dihydrocitrinin, were identified from *Penicillium* sp. GQ-7, an endophytic fungus associated with *Aegiceras corniculatum*. Their structures were elucidated on the basis of comprehensive spectral analysis. All the new compounds were evaluated for their cytotoxic effects on four cell lines by the MTT method. Penicillenols A_1 and B_1 showed cytotoxicities against HL-60 cell line with IC_{50} values of 0.76 μM and 3.20 μM, respectively.

Key words penicillenol; endophytic fungus; *Penicillium*; mangrove; *Aegiceras corniculatum*

In our search for biologically active metabolites from endophytic fungi, we found the culture broth of a fungus, authenticated as *Penicillium* sp. GQ-7, exhibiting cytotoxicity against the P388 cell line. Studies on the active constituents of this fungus led to the isolation of six new tetramic acids derivatives (**1**—**6**) (Fig. 1), together with citrinin, phenol A acid, phenol A, and dihydrocitrinin.

The naturally occurring tetramic acids were isolated from bacteria,[1,2] molds,[3] fungi[4,5] and sponges.[6] These isolated compounds exhibit a wide range of biological and pharmaceutical activities[7,8] (including potent antibiotic[6,9] and antiviral[10,11] properties, mycotoxicity,[12] and cytotoxicity[13—18]) and have attracted much attention of chemists, physicians, and biologists.[19,20] Biosynthetic studies have shown that the tetramic acid ring (2,4-pyrrolidinedione ring) is formed from a C_2 unit, and the nitrogen atom and the remaining two carbon atoms are derived from an amino acid.[21] All the 3-acyl tetramic acids can be considered as the results from the substitution of the hydrogens of C-5 with other functional groups and the substitution of the methyl group of C-7.[22] To date, hundreds of tetramic acids have been isolated, and to the best of our knowledge, among them neither 2-isooctyl nor oct-6-en-2-yl substituent at C-7 has been reported prior to our new penicillenols A_1, A_2, B_1, B_2, C_1, and C_2 (**1**—**6**). In this paper we report the isolation, structure elucidation, and cytotoxic activities of the six penicillenols (**1**—**6**).

Results and Discussion

The bioactive ethyl acetate extract of the *Penicillium* sp. was chromatographed on silica gel columns and extensive reversed phase preparative HPLC to give compounds (**1**—**6**). These compounds seemed to contain unsaturated alcohol moiety because they produced a purple color with $FeCl_3$ on a thin layer plate.

Penicillenol A_1 (**1**), obtained as a yellow oil, showed a *quasi*-molecular ion peak in the low-resolution ESI-MS at *m*/*z* 296 $[M-H]^-$, HR-ESI-MS *m*/*z* 296.1864 $[M-H]^-$ (Calcd for $C_{16}H_{26}NO_4$, 296.1862) indicated that **1** had a formula $C_{16}H_{27}NO_4$. The IR spectrum suggested the presence of OH group(s) (3436 cm^{-1}) and conjugated carbonyl group(s) (1700, 1650, 1605 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) disclosed the presence of four methyls (δ 3.00, H-17; 1.18, H-16; 1.15, H-7; 0.86, H-15) and three methine groups (δ 3.56, H-9; 3.80, H-5; 4.20, H-6). Furthermore, three multiplets (δ 1.21—1.32, H-11—H-14 approximately 8H; δ 1.47, H-10b 1H; δ 1.69, H-10a 1H) were observed in ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum (Table 2) revealed one *N*-methyl group (δ 27.2, C-17), three aliphatic methyl groups (δ 13.9, C-15; 17.0, C-16; 17.8, C-7), five methylene groups (δ 22.4, C-13; 27.1, C-11; 29.1, C-12; 31.5, C-14; 33.4, C-10), three methine groups (δ 68.7, C-5; 66.6, C-6; 36.3, C-9), and four quaternary carbons (δ 194.6, C-4; 192.6, C-8; 174.1, C-2; 100.9, C-3). The UV spectrum of **1** maxima absorption at 279, 223 nm indicating the conjugation between amide, ketone and enol,[23] along with the four quaternary carbon signals suggested that **1** possessed a 3-acyl tetramic acid ring system. In the HMBC spectrum (Fig. 2), the correlations H-17 to C-2 and C-5, H-5 to C-2 and C-4, and H-9 to C-8 and C-3 confirmed the 3-acyl tetramic acid moiety in **1**. Furthermore, in the ^{1}H–^{1}H COSY spectrum the correlations (Fig. 2) H-5 to H-6 and H-6 to H-7 indicated it carried a 1-hydroxyethyl group at the 5-position, and the correlations H-16 to H-9, H-9 to H-10 and H-14 to H-15 suggested a 2-

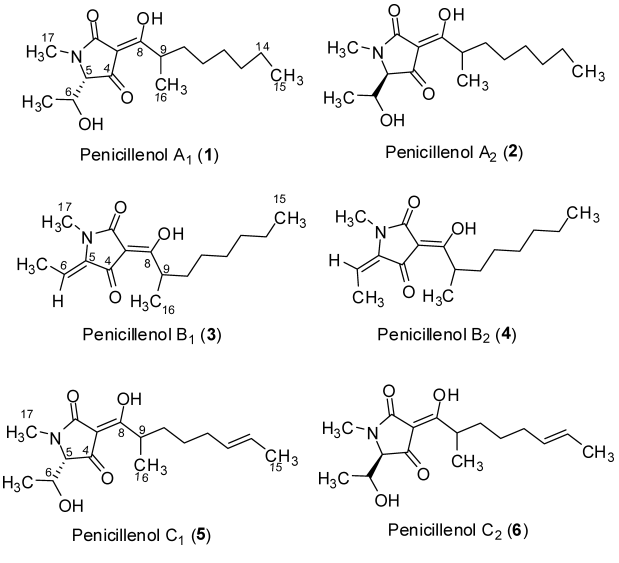

Fig. 1. Structures of Compounds **1**—**6**

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isooctyl group in **1**. The HMBC correlations H-9 to C-8, C-3, C-16, C-10 and C-11, indicated that the 2-isooctyl group attached to the 3-acyl tetramic acid moiety at C-8. So the planar structure of penicillenol A_1 was deduced to be **1** as shown in Fig. 1.

Penicillenol A_2 (**2**), also obtained as a yellow oil, showed a *quasi*-molecular ion peak in the negative low-resolution ESI-MS at *m*/*z* 296 $[M-H]^-$, HR-ESI-MS *m*/*z* 296.1853 $[M-H]^-$ (Calcd for $C_{16}H_{26}NO_4$, 296.1862) indicated that **2** had a formula $C_{16}H_{27}NO_4$ as **1**. Almost identically to **1**, the UV spectrum of **2** showed the same absorption which revealed a tetramic acid ring system, and the IR spectrum of **2** suggested the presence of OH group(s) (3444 cm^{-1}) and conjugated carbonyl group(s) (1711, 1650, 1613 cm^{-1}). The 1D NMR spectra of **2** (Tables 1, 2) also revealed a 3-acyl tetramic acid ring, a 2-isooctyl moiety, a 1-hydroxyethyl group, and an *N*-methyl group. Further comparison of the NMR spectral data (Tables 1, 2) of **2** with those of **1** showed **2** differed from **1** only at the chemical shifts of C-4 (δ_C 192.3, 2.3 ppm highfield shifting than **1**) and C-6 (δ_C 71.5, 6 ppm downfield shifting than **1**), suggesting that **2** owned the same plane structure as **1** with exception of the different sterochemistry of C-5. That indicated **2** was an isomer of **1** at C-5.

Both compounds **1** and **2** were designated as a common $\Delta^{3,8}$ *Z* stereochemistry for the tetramic acid moiety by the chemical shifts (δ 174.1 and δ 174.3, respectively) at C-2.[23] The absolute stereochemistry of the chiral center at C-5

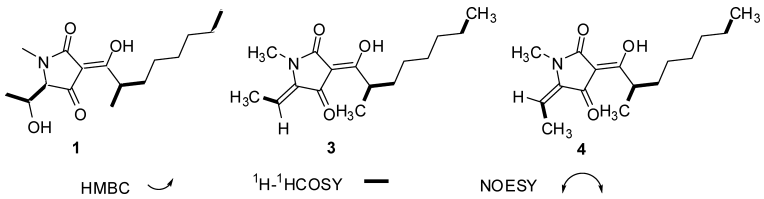

Fig. 2. Key HMBC, ^{1}H–^{1}H COSY and NOESY Correlations of Compounds **1**, **3** and **4**

Table 1. ^{1}H-NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds **1**—**6**

Position	Penicillenol A_1 (**1**)	Penicillenol A_2 (**2**)	Penicillenol B_1 (**3**)	Penicillenol B_2 (**4**)	Penicillenol C_1 (**5**)	Penicillenol C_2 (**6**)
5	3.80 d (4.2)	3.75 d (2.8)	—	—	3.80 d (4.5)	3.75 d (2.3)
6	4.20 m	4.31 dq (3.2, 6.4)	5.89 q (7.7)	5.38 q (7.8)	4.19 m	4.29 m
7	1.15 d (6.4)	1.16 d (6.8)	2.03 d (8.2)	2.23 d (7.8)	1.14 d (7.0)	1.16 d (6.4)
9	3.56 m	3.58 m	3.70 m	3.70 m	3.57 m	3.58 m
10a	1.69 m	1.67 m	1.69 m	1.70 m	1.68 m	1.67 m
10b	1.47 m	1.46 m	1.47 m	1.46 m	1.47 m	1.47 m
11	1.21—1.32 m	1.21—1.32 m	1.21—1.33 m	1.21—1.34 m	1.34 m	1.34 m
12					1.95 m	1.95 m
13					5.36 m	5.36 m
14					5.40 m	5.40 m
15	0.86 t (6.8)	0.86 t (7.1)	0.86 t (6.8)	0.86 t (7.4)	1.62 d (5.7)	1.62 d (5.5)
16	1.18 d (6.8)	1.21 d (6.4)	1.18 d (6.8)	1.18 d (6.9)	1.18 d (7.1)	1.21 d (6.4)
17	3.00 s	3.11 s	3.39 s	3.06 s	2.99 s	3.11 s

Spectra were recorded at 600 MHz for ^{1}H using TMS as internal standard.

Table 2. ^{13}C-NMR Spectroscopic Data (150 MHz, $CDCl_3$) for Compounds **1**—**6**

Position	Penicillenol A_1 (**1**)	Penicillenol A_2 (**2**)	Penicillenol B_1 (**3**) (Exo A)	Penicillenol B_2 (**4**) (Exo A)	Penicillenol C_1 (**5**)	Penicillenol C_2 (**6**)
2	174.1, qC	174.3, qC	172.5, qC[a]	170.8, qC[e]	174.1, qC	174.5, qC
3	100.9, qC	100.8, qC	99.6, qC[b]	101.3, qC[f]	101.1, qC	100.9, qC
4	194.6, qC	192.3, qC	191.2, qC[c]	190.8, qC[g]	194.9, qC	192.2, qC
5	68.7, CH	68.1, CH	136.7, qC	134.2, qC	68.4, CH	68.2, CH
6	66.6, CH	71.5, CH	107.5, CH	111.8, CH	66.7, CH	71.5, CH
7	17.8, CH_3	17.5, CH_3	11.4, CH_3	11.7, CH_3	17.9, CH_3	17.8, CH_3
8	192.6, qC	192.2, qC	181.0, qC[d]	182.8, qC[h]	192.7, qC	192.0, qC
9	36.3, CH	36.1, CH	35.9, CH	35.7, CH	36.3, CH	36.0, CH
10	33.4, CH_2	33.7, CH_2	33.7, CH_2	33.6, CH_2	33.0, CH_2	33.2, CH_2
11	27.1, CH_2	27.1, CH_2	27.3, CH_2	27.2, CH_2	27.1, CH_2	27.0, CH_2
12	29.1, CH_2	29.1, CH_2	29.2, CH_2	29.2, CH_2	32.4, CH_2	32.3, CH_2
13	22.4, CH_2	22.5, CH_2	22.6, CH_2	22.5, CH_2	130.8, CH	130.9, CH
14	31.5, CH_2	31.6, CH_2	31.7, CH_2	31.6, CH_2	125.2, CH	125.1, CH
15	13.9, CH_3	14.0, CH_3	14.1, CH_3	14.0, CH_3	17.6, CH_3	17.8, CH_3
16	17.0, CH_3	17.1, CH_3	17.2, CH_3	17.2, CH_3	17.2, CH_3	17.1, CH_3
17	27.2, CH_3	28.5, CH_3	27.9, CH_3	24.7, CH_3	27.1, CH_3	28.5, CH_3

Spectra were recorded at 150 MHz for ^{13}C using TMS as internal standard: chemical shifts in Exo B (a: 165.7, b: 101.8, c: 197.4, d: 185.0, e: 164.0, f: 103.7, g: 196.6, h: 187.3 ppm).

of the tetramic acid moiety was determined by the CD spectrum. The CD maxima [212 nm ($\Delta\varepsilon$=+60.74), 233 (−33.04), 268 (−4.14), 285 (−9.21)] of **1** was very similar to that seen for a simple tetramic acid, tenuazonic acid,[24] suggesting that the chirality of the asymmetric carbon in the tetramic acid ring of **1** was *S*. Compound **2** giving a opposite CD maxima [213 nm ($\Delta\varepsilon$=−37.26), 232 (+23.65), 321 (+3.42)] should have a *R* configuration at C-5.

Penicillenol B_1 (**3**), obtained as a yellow oil, showed a *quasi*-molecular ion peak in the positive and negative low resolution ESI-MS at *m/z* 280 $[M+H]^+$ and 278 $[M-H]^-$ respectively. Together with the consideration of ^{1}H- and ^{13}C-NMR data (Tables 1, 2), $C_{16}H_{25}NO_3$ was assigned as molecular formula which was also confirmed by high-resolution ESI-MS *m/z* 280.1902 $[M+H]^+$ (Calcd for $C_{16}H_{26}NO_3$, 280.1913). The IR spectrum suggested the presence of conjugated carbonyl group(s) (1712, 1669, 1611 cm^{-1}).The 1D NMR spectra of **3** also showed a 3-acyl tetramic acid ring, a 2-isooctyl moiety, and an *N*-methyl group (δ_C 27.9, C-17; δ_H 3.39, H-17). Differently to **1**, the 1D NMR spectra of **3** showed that two downfiled methine groups (δ_H 3.80, H-5; 4.20, H-6) disappeared in **1**, while increased one trisubstitued double bond carbon (δ_H 5.89, H-6 and δ_C 107.5, C-6) and one tetrasubstituted double bond carbon (δ_C 136.7, C-5) in **2**. The above result indicated that **3** was the 5,6-dehydrate derivative of **1**. This assumption was confirmed by the molecular formula of **3**, with a difference of a H_2O to that of **1** and the detailed analysis of the ^{1}H–^{1}H COSY spectra (Fig. 2). The NOESY correlation (Fig. 2) from H-17 to H-7 indicated a $\Delta^{5,6}$ *Z* configuration for **3**.

The structure elucidation of penicillenol B_2 (**4**) proceeded in a very straightforward manner. The HR-ESI-MS *m/z* 280.1926 $[M+H]^-$ (Calcd for $C_{16}H_{26}NO_3$, 280.1913) indicated **4** that had a formula $C_{16}H_{25}NO_3$, which was isomer of **3**. The IR spectrum suggested the presence of conjugated carbonyl group(s) (1700, 1650, 1611 cm^{-1}). The 1D NMR spectra of **4** also showed a 3-acyl tetramic acid ring, a 2-isooctyl moiety, an *N*-methyl group and a double bond (Tables 1, 2). In comparison to **3**, the double bond carbon C-5 at δ 134.2 highfield shifted by 2.5 ppm, and C-6 at 118.3 ppm downfield shifted by 4.3 ppm, implying that **4** contained the double bond with a different configuration. In the NOESY spectrum the correlation (Fig. 2) from H-17 to H-6 suggested a $\Delta^{5,6}$ *E* configuration for **4**, which was opposite to that of **3**.

Similar to the observation reported for melophlins A and B,[23] the ^{1}H- and ^{13}C-NMR spectra of **3** and **4** revealed that both **3** and **4** existed as 7 : 5 mixtures of two tautomers, designated as exo A and exo B (NMR data were shown in Table 2) form of the tetramic acid moiety. It was reported that the two forms interconvert at room temperature.[23] Thus, both **3** and **4** were respectively mixtures of two diastereomers.

Penicillenol C_1 (**5**) and penicillenol C_2 (**6**) were also obtained as yellow oil. The high resolution-electrospray ionization mass spectra HR-ESI-MS *m/z* 296.1869 $[M+H]^+$, *m/z* 296.1852 $[M+H]^+$ respectively (Calcd for $C_{16}H_{26}NO_4$, 296.1862) indicated both of them to have the formula $C_{16}H_{25}NO_4$, This was 2 amu smaller than **1**, with a difference of a H2 in the molecular formula. The IR spectrum suggested the presence of OH group(s) (3390 cm^{-1} in **5**, 3417 cm^{-1} in **6**) and conjugated carbonyl group(s) (1697, 1654, 1611 cm^{-1} in **5**; 1685, 1650, 1607 cm^{-1} in **6**).

Detailed analysis of the 1D NMR data revealed that compound **5** showed a 3-acyl tetramic acid ring and a 1-hydroxyethyl group, which were identical to the respective signals present in **1**. Moreover, a downfield shift doublet methyl group (δ_C 17.6, C-15 and δ_H 1.62, H-15) in **5** instead of a terminal methyl group (δ_C 13.9, C-15 and δ_H 0.86, H-15) in **1** was observed, and was considered to be attached to a double bond (δ_C 130.8, C-13; 125.2, C-14 and δ_H 5.36, H-13; 5.40, H-14) observed in **5**. This indicated that there was an oct-6-en-2-yl substituent group at C-8 in **5**. So compound **5** was deduced to be the 13,14-dehydro derivative of **1**, which was consistent with the molecular formula. The *E* configuration was assigned to the C-13–C-14 double bond by comparison of the chemical shifts of C-12 (δ_C 32.4) and C-15 (δ_C 17.6) with the reported calculated values (δ_C (*E*) 33.0, (*Z*) 27.0 for C-12; δ_C (*E*) 17.0, (*Z*) 11.0 for C-15).[25] The 1D NMR data of **6** revealed the same 3-acyl tetramic acid ring and 1-hydroxyethyl group as **2** (Tables 1, 2), and an identical oct-6-en-2-yl substituent group to **5** (Tables 1, 2). It could be easily shown that compound **6** should be the 13,14-dehydro derivative of **2**.

The fact that **5** and **1** showed similar CD spectra [194 nm ($\Delta\varepsilon$=−12.19), 212 (+17.45), 235 (−6.79), 266 (+0.45), 285 (−3.91) for **5**], moreover, ^{1}H-NMR data (Table 1) showed the same proton–proton coupling constants between the C-5 proton and C-6 proton, suggested that the configuration at C-5 remain the same in both compounds. So, compound **5** had an *S* configuration at C-5. Compounds **6** and **2** also showed similar CD spectra [193 nm ($\Delta\varepsilon$=+35.13), 212 (−18.87), 235 (+11.33), 270.4 (−3.6), 315.6 (+1.59) for **6**] and the same H-5, H-6 coupling constants (Table 1), and remained the same configuration at C-5. Thus the stereo chemical detail of **6** was determined as *R* at C-5.

Compounds **1**—**6** were evaluated for their cytotoxicities against the A-549, BEL-7402, P388 and HL-60 cell lines by the MTT method.[26] Compounds **1**, **2**, **3** and **4** showed cytotoxicities toward HL-60 cell lines, with IC_{50} values ranging from 0.76 to 16.26 μM (Table 3). Despite their slight structural difference of the fatty chain from compounds **1**—**4**, compounds **5** and **6** showed no cytotoxicity. This result had a significant implication that the presence of a saturated fatty chain at C-8 might be an essential part of the pharmacophore of penicillenols. Similar results have been shown in recent works that Oda T. *et al.* examined the effects of different saturated fatty chain-type melophlins on the V79 and L1210 cells, and the investigation resulted in the observing some structure–activity relationships of these compounds, *i.e.*, compounds possessing *iso*-type or *anteiso*-type chains with a chain-length of less than 12 carbons showed stronger activ-

Table 3. Cytotoxicities of Compounds **1**—**6** in Four Cancer Cell Lines

Compounds	Cytotoxicity (IC_{50}, μM)			
	A-549 cells	BEL-7402 cells	P388 cells	HL-60 cells
1	23.8	13.03	8.85	0.76
2	>100	>100	>100	16.26
3	>100	>100	>100	3.20
4	>100	>100	>100	7.65
5	>100	>100	>100	>100
6	>100	>100	>100	>100

ity.[27] In addition, it is interesting to note that the structures of compounds **1** and **2** were only configuration difference at C-5 of the tetramic acid ring, while their cytotoxicities against A-549, BEL-7402 and P388 cell lines were obviously variance. This result prompted the 1-hydroxyethyl group at the 5*S*-position might be essential to the activity. Therefore, further antitumor activity of penicillenols should be performed to elucidate the structure–activity relationships and action mechanism of them.

Experimental

General Experimental Procedures Optical rotations were obtained on a JASCO P-1020 digital polarimeter. CD spectra were obtained on a JASCO J-810 spectropolarimeter. UV spectra were recorded on a Beckmen DU 640 spectrophotometer. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer in KBr disks. ^{1}H-, ^{13}C-NMR and DEPT spectra and 2D NMR were recorded on a JEOL Eclips-600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. NOESY experiments were carried out using a mixing time of 0.5 s. 1D NOE spectra were obtained on a Varian INOVA-400 spectrometer. ESI-MS was measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS (A), 10×250 mm, 5 μm, 4 ml/min].

Fungal Material A sample of *Penicillium* sp. GQ-7 was isolated from the inner bark of *Aegiceras corniculatum* collected in Gaoqiao, China. The outer bark was removed from a branch of mangrove plant, the surface were sterilized by sequential washes in 0.53% sodium hypochlorite (2 min) and 75% ethanol (2 min) and then rinsed with sterile distilled water and allowed to surface dry under aseptic conditions. The surface-sterilized branch cut into several segments, which were placed on 2% malt extract agar in Petri dishes. All plates were incubated at room temperature for a maximum of 3 weeks. Fungi growing out from the plant tissues were transferred to potato dextrose agar slants and stored at 4 °C. A similar procedure without surface sterilization was conducted as a negative control to check the surface contaminating microbes.

Fermentation and Extraction The fungus was grown under static conditions at 24 °C for 30 d in 70 1000-ml conical flasks containing the liquid medium (300 ml/flask) composed of glucose (10 g/l), maltose (20 g/l), mannitol (20 g/l), monosodium glutamate (10 g/l), KH_2PO_4 (0.5 g/l), $MgSO_4 \cdot 7H_2O$ (0.3 g/l), corn steep liquor (1 g/l) and yeast extract (3 g/l) and seawater after adjusting its pH to 7.0. The fermented whole broth (21 l) was filtered through cheesecloth to separate into supernatant and mycelia. The former was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with ethyl acetate to give an ethyl acetate solution, while the latter was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with ethyl acetate to give another ethyl acetate solution. Both ethyl acetate solutions were combined and concentrated under reduced pressure to give a crude extract (15.0 g).

Purification The crude extract (15.0 g) showing cytotoxicity against P388 cells at 100 μg/ml was separated into 20 fractions on a silica gel column using a step gradient elution of petroleum ether/acetone. The fraction 10, eluted with 6 : 4 petroleum ether/acetone (160 mg), was further separated by reversed-phase column (MeOH–H_2O=3 : 2→4 : 1→MeOH) to afford fr. 10-1 (4.0 mg), fr. 10-2 (20 mg) and fr. 10-3 (60 mg). Then, the fr. 10-1 was separated by extensive PHPLC (85% MeOH containing 0.1% TFA, 4.0 ml/min) to yield compound **3** (5.0 mg, t_R 15 min) and compound **4** (2.6 mg, t_R 17 min). The fr. 10-2 and fr. 10-3 were purified by extensive PHPLC (75% MeOH containing 0.1% TFA, 4.0 ml/min) to give compound **1** (10.0 mg, t_R 15 min), compound **2** (15.0 mg. t_R 16 min), compound **5** (5 mg, t_R 10 min) and compound **6** (3 mg, t_R 11 min).

Penicillenol A_1 (**1**): Obtained as yellow oil; $[\alpha]_D^{25}$ −864.5° (c=0.155, MeOH); UV (MeOH) λ_{max} 223, 279 nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 195 (−52.39), 212 (+60.74), 233 (−33.04), 268 (−4.14), 285 (−9.21) nm; IR (KBr) ν_{max} 3436, 2959, 2931, 2859, 1700, 1650, 1605, 1482, 1458, 1378, 1341, 1089 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Tables 1, 2); HR-ESI-MS m/z 296.1864 $[M-H]^-$ (Calcd for $C_{16}H_{26}NO_4$, 296.1862).

Penicillenol A_2 (**2**): Obtained as yellow oil; $[\alpha]_D^{25}$ +386.7° (c=0.135, MeOH); UV (MeOH) λ_{max} 223, 279 nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 193 (+60.43), 213 (−37.26), 232 (+23.65), 276 (−6.15), 279 (−5.10), 284 (−6.24), 321 (+3.42) nm; IR (KBr) ν_{max} 3444, 2958, 2930, 2859, 1711, 1650, 1613, 1489, 1459, 1338 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Tables 1, 2); HR-ESI-MS m/z 296.1853 $[M-H]^-$ (Calcd for $C_{16}H_{26}NO_4$, 296.1862).

Penicillenol B_1 (**3**): Obtained as yellow oil; $[\alpha]_D^{25}$ −7.8° (c=0.230, MeOH); UV (MeOH) λ_{max} 256 nm; IR (KBr) ν_{max} 2963, 2928, 2854, 1712, 1669, 1611, 1452, 1320, 1164, 994 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Tables 1, 2); HR-ESI-MS m/z 280.1902 $[M+H]^+$ (Calcd for $C_{16}H_{26}NO_3$, 280.1913).

Penicillenol B_2 (**4**): Obtained as yellow oil; $[\alpha]_D^{25}$ −15.9° (c=0.130, MeOH); UV (MeOH) λ_{max} 256 nm; IR (KBr) ν_{max} 2955, 2924, 2854, 1700, 1650, 1611, 1444, 1083, 831 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Tables 1, 2); HR-ESI-MS m/z 280.1926 $[M+H]^+$ (Calcd for $C_{16}H_{26}NO_3$, 280.1913).

Penicillenol C_1 (**5**): Obtained as yellow oil; $[\alpha]_D^{25}$ −47.2° (c=0.125, MeOH); UV (MeOH) λ_{max} 224, 280 nm, CD (MeOH) λ_{max} ($\Delta\varepsilon$) 194 (−12.19), 212 (+17.45), 235 (−6.79), 266 (+0.45), 285 (−3.91) nm; IR (KBr) ν_{max} 3390, 2956, 2928, 2651, 1697, 1654, 1611, 1475, 1448, 1335, 1203, 1122, 959 cm^{-1}, ^{1}H- and ^{13}C-NMR (see Tables 1, 2).ESI-MS m/z 296 $[M+H]^+$, HR-ESI-MS m/z 296.1869 $[M+H]^+$ (Calcd for $C_{16}H_{26}NO_4$, 296.1862).

Penicillenol C_2 (**6**): Obtained as yellow oil; $[\alpha]_D^{25}$ +32° (c=0.20, MeOH); UV (MeOH) λ_{max} 224, 280 nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 193 (+35.13), 212 (−18.87), 235 (+11.33), 270.4 (−3.6), 315.6 (+1.59) nm; IR (KBr) ν_{max} 3417, 2971, 2924, 2850, 1685, 1650, 1607, 1483, 1444, 1207, 1130, 966 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Tables 1, 2); HR-ESI-MS m/z 296.1852 $[M+H]^+$ (Calcd for $C_{16}H_{26}NO_4$, 296.1862).

Biological Assays Cytotoxic activity was evaluated by the MTT method using A-549, BEL-7402, P388 and HL-60 cell lines. The cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Those cell suspensions (200 μl) at a density of 5×10^4 cell ml^{-1} were plated in 96-well microtiter plates and incubated for 24 h at the above condition. The test compound solution (2 μl in DMSO) at different concentrations was added to each well and further incubated for 72 h in the same condition. Then 20 μl of the MTT solution (5 mg/ml in RPMI-1640 medium) was added to each well and incubated for 4 h. The old medium containing MTT (150 μl) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm.

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