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Chemical Constituents and Their Bioactivities of "Tongling White Ginger" (*Zingiber officinale*)

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S Supporting Information

ABSTRACT: Gingerols and their corresponding dehydration products shogaols were considered as the active principles of ginger, the rhizome of the plant *Zingiber officinale*, for its antioxidant, anti-inflammatory, and antitumor activities. Ginger (*Z. officinale*) has been cultivated for thousands of years as a spice and for medicinal purposes in China. Tongling (Anhui province, China) has traditionally been regarded as an ideal cultivation place. "Tongling White Ginger" enjoys a reputation for being one of the top gingers in China for its thin white peel, tender flesh, rich juice, and flavor. In this study, we have isolated and identified two novel gingerdione dimers, bisgingerdiones A (**1**) and B (**2**); two new gingerol derivatives, (5*R*)-5-acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one (**3**) and methyl (*Z*)-neral acetal-[6]-gingerdiol (**4**); and 38 known compounds (**5**−**42**) from rhizomes of *Zingiber officinale* collected from Tongling, China. Their structures were elucidated by means of spectroscopic methods. Compounds **1**−**4** showed weak cytotoxic and anti-HIV-1 activities. Compounds **6**, **8**, and **26** showed inhibitory activities against human and mouse 11β-HSD1 (11β-hydroxysteroid dehydrogenases) with IC_{50} values between 1.09 and 1.30 μM.

KEYWORDS: *Zingiber officinale*, Tongling White Ginger, gingerdione dimers, gingerol derivatives, 11β-HSD

INTRODUCTION

Ginger, the rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae), as one of the most popular spices has been cultivated for thousands of years for medicinal uses in China. It has been used in both fresh and dried forms for the treatment of cough, rheumatism, asthma, stroke, and diabetes.[1] A number of gingerol and diarylheptanoid derivatives and their bioactivities have been reported.[2−6] Tongling (Anhui province, China) has traditionally been regarded as an ideal cultivation place (Figure 1). Although "Tongling White Ginger" enjoys a reputation for being one of the top gingers in China for its thin white peel, tender flesh, rich juice, and flavor,[7−10] no details of the chemical profile of "Tongling White Ginger" have been reported previously except its essential oil.[10] According to a crude extract HPLC analysis, we found that the gingerol derivatives are abundant in "Tongling White Ginger" (Figure 2). Further studies on this extract led to the isolation of four new gingerol derivatives, bisgingerdiones A (**1**) and B (**2**), (5*R*)-5-acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one (**3**), and methyl (*Z*)-neral acetal-[6]-gingerdiol (**4**) (Figure 3), together with 38 known compounds (**5**−**42**) (Table 1). Their structures were elucidated and identified on the basis of extensive spectroscopic methods including 1D or 2D NMR, MS, UV, IR, OR, and CD. Compounds **1**−**4** showed weak cytotoxic and anti-HIV-1 activities. Compounds **6**, **8**, and **26** showed inhibitory activities against human and mouse 11β-HSD1 (11β-hydroxysteroid dehydrogenases) with IC_{50} values between 1.09 and 1.30 μM.

Figure 1. "Tongling White Ginger" locale.

MATERIALS AND METHODS

Instrumentation. Optical rotations (OR) were measured with a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). Circular dichroism (CD) spectra were detected by a digital Applied Photophysics instrument (Agilent). Ultraviolet absorption (UV) spectra were recorded by a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). Infrared spectroscopy (IR) spectra were obtained from a Bio-Rad FtS-135 spectrometer (Bio-Rad, Hercules, CA). Nuclear magnetic resonance (NMR) spectra were measured on Bruker AV400, Bruker

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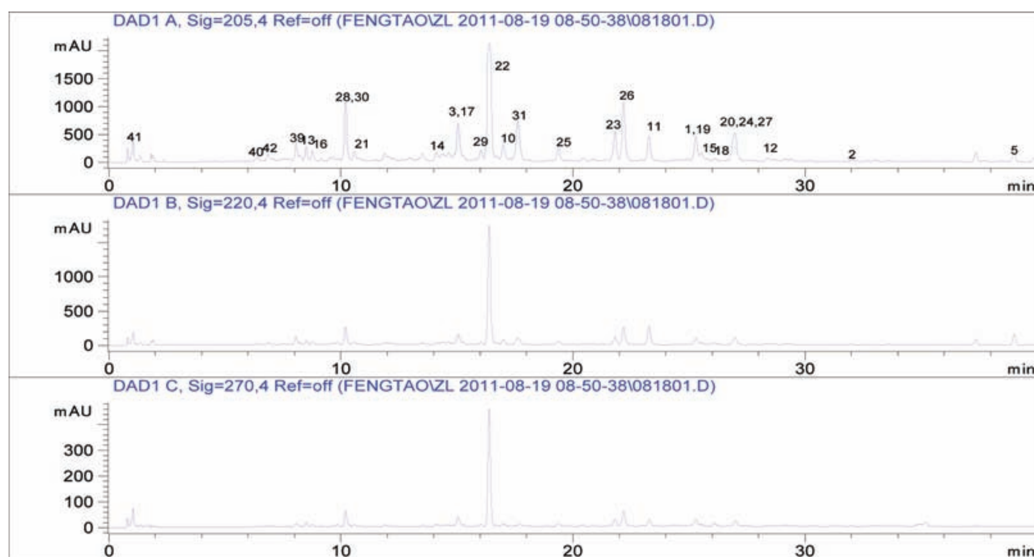

Figure 2. HPLC analysis on the crude extract of Tongling White Ginger (analysis conditions: MeCN/H_2O, from 20:80 to 100:0 in 40 min).

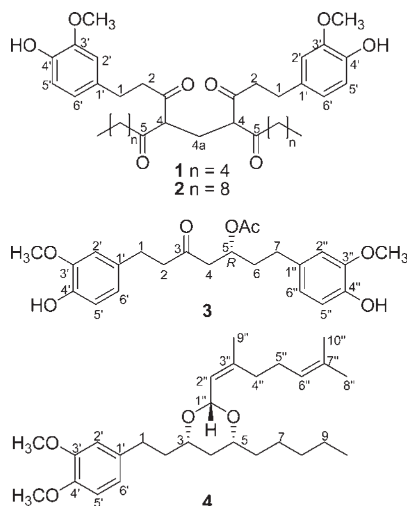

Figure 3. Structures of the new compounds **1**−**4**.

DRX500, and Bruker AV600 MHz spectrometers with tetramethylsilane as the internal standard. Electrospray ionization mass spectrometry (ESI-MS) and high-resolution (HR) ESI-MS were recorded with an APIQSTAR Pulsar 1 spectrometer (Advanced Biomics, Los Angeles, CA). Column chromatography was performed with normal-phase silica gel (200−300 mesh, Merck) and reversed-phase C18 silica gel (20−45 μm, Fuji Silysia Chemical Ltd., Aichi, Japan). A BUCHI C-615 instrument was used for middle-performance liquid chromatography (MPLC) preparation equipped with C18 silica gel columns. An Agilent 1100 series instrument equipped with Agilent ZORBAX SB-C18 column (5 μm, 4.6 mm × 150 mm) was used for high-performance liquid chromatography (HPLC) analysis, and a semipreparative Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 150 mm) was used for the sample preparation. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China), and spots were visualized by heating TLC plates sprayed with 10% H_2SO_4 in ethanol.

Plant Material. The gingers of *Zingiber officinale* Roscoe were collected in Tongling, Anhui province, People's Republic of China, and identified by Mr. Yi Chen of Kunming Institute of Botany, Chinese Academy of Sciences. A sample (20101008) was deposited in Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The fresh gingers (5.2 kg) were cut into pieces and extracted in 90% Me_2CO (3 × 5 L, each 1 day). After removal of acetone under reduced pressure, the viscous concentrate was partitioned between EtOAc (2 L) and H_2O (2 L) thrice. The EtOAc extract (24 g) was chromatographed on a silica gel column, eluting with a gradient of petroleum ether/Me_2CO (from 1:0 to 0:1, v/v). Five fractions (A−E) were obtained on the basis of TLC plate analysis. Fraction A (4.3 g) was separated repeatedly by silica gel eluting with petroleum ether/Me_2CO (from 10:1 to 5:1, v/v) to afford **4** (10 mg), **6** (8 mg), **7** (6 mg), **8** (55 mg), **9** (4 mg), **37** (30 mg), and **38** (6 mg). The gingerol derivatives including **1** (10 mg), **2** (11 mg), **5** (17 mg), **10** (4 mg), **11** (11 mg), and **12** (3 mg) from fraction B (2.4 g) were prepared by HPLC eluted with MeCN/H_2O (from 3:2 to 1:0, v/v). In addition, compounds **32** (210 mg), **34** (14 mg), **35** (7 mg), and **36** (13 mg) were crystals and precipitated during the process of the isolation of fraction B. Fraction C (9.2 g) was separated first by MPLC eluted with MeOH/H_2O (from 1:4 to 1:0, v/v) to afford **22** (5.3 g), **39** (31 mg) and a mixture (800 mg). The mixture was purified further by HPLC eluting with MeCN/H_2O (from 2:3 to 4:1) to afford **19** (50 mg), **20** (9 mg), **21** (22 mg), **23** (15 mg), **24** (7 mg), **25** (8 mg), **26** (25 mg), and **27** (7 mg). Fraction D (760 mg) was separated by MPLC eluted with MeOH/H_2O (from 1:4 to 1:1) to afford **40** (13 mg), **42** (6 mg), and a mixture (520 mg). The mixture was prepared by HPLC eluting with MeCN/H_2O (from 1:4 to 3:2) to afford **13** (11 mg), **14** (110 mg), **15** (12 mg), **16** (11 mg), **17** (50 mg), **18** (8 mg), **28** (45 mg), **31** (62 mg), and a two-compound mixture (60 mg). The latter was separated further by silica gel eluted with $CHCl_3$/Me_2CO (10:1) to afford **3** (15 mg) and **29** (10 mg). Fraction E (440 mg) was separated by silica gel eluting with $CHCl_3$/Me_2CO (from 10:1 to 5:1) to give **33** (133 mg) and a mixture (122 mg). The latter was separated by HPLC eluting with MeCN/H_2O (from 1:4 to 1:1) to afford **30** (18 mg) and **41** (33 mg).

*Physical Data for Bisgingerdione A (**1**).* Colorless oil. IR (KBr): ν_{max} 3447, 2920, 2932, 1724, 1699, 1516, 1271, 1034 cm^{-1}. UV (MeOH): λ_{max} (log ε) 282 (3.25), 218 (3.55) nm. For ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2. ESIMS: m/z 619 $[M + Na]^+$. HRESIMS: m/z 619.3260 $[M + Na]^+$, calcd for $C_{35}H_{48}O_8Na$, 619.3246.

*Physical Data for Bisgingerdione B (**2**).* Colorless oil. IR (KBr): ν_{max} 3445, 2926, 1725, 1700, 1516, 1271, 1035 cm^{-1}. UV (MeOH): λ_{max} (log ε) 282 (3.19), 218 (3.51) nm. For ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR

Table 1. Thirty-Eight Known Compounds from "Tongling White Ginger" (*Zingiber officinale*)

Structure	Cpd	n	R	Name
	5			(2*Z*)-Neral acetal-[6]-gingerdiol
	6			[6]-Paradol
	7	2		(*E*)-[4]-Shogaol
	8	4		(*E*)-[6]-Shogaol
	9	8		(*E*)-[10]-Shogaol
	10	4		5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one
	11	6		5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-4-dodecen-3-one
	12	8		5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-4-tetradecane-3-one
	13	2		(3*S*,5*R*)-[4]-Gingerdiol
	14	4		(3*S*,5*R*)-[6]-Gingerdiol
	15	8		(3*S*,5*R*)-[10]-Gingerdiol
	16	2		(3*R*,5*S*)-[4]-Gingerdiol
	17	4		(3*R*,5*S*)-[6]-Gingerdiol
	18	8		(3*R*,5*S*)-[10]-Gingerdiol
	19	4	H	(3*R*,5*S*)-Diacetoxy-[6]-gingerdiol
	20	4	CH_3	(3*R*,5*S*)-Methyl diacetoxy-[6]-gingerdiol
	21	2	H	(5*S*)-[4]-Gingerol
	22	4	H	(5*S*)-[6]-Gingerol
	23	6	H	(5*S*)-[8]-Gingerol
	24	8	H	(5*S*)-[10]-Gingerol
	25	4	CH_3	(5*S*)-Methyl [6]-gingerol
	26	4		(5*R*)-Acetoxy-[6]-gingerol
	27	6		(5*R*)-Acetoxy-[8]-gingerol
	28			(5*R*)-1,7-Bis-(4′-hydroxy-3′-methoxyphenyl)-5-hydroxyheptan-3-one
	29			(*E*)-1,7-Bis-(4′-hydroxy-3′-methoxyphenyl)-4-hepten-3-one
	30		H	(3*R*,5*S*)-3,5-Dihydroxy-1,7-bis-(4′-hydroxy-3′-methoxyphenyl)-heptane
	31		Ac	(3*R*,5*S*)-3,5-Diacetoxy-1,7-bis-(4′-hydroxy-3′-methoxyphenyl)-heptane
	32		H	*β*-Sitosterol
	33		Glc	Daucosterol
	34		=O	Stigmast-4-en-3,6-dione
	35		OH	6*β*-Hydroxystigmast-4-en-3-one
	36		H	Stigmast-4-en-3-one
	37			(*E*)-Citral
	38			(*Z*)-Citral
	39		CHO	8-Hydroxy-2,6-dimethyl-2,6-octadienal
	40		CH_2OH	8-Hydroxygeraniol
	41		COOH	Foliamenthic acid
	42			2,6-Dimethyl-2-octene-1,8-diol

(100 MHz, $CDCl_3$) data, see Table 2. ESIMS: m/z 731 $[M + Na]^+$. HRESIMS: m/z 731.4516 $[M + Na]^+$, calcd for $C_{43}H_{64}O_8Na$, 731.4498.

*Physical Data for (5R)-5-Acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one (**3**).* Colorless oil; $[\alpha]_D^{25}$ −3.3 (c 0.25, $CHCl_3$). IR (KBr): ν_{max} 3443, 2921, 1720, 1638, 1521, 1272, 1128, 1034 cm^{-1}. UV (MeOH): λ_{max} (log ε) 281 (3.08), 223 (3.49), 204 (3.87) nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.79 (2H, m, H-1), 2.68 (2H, m, H-2), 2.73 and 2.57 (each 1H, m, H-4), 5.24 (1H, m, H-5), 1.82 (2H, m, H-6), 2.60 and 2.53 (each 1H, m, H-7), 6.65 and 6.66 (each 1H, d, J = 1.8 Hz, H-2′), 6.80 and 6.81 (each 1H, d, J = 8.0 Hz, H-5′), 6.62 and 6.63 (each 1H, dd, J = 8.0, 1.8 Hz, H-6′), 3.87 (3H, s, OMe), 3.85 (3H, s, OMe), 2.00 (3H, s, $OOCCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 29.4 (t, C-1), 45.3 (t, C-2), 207.2 (s, C-3), 47.5 (t, C-4), 70.0 (d, C-5), 36.2 (t, C-6), 31.4 (t, C-7), 132.9 and 133.1 (each 1C, s, C-1′), 111.0 (2C, d, C-2′), 146.5 (2C, s, C-3′), 143.9

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of 1 and 2 in $CDCl_3$ (δ in ppm)

	1		2	
no.	δ_H (J in Hz)	δ_C mult	δ_H (J in Hz)	δ_C mult
1	2.78, m	29.1, t	2.78, m	28.9, t
2	2.72, m	44.1, t	2.72, m	44.1, t
3		205.2, s		205.2, s
4	3.47, t (6.6)	63.8, d	3.47, t (6.9)	63.7, d
4a	2.15, m	25.2, t	2.14, m	25.1, t
5		205.7, s		205.8, s
6	2.27, t (7.3)	42.5, t	2.28, t (7.2)	42.5, t
7	1.24, m	22.9, t	1.22, m	23.3, t
8	1.17, m	31.1, t	1.43, m	31.8, t
9	1.24, m	22.3, t	1.24, overlapped	29.3, t[a]
10	0.85, t (7.3)	13.8, q	1.24, overlapped	29.3, t[a]
11			1.24, overlapped	29.2, t[a]
12			1.24, overlapped	29.1, t[a]
13			1.27, m	22.6, t
14			0.86, t (7.0)	14.0, q
1′		132.3, s		132.2, s
2′	6.67, d (1.7)	111.1, d	6.67, d (1.6)	111.0, d
3′		146.4, s		146.3, s
4′		144.0, s		143.9, s
5′	6.80, d (8.0)	114.3, d	6.80, d (8.0)	114.2, d
6′	6.63, dd (8.0, 1.7)	120.9, d	6.61, dd (8.0, 1.7)	120.8, d
OCH_3	3.87, s	55.9, q	3.86, s	55.8, q

[a] Interchangeable.

and 144.0 (each 1C, s, C-4′), 114.4 (2C, d, C-5′), 120.9 (2C, d, C-6′), 56.0 (2C, q, OMe), 170.7 (s, $OOCCH_3$), 21.3 (q, $OOCCH_3$). ESIMS: m/z 439 $[M + Na]^+$. HRESIMS: m/z 439.1720 $[M + Na]^+$, calcd for $C_{23}H_{28}O_7Na$, 439.1732.

*Physical Data for Methyl (Z)-Neral Acetal-[6]-gingerdiol (**4**).* Colorless oil; $[\alpha]_D^{25}$ + 8.5 (c 0.25, MeOH). IR (KBr): ν_{max} 3425, 2931, 1729, 1516, 1454, 1262, 1237, 1003 cm^{-1}. UV (MeOH): λ_{max} (log ε) 279 (2.80), 228 (3.26) nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.63 and 2.71 (each 1H, m, H-1), 1.88 and 1.70 (each 1H, m, H-2), 3.60 (1H, m, H-3), 1.63 and 1.42 (each 1H, m, H-4), 3.62 (1H, m, H-5), 1.49 and 1.31 (each 1H, m, H-6), 1.40 and 1.33 (each 1H, m, H-7), 1.25 (2H, m, H-8), 1.28 (2H, m, H-9), 0.86 (3H, H-10), 6.73 (1H, s, H-2′), 6.77 (1H, d, J = 8.0 Hz, H-5′), 6.72 (1H, d, J = 8.0 Hz, H-6′), 5.17 (1H, d, J = 6.0 Hz, H-1″), 5.34 (1H, d, J = 6.0 Hz, H-2″), 2.03 (2H, m, H-4″), 2.12 (2H, m, H-5″), 5.09 (1H, t, J = 5.8 Hz, H-6″), 1.68 (3H, s, H-8″), 1.73 (3H, s, H-9″), 1.59 (3H, s, H-10″), 3.86 (3H, s, OMe), 3.86 (3H, s, OMe). ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.7 (t, C-1), 37.7 (t, C-2), 76.4 (d, C-3), 35.9 (t, C-4), 75.4 (d, C-5), 36.8 (t, C-6), 24.6 (t, C-7), 31.7 (t, C-8), 22.5 (t, C-9), 14.0 (q, C-10), 134.5 (s, C-1′), 111.0 (d, C-2′), 148.6 (s, −3′), 147.0 (s, C-4′), 111.6 (d, C-5′), 120.1 (d, C-6′), 98.5 (d, C-1″), 122.3 (d, C-2″), 142.3 (s, C-3″), 39.2 (t, C-4″), 26.0 (t, C-5″), 123.8 (d, C-6″), 131.7 (s, C-7″), 25.6 (q, C-8″), 17.3 (q, C-9″), 17.6 (q, C-10″), 55.7 (q, OMe), 55.8 (q, OMe). ESIMS: m/z 445 $[M + H]^+$. HRESIMS: m/z 445.3323 $[M + H]^+$, calcd for $C_{28}H_{45}O_4$, 445.3317.

Cytotoxic Assay. Compounds **1**−**4** were evaluated for their cytotoxicity against five human cancer cell lines, breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells. Cells were cultured in RPMI-1640 or in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method in 96-well microplates.[11] Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h, and all tests were done twice with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.[12]

***In Vitro* Anti-HIV-1 Assay.** Compounds **1**−**4** were evaluated for their anti-HIV-1_{IIIB} activity by microtiter syncytium formation infectivity assay, using the method previously described, with 3′-azido-3′-deoxythymidine (AZT) as a positive control.[13] The assays included cytotoxicity (CC_{50}) in C8166 cell and inhibition (EC_{50}) of syncytium formation in HIV-1_{IIIB}-infected C8166 cell in vitro. The cytotoxicity was determined by the MTT method. The absorbance at 595 nm/630 nm ($A_{595/630}$) was read in an ELISA reader (Elx800, Bio-Tek Instrument Inc., Highland City, FL, USA). The minimum cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined from the dose response curve. In the presence or absence of various concentrations of test compounds, 3 × 104 C8166 cells were exposed to HIV-1_{IIIB} at a multiplicity of infection (MOI) of 0.01. The cells were incubated in 96-well plates or 24-well plates at 37 °C in 5% CO_2 for 3 days. AZT (3′-azido-3′-deoxythymidine, Sigma) was used as a positive control. At 3 days postinfection, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cells) in each well of 96-well plates under an inverted microscope, and HIV-1_{IIIB} p24 antigen in the culture supernatants was determined by an enzyme-linked immunosorbent assay (ELISA). The virus particles in the culture supernatant of each well of 96-well plates were precipitated by 30% PEG (M_w 8000) and lysed with lysis buffer, and the activity of reverse transcriptase (RT) was measured by a commercial RT kit (Roche Molecular Biochemicals). The minimum inhibitory concentrations that reduced CPE, HIV-1_{IIIB} p24 antigen, and RT production by 50% (EC_{50}) were interpolated from plots generated from the data. The therapeutic index (TI) was calculated from the ratio of CC_{50}/EC_{50}.

Inhibitory Activities Against 11β-HSD Assay. The inhibitory activities of the compounds on human or mouse 11β-HSD1 and 11β-HSD2 were determined using the scintillation proximity assay (SPA). Microsomes containing 11β-HSD1 or 11β-HSD2 were used according to our previous studies.[14] The full-length cDNAs of human or murine 11β-HSD1 and 11β-HSD2 were isolated from the cDNA libraries provided by NIH Mammalian Gene Collection. The cDNAs were cloned into pcDNA3 expression vectors. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in the presence of 700 μg/mL of G418. The microsomal fraction overexpressing 11β-HSD1 or 11β-HSD2 was prepared from the HEK-293 cells, which were stably transfected with 11β-HSD1 or 11β-HSD2. The fraction was then used as the enzyme source for SPA. Microsomes containing human or mouse 11β-HSD1 were incubated with NADPH and [^{3}H]cortisone. The product, [^{3}H]cortisol, was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. The 11β-HSD2 screening was performed by incubating 11β-HSD2 microsomes with [^{3}H]cortisol and NAD^+ and monitoring substrate disappearance. All tests were done twice with glycyrrhizinic acid as a positive control. IC_{50} ($X \pm$ SD, n = 2) values were calculated by using Prism Version 4 (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

Compound **1**, isolated as a colorless oil, possesses a molecular formula $C_{35}H_{48}O_8$ with 12 degrees of unsaturation as established

by the HRESIMS at m/z 619.3260 [M + Na]$^+$. The UV spectrum indicated the existence of aromatic rings based on the maximum absorption bands at 282 and 218 nm, while the FTIR spectrum exhibited absorption bands for carbonyl (1724 and 1699 cm^{-1}) and hydroxy (3447 cm^{-1}) groups.

In the ^{1}H NMR spectrum, three downfield signals at δ_H 6.63 (1H, dd, J = 8.0, 1.7 Hz, H-6′), 6.67 (1H, d, J = 1.7 Hz, H-2′), and 6.80 (1H, d, J = 8.0 Hz, H-5′) suggested the existence of a 1,2,4-substituted benzene ring (Table 2). The ^{13}C NMR spectrum displayed 18 carbon resonances ascribable to one methyl, one methoxy, seven methylenes, four methines, three aromatic quaternary carbons, and two carbonyl carbons on the basis of HSQC spectrum (Table 2). Two fragments **a** = $-CH_2CH_2-$ and **b** = $-CH_2CH_2CH_2CH_2CH_3$ were established readily according to the ^{1}H−^{1}H COSY spectrum (Figure 4), and they were connected by a phenyl and two carbonyl groups as supported by the HMBC spectrum including the correlations of δ_H 2.78 (2H, m, H-1) with δ_C 132.3 (s, C-1′), δ_H 2.72 (2H, m, H-2) with δ_C 205.2 (s, C-3), and δ_H 2.27 (2H, t, J = 7.3 Hz, H-6) with δ_C 205.7 (s, C-5) (Figure 4). The above information suggested that compound **1** had a similar structure to the known compound [6]-gingerdione.[15] Differently, the ^{1}H−^{1}H COSY spectrum displayed a cross peak of $-CHCH_2-$ and constructed an additional fragment **c** (Figure 4). This information led to a hypothesis that **1** was a [6]-gingerdione dimer. In the HMBC spectrum, the correlation of a methine proton at δ_H 3.47 (1H, t, J = 6.6 Hz, H-4) with two carbonyl carbons at δ_C 205.2 and 205.7 suggested the methine to be CH-4 (Figure 4). Therefore, compound **1** should be a [6]-gingerdione dimer linked by an additional methylene (δ_H 2.15, 2H, m; δ_C 25.2, t) connected to C-4. The lack of optical activity and no CD absorption band suggested that compound **1** might be a mesomer.[16,17] Therefore, compound **1** was established as shown in Figure 3 and named as bisgingerdione A.

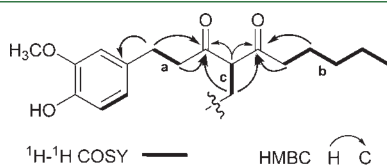

Figure 4. Key 2D NMR correlations of **1**.

Table 3. Cytotoxicity of Compounds 1−4 (IC_{50}, μM)

entry	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	14.1	14.5	21.0	15.5	21.1
2	>40.0	>40.0	>40.0	>40.0	>40.0
3	10.0	12.6	17.1	11.8	15.6
4	22.7	>40.0	>40.0	>40.0	>40.0
cisplatin	1.1	14.5	12.2	17.8	22.5

Table 4. Anti-HIV-1_{IIIB} Activity of 1−4

entry	CC_{50}[a] (μM)	EC_{50}[b] (μM)	CC_{50}/EC_{50}[c]
1	51.2	12.4	4.1
2	174.2	13.5	4.8
3	58.0	10.9	8.2
4	21.0	9.5	2.2
AZT	1390.0	0.0033	4221196.9

[a] Cytotoxicity. [b] Anti-HIV-1_{IIIB} activity. [c] Therapeutic index (TI).

HRESIMS of compound **2** established the molecular formula $C_{43}H_{64}O_8$ on the basis of an [M + Na]$^+$ peak at 731.4516 (calcd for $C_{43}H_{64}O_8Na$, 731.4498). The UV absorption bands at 282 and 218 nm suggested the existence of an aromatic system, while the IR absorption bands at 3445, 1725, and 1700 cm^{-1} corresponded to hydroxy and carbonyl groups. The ^{1}H and ^{13}C NMR spectra displayed similar patterns to those of **1**, except for additional signals at δ_H 1.24 (8H, overlapped) and δ_C 29.1−29.3 (four carbons), corresponding to four additional methylenes in **2**. These data suggested that compound **2** should be a [10]-gingerdione dimer linked by an additional methylene (δ_H 2.14, 2H, m; δ_C 25.1, t) connected to C-4. Detailed analysis of 1D and 2D (HSQC, HMBC) NMR and MS data supported the structure elucidated above. The lack of optical activity also suggested compound **2** to be a mesomer. Therefore, compound **2** was established and named as bisgingerdione B. It is noted that bisgingerdiones A (**1**) and B (**2**) are two novel [6]-gingerdione dimers connected by a methylene group, which have not been found previously.

Compound **3** was isolated as a colorless oil. The molecular formula $C_{23}H_{28}O_7$ was established on the basis of HRESIMS at m/z 439.1720 [M + Na]$^+$. Preliminary analysis of ^{1}H and ^{13}C NMR data suggested that **3** was a diarylheptanoid analogue. A carbonyl carbon at δ_C 207.2, a methine carbon at δ_C 70.0, and an acetoxy group at δ_C 170.7 and 21.3 suggested that compound **3** might be (5*S*)-5-acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one.[18] Detailed analysis of 2D NMR (HSQC, HMBC) spectra confirmed the planar structure as elucidated above. The negative optical rotation ($[\alpha]_D^{20}$ −3.3) of **3** indicated *R* configuration of C-5 in **3**, rather than *S* configuration of that in (5*S*)-5-acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one ($[\alpha]_D^{24}$ + 3.0).[18−21] Therefore, compound **3** was elucidated as (5*R*)-5-acetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one.

Compound **4** was isolated as a colorless oil. HRESIMS displayed an [M + H]$^+$ peak at m/z 445.3323, corresponding to a molecular fomula $C_{28}H_{45}O_4$. Complete analysis of 1D, 2D NMR, and MS data suggested that **4** was an analogue of the

Table 5. Inhibitory Activities of Compounds 6, 8, and 26 against Isozymes of 11β-Hydroxysteroid Dehydrogenases

	mouse			human		
	IC_{50} (μM)			IC_{50} (μM)		
entry	11β-HSD1	11β-HSD2	HSD2/HSD1	11β-HSD1	11β-HSD2	HSD2/HSD1
6	1.09	>100	>91.68	1.19	<100	<83.80
8	1.12	>100	>89.38	1.21	16.60	13.77
26	1.30	>100	>76.83	1.23	<100	<81.50
glycyrrhizinic acid[a]				5.95	0.42	0.07

[a] IC_{50} values in nM.

known compound **5**,[22] except for an additional methoxy placed at C-4′, which was elucidated by the HMBC correlation of δ_H 3.86 (3H, s, OMe) with δ_H 147.0 (s, C-4′). The stereoconfiguration was elucidated by the ROESY experiment. The cross peaks among H-3, H-5, and H-1″ suggested that they were on the same side. The ROESY correlation of H-2″ with H-9″ suggested *Z*-form of the double bond C-2″=C-3″. Therefore, compound **4** was established as depicted.

Systemic study on the chemical constituents of "Tongling White Ginger" led to the isolation of other 38 known compounds including 27 gingerol derivatives (**5**−**31**), five steroids (**32**−**36**), and six monoterpenoids (**37**−**42**) (Table 1). Their structures were elucidated on the basis of 1D NMR, MS, and OR spectroscopic analysis.

Compounds **1**−**4** were evaluated for their cytotoxic and anti-HIV-1_{IIIB} activities, and all compounds (**1**−**42**) were tested for their inhibitory activities against two isozymes of 11β-hydroxysteroid dehydrogenases (11β-HSD1 and 11β-HSD2). Compounds **1**−**4** showed weak cytotoxicities against all of the tested five human cancer cell lines (Table 3). Compounds **1**−**4** showed weak anti-HIV-1_{IIIB} activity with therapeutic index (TI) < 10 (Table 4). Compounds **6**, **8**, and **26** demonstrated significant inhibitory activities against mouse and human 11β-HSD1 (Table 5). However, they showed no selectivity against mouse and human 11β-HSD2 (Table 5).

ASSOCIATED CONTENT

Supporting Information. NMR and MS of compounds **1**−**42**. This material is available free of charge via the Internet at http://pubs.acs.org.

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