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Iridoid Glycosides from *Barleria lupulina*

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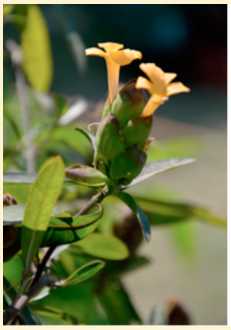

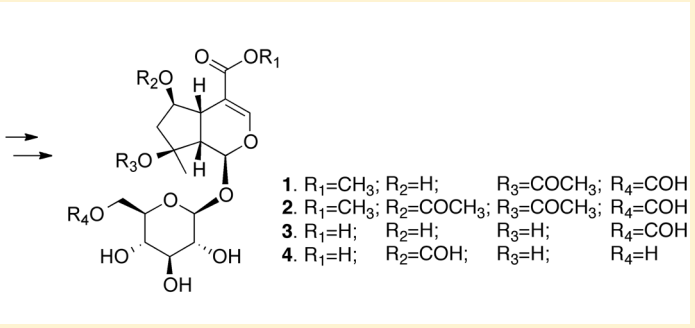

1. R_1=CH_3; R_2=H; R_3=$COCH_3$; R_4=COH
2. R_1=CH_3; R_2=$COCH_3$; R_3=$COCH_3$; R_4=COH
3. R_1=H; R_2=H; R_3=H; R_4=COH
4. R_1=H; R_2=COH; R_3=H; R_4=H

ABSTRACT: Phytochemical investigation of an extract of the aerial part of *Barleria lupulina* resulted in the identification of four new iridoid glycosides (**1**−**4**), together with 14 known analogues (**5**−**18**). The structures of **1**−**4** were determined through 1D and 2D NMR spectroscopic data analysis, HRMS, and acid hydrolysis. This is the first report of iridoid glycosides with a formate group. The free-radical scavenging activity of compounds **9**, **12**, and **15**−**17** was assessed using the DPPH assay. Compounds **16** and **17** scavenged DPPH radicals weakly with IC_{50} values of 97.5 and 78.6 μg/mL, respectively.

Barleria lupulina Lindl. (Acanthaceae) is a small shrub, distributed in Southeast Asia. *B. lupulina* has been traditionally used in folk medicine for mental stress, diabetes, rheumatoid arthritis, and snake bites.[1] This plant has also been used in Thai traditional medicine as an anti-inflammatory agent for insect bites and as remedies for herpes simplex and varicella zoster lesions[2] and is categorized as one of the essential medicinal plants for primary healthcare by the Thai Ministry of Public Health.[2] The essential oil from *B. lupulina* showed in vitro antibacterial activity.[3] A *B. lupulina* extract exhibited potent antiviral activity against herpes simplex virus type 2 (HSV-2).[4] In addition, the organic solvent extracts of *B. lupulina* were recently shown to have a protective effect against experimental gastric and duodenal ulcer formation and anti-inflammatory effects associated with reduced neutrophil migration.[5,6] Phytochemical studies on aerial parts and leaves of this plant revealed the presence of a number of iridoid glucosides.[7−11] Some of the iridoid glucosides showed antiviral effects against herpes simplex virus type 1 (HSV-1) and alkaline phosphatase (ALP)-enhancing activity in MC3T3-E1 osteoblast cells.[10,11]

In the search for new bioactive constituents of the aerial parts of *B. lupulina*, four new iridoid glycosides, barlupulins A−D (**1**−**4**), together with 14 known analogues (**5**−**18**), were isolated from the EtOAc-soluble fraction of its water extracts. In this paper, the isolation and structural elucidation of these new iridoid glycosides together with the bioactivity against cancer cells and the antioxidant effect of some of the isolates are reported.

Compound **1** was obtained as an amorphous powder with a specific rotation of $[\alpha]_D^{25}$ −17. Its molecular formula, $C_{20}H_{28}O_{13}$, was deduced by the ^{13}C NMR data and the molecular ion $[M + Na]^+$ at m/z 499.1431 (calcd for $C_{20}H_{28}O_{13}Na$, 499.1428) in the positive-ion HR-ESIMS. The IR spectrum showed absorption bands for hydroxy (3398 cm^{-1}) and carbonyl (1707 cm^{-1}) groups and an enol ether system (1595 cm^{-1}). The ^{13}C NMR spectrum (Table 1) showed 20 carbon signals including seven in a glucopyranosyl unit, three carbonyl groups, a methoxy group, and the remaining nine in the iridoid moiety. The ^1H and ^{13}C NMR data of **1** displayed signals characteristic of an iridoid glycoside, which were similar to those reported for barlerin (**12**) isolated from this plant.[12,13] The only difference between **1** and **12** was

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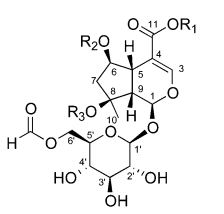
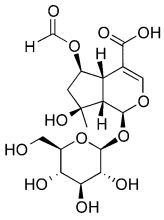


1 R_1 = CH_3 R_2 = H R_3 = Ac
2 R_1 = CH_3 R_2 = Ac R_3 = Ac
3 R_1 = H R_2 = H R_3 = H



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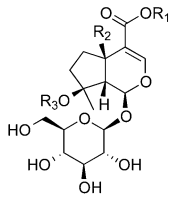
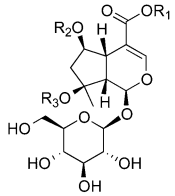


13 R_1 = CH_3 R_2 = OH R_3 = Ac
18 R_1 = H R_2 = H R_3 = H



	R_1	R_2	R_3
5	CH_3	*p*-OMe-*trans*-cinnamoyl	Ac
6	CH_3	*p*-OMe-*cis*-cinnamoyl	Ac
7	H	*p*-OMe-*trans*-cinnamoyl	Ac
8	H	*p*-OMe-cis-cinnamoy	Ac
9	CH_3	*p-trans*-coumaroyl	Ac
10	CH_3	*p-cis*-coumaroyl	Ac
11	CH_3	Ac	Ac
12	CH_3	H	Ac
14	H	H	Ac
15	CH_3	Ac	H
16	CH_3	H	H
17	H	H	H

that **1** showed resonances due to a formate group [δ_H 8.17 (s); δ_C 163.4]. The deshielded H-6′ chemical shifts (δ_H 4.49 and 4.33 from δ_H 3.91 and 3.64) suggested the attachment of the formate group to the C-6 hydroxy group of the glucose unit. The assumption was confirmed by the gHMBC correlations from H_2-6′ (δ_H 4.49 and 4.33) to the formate carbon (δ_C 163.4) and from the formate hydrogen (δ_H 8.17) to C-6′ (δ_C 64.2) (Figure 1). The relative configuration of **1** was established by analysis of the NOESY spectrum (Figure 2). In the NOESY spectrum, correlations between H-9 and H-5/H-7β indicated that H-5 and H-9 are both β-oriented. NOESY correlations between CH_3-10 and H-7α/H6/H-1 supported that H-1, H-6, and CH_3-10 are all α-oriented. Acid hydrolysis of **1** afforded D-glucose, which was identified by TLC comparison with an authentic sample,[14] and the configuration was determined by comparison of optical rotation data. The β-anomeric configuration for the glucosyl unit was established from its ^3J coupling constant (d, J = 8.0 Hz). The complete assignments of the proton and carbon signals in **1** were accomplished by analysis of the ^{1}H−^{1}H COSY, TOCSY, gHSQC, and gHMBC spectra (Table 1). Therefore, barlupulin A was characterized as **1**. This is the first report of an iridoid glycoside with the formate group as a side functional group.

Compound **2** was isolated as an amorphous powder, and its molecular formula was established as $C_{22}H_{30}O_{14}$ on the basis of its ^{13}C NMR data and the molecular ion $[M + Na]^+$ at m/z 541.1525 (calcd for $C_{22}H_{30}O_{14}Na$, 541.1533) in the HR-ESIMS. Comparison of the spectroscopic data (Table 1) of **2** with those of **1** showed that **2** carried an additional acetyl group [δ_H 2.04 (s); δ_C 172.5]. In the gHMBC spectrum, a correlation from H-6 (δ_H 5.23) to the acetyl carbonyl carbon (δ_C 172.5) assigned the additional acetyl group to C-6 (Figure 1). The relative configuration of **2** was established to be identical to **1** by the coupling constants and the NOESY data of **2**. The absolute configuration of the glucose moiety was similarly established as D. Thus, the structure of barlupulin B was characterized as **2**.

Compound **3**, an amorphous powder, had the molecular formula $C_{17}H_{24}O_{12}$ established from its ^{13}C NMR data and the molecular ion $[M + Na]^+$ at m/z 443.1167 (calcd for $C_{17}H_{24}O_{12}Na$, 443.1165) in the HR-ESIMS. Its NMR data were similar to those of shanzhiside (**17**) isolated from this plant,[15] except for the presence of a formate group [δ_H 8.14 (s); δ_C 163.3] in **3**. The C-6′ location of the formate group was confirmed by gHMBC correlations from H_2-6′ (δ_H 4.50 and 4.33) to the formate carbonyl carbon (δ_C 163.3) and from the formate hydrogen (δ_H 8.14) to C-6′ (δ_C 64.3) (Figure 1). The

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 HMz) Data of Barlupulins A (1) and B (2)

	1		**2**	
position	δ_C (methanol-d_4)[a]	δ_H (J in Hz)	δ_C (methanol-d_4)[a]	δ_H (J in Hz)
1	95.7 d	5.74, d (2.5)	95.7 d	5.67, d (4.0)
3	153.8 d	7.42, d (1.0)	154.0 d	7.48, d (1.0)
4	110.1 s		109.0 s	
5	42.6 d	3.03, d (8.5)	40.2 d	3.23, dd (7.0, 2.0)
6	76.6 d	4.26, m	79.2 d	5.23, m
7α	47.9 t	2.18, d (15.0)	45.2 t	2.33, d (15.0)
7β		2.05, dd (15.0, 6.0)		2.09, dd (15.0, 6.0)
8	89.6 s		89.5 s	
9	50.0 d	2.96, dd (8.5, 2.5)	50.5 d	2.90, dd (7.0, 4.0)
10	22.3 q	1.49, s	22.0 q	1.53, s
11	169.3 s		168.7 s	
OMe	52.1 q	3.71, s	52.1 q	3.68, s
6-OAc			172.5 s	2.04, s
8-OAc	172.7 s	1.98, s	172.5 s	1.99, s
1′	100.6 d	4.62, d (8.0)	100.5 d	4.66, d (7.0)
2′	74.9 d	3.17, m	74.9 d	3.18, m
3′	78.0 d	3.36, m	78.0 d	3.35, m
4′	71.8 d	3.32, m	71.8 d	3.31, m
5′	75.8 d	3.51, m	75.8 d	3.51, m
6′a	64.2 t	4.49, dd (11.5, 2.0)	64.2 t	4.52, dd (12.0, 2.0)
6′b		4.33, dd (11.5, 6.0)		4.34, dd (12.0, 5.5)
6′-COH	163.4 s	8.17, s	163.4 s	8.17, s

[a]The assignments were based on ^{1}H−^{1}H COSY, gHSQC, TOCSY, and gHMBC experiments.

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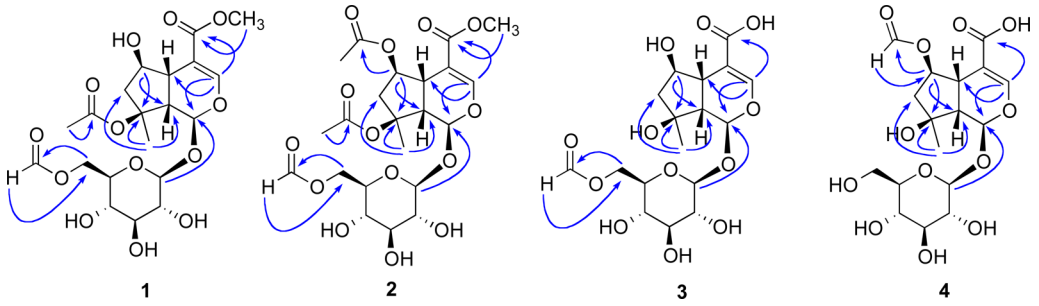

Figure 1. Key HMBC correlations of **1**–**4**.

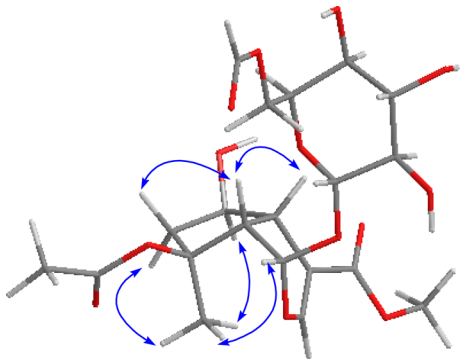

Figure 2. Key NOESY correlations of **1**.

relative configuration of **3** was confirmed to be identical to shanzhiside by the coupling constants and its NOESY data. Acid hydrolysis of **3** also afforded D-glucose. From these data, the structure of compound **3** (barlupulin C) was established as shown.

Compound **4** exhibited the same molecular formula, $C_{17}H_{24}O_{12}$, as **3**, which was established by its ^{13}C NMR data and the molecular ion $[M + Na]^+$ at m/z 443.1164 (calcd for $C_{17}H_{24}O_{12}Na$, 443.1165) in the HR-ESIMS. The NMR spectroscopic data of **4** were similar to those of **3** except that the formate group was located at C-6 instead of C-6′. This was confirmed by gHMBC correlations between H-6 (δ_H 5.19) and the carbonyl carbon of the formate group (δ_C 163.1) and between the formate hydrogen (δ_H 8.07) and C-6 (δ_C 79.5) (Figure 1), together with ^1H–^1H COSY correlations of H-5/H-6/H-7. The relative configuration of **4** was determined to be identical to **3** by the coupling constants and its NOESY data. Therefore, the structure of barlupulin D was characterized as **4**.

The known compounds were identified as 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester (**5**),[8] 6-*O*-*p*-methoxy-*cis*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester (**6**),[8] 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside (**7**),[11] 6-*O*-*p*-methoxy-*cis*-cinnamoyl-8-*O*-acetylshanzhiside (**8**),[11] 6-*O*-*p*-*trans*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (**9**),[8] 6-*O*-*p*-*cis*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (**10**),[8] acetylbarlerin (**11**),[12,13] barlerin (**12**),[12,13] ipolamiidoside (**13**),[16] 8-*O*-acetylshanzhiside (**14**),[17] 6-*O*-acetylshanzhiside methyl ester (**15**),[18] shanzhiside methyl ester (**16**),[18] shanzhiside (**17**),[15] and mussaenosidic acid (**18**)[19] by comparison of their physical data with reported values. LC/MS analysis showed the presence of compounds **1**–**4** in the crude extract of *B. lupulina*, indicating that they are not artifacts produced during the separation process.

The in vitro cytotoxic activity of compounds **1**–**4**, **7**, **8**, and **11**–**18** was tested at 20 μg/mL against two Stat3-activated cancer cell lines, MDA-MB-231 (breast cancer) and U251 MG (glioblastoma), and control cell line NIH3T3. However, after 72 h incubation, none of the 14 iridoids showed significant effects on growth of the cell lines. Iridoid glucosides are known for their radical-scavenging activity.[20−23] Thus, compounds **9**, **12**, and **15**–**17** were tested for radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Compounds **16** and **17** showed a low level of free-radical-scavenging activity (antioxidant activity) in the DPPH assay (IC_{50} < 100 μg/mL). The IC_{50} values of **9**, **12**, and **15**–**17** were found to be 486.5, 110.3, 286.4, 97.5, and 78.6 μg/mL, respectively, compared to 7.63, 2.79, and 9.52 μg/mL for ascorbic acid, gallic acid, and quercetin, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a Jasco P-1010 polarimeter. UV spectra were recorded on an Amersham Biosciences Ultrospec 5300 Pro spectrophotometer, and IR spectra were measured on a Bruker Alpha-P spectrometer. All NMR experiments were carried out on a Varian INOVA 600 NMR spectrometer. ESIMS spectra were obtained by LC/MS analysis, which was performed on an Agilent 1200 Series HPLC/6130 Series mass spectrometer. High-resolution mass spectra were obtained on a Waters Micromass Q-Tof Ultima ESI-TOF mass spectrometer. All the compounds were purified on an Agilent 1100 series HPLC (Agilent Technologies) using a Phenomenex Luna phenyl-hexyl column (25 cm × 10 mm, 5 μm particle size), a Phenomenex Luna phenyl-hexyl column (250 × 21.2 mm, 10 μm particle size), and a Phenomenex Luna C_{18} HPLC column (250 × 21.2 mm, 5 μm particle size).

Plant Material. The aerial part of *B. lupulina* was purchased in Vung Tau, Vietnam, in March 2012. A voucher specimen (No. 101) was deposited at BIDMC, Harvard Medical School.

Extraction and Isolation. The dried aerial parts (2 × 200 g) of *B. lupulina* were sliced, and each 200 g was separately boiled in water (1.2 L) for 4–5 h, over which time the volume was reduced to 100 mL. The residual aqueous extracts were centrifuged at 10000*g* for 30 min and filtered/sterilized. The combined extracts (200 mL) were suspended in H_2O (100 mL) and successively extracted with EtOAc and *n*-BuOH to afford 0.52, 9.0, and 20.0 g of residues, respectively. The EtOAc-soluble fraction (0.52 g) was fractionated by preparative HPLC (C_{18} column, Phenomenex Luna, 250 × 21.2 mm, 5 μm) using 23% aqueous MeCN (+0.1% formic acid) for 20 min, 100% MeCN (+0.1% formic acid) over the next 10 min, and 100% MeCN (+0.1% formic acid) for the next 10 min (flow rate: 10 mL/min) to give eight fractions (A–H) according to HPLC analysis. Fraction H was further separated by preparative Phenomenex Luna C_{18} HPLC using 40% aqueous MeCN (+0.1% formic acid) for 20 min, 60% MeCN (+0.1% formic acid) over the next 10 min, and 100% MeCN (+0.1% formic acid) for the next 10 min (flow rate: 10 mL/min) to yield 39 fractions according to HPLC analysis. Fraction H-24 was subsequently purified by a semipreparative Phenomenex Luna HPLC phenyl-hexyl column (25 cm × 10 mm, 5 μm; 2 mL/min) with the isocratic solvent of 40% aqueous MeCN (+0.1% formic acid) to give compound **5** (t_R 21.0 min, 2.6 mg). Fractions H-11, H-14, and H-21 were purified using a

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semipreparative Phenomenex Luna HPLC phenyl-hexyl column to yield compounds **6** (from H-21, 40% MeCN, t_R 19.0 min, 0.7 mg), **7** (from H-14, 32% MeCN, t_R 32.6 min, 0.5 mg), **8** (from H-14, 32% MeCN, t_R 26.4 min, 0.5 mg), **9** (from H-11, 30% MeCN, t_R 30.7 min, 4.0 mg), and **10** (from H-11, 30% MeCN, t_R 27.5 min, 1.6 mg). The combined mixture of fractions from H-5 to H-9 (assigned as A′) was further separated by a preparative Phenomenex Luna phenyl-hexyl column (250 × 21.2 mm, 10 μm particle size) using 10% aqueous MeCN (+0.1% formic acid) for 30 min, 100% MeCN (+0.1% formic acid) over the next 5 min, and 100% MeCN (+0.1% formic acid) for the next 10 min (flow rate: 10 mL/min) to yield 39 subfractions (A1′−A39′), comprising pure compounds **14** (A28′, t_R 30.5 min, 9.6 mg), **15** (A23′, t_R 26.0 min, 12.4 mg), and **16** (A13′, t_R 15.8 min, 30.5 mg). Further purification of the subfractions A37′ and A38′ using a Phenomenex Luna HPLC phenyl-hexyl column afforded compounds **1** (from A38′, 23% MeCN, t_R 15.9 min, 1.4 mg), **2** (from A37′, 21% MeCN, t_R 24.1 min, 1.7 mg), **11** (from A37′, 21% MeCN, t_R 23.2 min, 7.6 mg), **12** (from A37′, 21% MeCN, t_R 11.5 min, 5.5 mg), and **13** (from A37′, 21% MeCN, t_R 15.8 min, 2.5 mg). Subfraction A7′ was further separated by a Phenomenex Luna HPLC phenyl-hexyl column (8% MeCN) to give compounds **3** (t_R 25.8 min, 2.3 mg), **4** (t_R 16.0 min, 1.3 mg), and **17** (t_R 12.9 min, 8.9 mg). Subfraction A4′ yielded compound **18** (t_R 14.0 min, 0.8 mg) by separation with a Phenomenex Luna HPLC phenyl-hexyl column (10% MeCN).

*Barlupulin A (**1**):* amorphous powder; $[\alpha]_D^{25}$ −17 (*c* 0.04, MeOH); IR (KBr) ν_{max} 3398, 2920, 1707, 1595, 1439, 1354, 1283, 1187, 1026 cm^{-1}; UV (MeOH) λ_{max} (log ε) 236 (3.56) nm; ^1H (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) data, see Table 1; positive HR-ESIMS *m*/*z* 499.1431 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_{13}Na$, 499.1428).

*Barlupulin B (**2**):* amorphous powder; $[\alpha]_D^{25}$ −32 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3366, 2924, 1710, 1592, 1438, 1354, 1277, 1168, 1022 cm^{-1}; UV (MeOH) λ_{max} (log ε) 236 (3.58) nm; ^1H (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) data, see Table 1; positive HR-ESIMS *m*/*z* 541.1525 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_{14}Na$, 541.1533).

*Barlupulin C (**3**):* amorphous powder; $[\alpha]_D^{25}$ −43 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3375, 2925, 1658, 1597, 1453, 1352, 1276, 1170, 1025 cm^{-1}; UV (MeOH) λ_{max} (log ε) 236 (3.56) nm; ^1H (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) data, see Table 2; positive HR-ESIMS *m*/*z* 443.1167 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_{12}Na$, 443.1165).

*Barlupulin D (**4**):* amorphous powder; $[\alpha]_D^{25}$ −34 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3350, 2924, 1651, 1597, 1547, 1401, 1352, 1076 cm^{-1}; UV (MeOH) λ_{max} (log ε) 236 (3.56) nm; ^1H (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) data, see Table 2; positive HR-ESIMS *m*/*z* 443.1164 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_{12}Na$, 443.1165).

Acid Hydrolysis of 1−4. Each compound (0.5 mg) was individually refluxed in 6% HCl (1.0 mL) at 80 °C for 2 h. Each reaction mixture was extracted with $CHCl_3$ (3 × 6 mL), and the H_2O phase was dried by using a SpeedVac concentrator. The residues were separately subjected to column chromatography over silica gel with EtOAc−EtOH−H_2O (7:4:1) as eluent to yield glucose (0.1 mg) from **1**, $[\alpha]_D^{25}$ +46 (*c* 0.01, H_2O), glucose (0.1 mg) from **2**, $[\alpha]_D^{25}$ +42 (*c* 0.01, H_2O), glucose (0.2 mg) from **3**, $[\alpha]_D^{25}$ +52 (*c* 0.02, H_2O), and glucose (0.1 mg) from **4**, $[\alpha]_D^{25}$ +43 (*c* 0.01, H_2O), respectively. Identification of glucose was analyzed by silica gel co-TLC with an authentic sample [solvent system ($CHCl_3$−MeOH−H_2O, 8:5:1), R_f of glucose, 0.30].[14]

Cytotoxicity Assays. Viability of normal mouse fibroblasts (NIH3T3) and two Stat3-activated cancer cell lines, MDA-MB-231 (breast cancer) and U251 MG (glioblastoma), was determined using the CyQuant assay according to the manufacturer's instructions (Life Technologies, CA, USA).[24,25] Briefly, cells were cultured in 96-well plates at 1000 cells per well for 24 h and subsequently treated with compounds (20 μg/mL) for 72 h and analyzed. Relative viability of the treated cells was normalized to the DMSO-treated control cells.[24,25]

DPPH Assay. The DPPH radical-scavenging effect was assessed by the decoloration of a MeOH solution of DPPH. The method used by Kumarasamy and co-workers was adopted with suitable modification.[26]

Table 2. ^1H (600 MHz) and ^{13}C NMR (150 HMz) Data of Barlupulins C (3) and D (4)

	3		4	
position	δ_C (methanol-d_4)[a]	δ_H (*J* in Hz)	δ_C (methanol-d_4)[a]	δ_H (*J* in Hz)
1	95.3 d	5.38, d (3.5)	95.1 d	5.54, d (3.5)
3	152.9 d	7.40, s	153.8 d	7.45, s
4	112.0 s		110.0 s	
5	42.1 d	2.98, dd (10.0, 4.0)	39.3 d	3.34, obsc[b]
6	78.0 d	4.03, m	79.5 d	5.19, m
7α	49.4 t	2.01, dd (13.0, 6.0)	48.0 t	2.24, dd (14.0, 7.0)
7β		1.82, dd (14.0, 6.0)		1.86, dd (14.0, 4.0)
8	79.4 s		79.4 s	
9	52.0 d	2.55, dd (10.0, 3.5)	51.8 d	2.56, dd (9.0, 3.5)
10	24.9 q	1.25, s	25.7 q	1.32, s
11	171.4 s		170.4 s	
6-COH			163.1 s	8.07, s
1′	100.0 d	4.66, d (8.0)	100.2 d	4.68, d (8.5)
2′	74.8 d	3.17, m	74.9 d	3.19, m
3′	78.0 d	3.36, m	78.2 d	3.37, m
4′	71.6 d	3.31, m	71.9 d	3.25, m
5′	75.6 d	3.50, m	78.6 d	3.32, m
6′a	64.3 t	4.50, dd (12.0, 2.0)	63.3 t	3.91, dd (12.5, 2.0)
6′b		4.33, dd (12.0, 6.0)		3.64, dd (12.0, 6.5)
6′-COH	163.3 s	8.14, s		

[a]The assignments were based on ^1H−^1H COSY, gHSQC, TOCSY, and gHMBC experiments. [b]Obsc: signal obscured.

A MeOH solution (100 mL) of the compounds at various concentrations was added to a DPPH−MeOH (78 mg/mL) solution, and the absorbance of the remaining DPPH was measured on a spectrophotometer at 517 nm after 10 min incubation in a dark area. The radical-scavenging activity was determined by comparing the absorbance with that of the blank (100%) containing DPPH and solvent.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra of **1**−**4**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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