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Isoprenylated Flavonoid and Adipogenesis-Promoting Constituents of *Dodonaea viscosa*

Lai-Bin Zhang,[†] Jun Ji,[†] Chun Lei,[†] He-Yao Wang,[‡] Qin-Shi Zhao,[§] and Ai-Jun Hou*[,†]

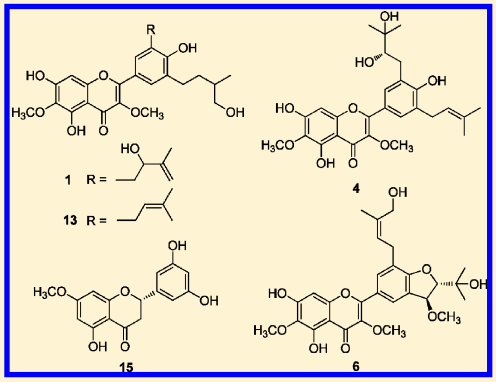
[†]Department of Pharmacognosy, School of Pharmacy, Fudan University, 826 Zhang Heng Road, Shanghai 201203, People's Republic of China

[‡]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, People's Republic of China

[§]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China

Supporting Information

ABSTRACT: Ten new isoprenylated flavonol derivatives, dodoviscins A–J (**1**–**10**), and seven known compounds (**11**–**17**) were isolated from the aerial parts of *Dodonaea viscosa*. Compounds **1**, **2**, **4**, **5**, **7**–**9**, 5,7,4′-trihydroxy-3′,5′-bis(3-methyl-2-buten-1-yl)-3-methoxyflavone (**11**), 5,7,4′-trihydroxy-3′,5′-bis(3-methyl-2-buten-1-yl)-3,6-dimethoxyflavone (**12**), 5,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methyl-2-buten-1-yl)-3,6-dimethyoxyflavone (**13**), sakuranetin (**14**), and blumeatin (**15**) promoted adipocyte differentiation as characterized by increased triglyceride levels in 3T3L1 cells. Compounds **1**, **13**, and **15** also enhanced the accumulation of lipid droplets and induced upregulation of the expression of the adipocyte-specific genes *aP2* and *GLUT4*.



The genus *Dodonaea* (Sapindaceae) comprises about 50 species, with many of these distributed in Australia. In mainland China, there is only one species, *Dodonaea viscosa* Jacq., which is a shrub that occurs in tropical and subtropical areas. This plant has been used as a folk medicine for the treatment of fever, skin diseases, stranguria, and toothache.[1] Flavonoids, diterpenoids, triterpenoids, and saponins were isolated from *D. viscosa* previously, and some of these compounds showed antibacterial, antioxidant, and antiproliferative activities.[2−6]

Adipocytes are regarded as a potential target for obesity and type-2 diabetes. Adipogenesis is a process from fibroblast-like preadipocytes to mature adipocytes. In the final stage, the differentiated cells show markers of mature adipocytes, such as gene expression of fatty acid-binding protein (*aP2*) and glucose transporter 4 (*GLUT4*) and the massive accumulation of triglycerides inside the cells.[7] The increased expression of *GLUT4* may promote insulin-stimulated glucose uptake in the adipose tissue and skeletal muscles and reduce the peripheral glucose level.[8] Recently, we reported that some isoprenylated flavonoids from the genus *Morus* promoted adipogenesis and increased the gene expression of *GLUT4*.[9,10]

In our continuing research on the discovery of antidiabetic agents from natural products, the chemical constituents of the aerial parts of *D. viscosa* have been investigated. Isolation work from an ethanol extract afforded 10 new isoprenylated flavonol derivatives, dodoviscins A–J (**1**–**10**), and seven known compounds, 5,7,4′-trihydroxy-3′,5′-bis(3-methyl-2-buten-1-yl)-3-methoxyflavone (**11**), 5,7,4′-trihydroxy-3′,5′-bis(3-methyl-2-buten-1-yl)-3,6-dimethoxyflavone (**12**), 5,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methyl-2-buten-1-yl)-3,6-dimethyoxyflavone (**13**), sakuranetin (**14**), blumeatin (**15**), 5,7,4′-trihydroxy-3,6-dimethoxyflavone (**16**), and isokaempferide (**17**). The structure elucidation of compounds **1**–**10** and the biological evaluation of 14 of the isolated compounds on adipogenesis are present herein.

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{27}H_{32}O_9$ by HREIMS. The IR spectrum showed absorptions for OH (3386 cm^{-1}), carbonyl (1653 cm^{-1}), and aromatic (1611 and 1465 cm^{-1}) moieties. The UV spectrum resembled that of a flavonol derivative.[2] The ^1H NMR spectrum of **1** displayed a hydrogen-bonded hydroxy group signal at δ_H 13.04 (1H, s, OH-5) and resonances for two hydroxy groups at δ_H 9.12 (1H, brs, OH-7) and 9.74 (1H, brs, OH-4′), two *meta*-coupled aromatic protons at δ_H 7.78 (1H, d, J = 2.0 Hz, H-2′) and 7.86 (1H, d, J = 2.0 Hz, H-6′), an aromatic singlet at δ_H 6.57 (1H, s, H-8), and two methoxy groups at δ_H 3.86 (3H, s, OMe-3) and 3.88 (3H, s, OMe-6). The ^1H NMR spectrum also showed signals for two isoprenoid groups, a 2-hydroxy-3-methyl-3-butenyl group at δ_H 2.98 (1H, brd, J = 14.8 Hz, H-1″a), 3.04 (1H, dd, J = 7.8, 14.8 Hz, H-1″b), 4.51 (1H, brd, J = 7.8 Hz, H-2″), 4.83 and 5.03 (each 1H, brs, H-4″a, H-4″b), 1.83

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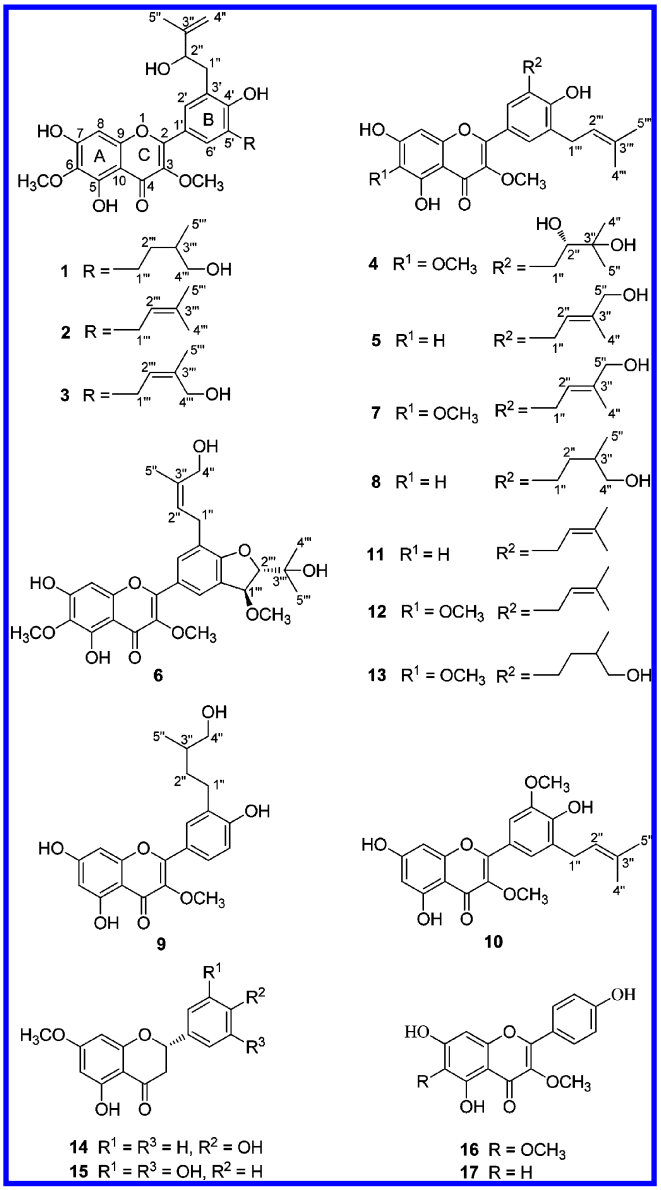

(3H, brs, H_3-5″), and 5.89 (1H, brs, OH-2″), and a 4-hydroxy-3-methylbutyl group at δ_H 2.71 and 2.80 (each 1H, m, H-1‴a, H-1‴b), 1.47 and 1.81 (each 1H, m, H-2‴a, H-2‴b), 1.67 (1H, m, H-3‴), 3.43 and 3.50 (each 1H, m, H-4‴a, H-4‴b), 0.99 (3H, d, J = 6.7 Hz, H_3-5‴), and 3.57 (1H, brt, J = 5.0 Hz, OH-4‴) (Table 1). The ^{13}C NMR spectrum exhibited 27 carbon signals (Table 3), corresponding to a flavonol derivative with two isoprenoid units and two methoxy groups. Location of the substituents was determined by the HMBC experiment (Figure 1). The signals at δ_H 13.04, 9.12, and 9.74 were

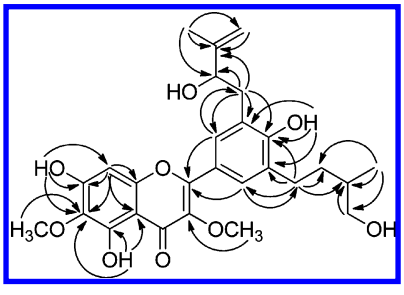

Figure 1. Key HMBC correlations of **1**.

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−5 (J in Hz)

position	1[a]	2[a]	3[b]	4[a]	5[a]
6					6.24, d (1.2)
8	6.57, s	6.55, s	6.56, s	6.55, s	6.46, d (1.2)
2′	7.78, d (2.0)	7.78, d (2.0)	7.81, brs	7.78, d (2.0)	7.82, s
6′	7.86, d (2.0)	7.85, d (2.0)	7.86, brs	7.85, d (2.0)	7.82, s
1″	2.98, brd (14.8)	2.96, dd (2.3, 14.9)	2.97, brd (14.8)	2.87, dd (9.0, 14.1)	3.50, brd (7.4)
	3.04, dd (7.8, 14.8)	3.06, dd (8.2, 14.9)	3.03, dd (8.0, 14.8)	3.00, brd (14.1)	
2″	4.51, brd (7.8)	4.50, brd (8.2)	4.49, brs	3.72, brd (9.0)	5.68, brt (7.4)
4″	4.83, brs	4.84, brs	4.83, brs	1.28, s[c]	1.77, brs
	5.03, brs	5.04, brs	5.02, brs		
5″	1.83, brs	1.84, brs	1.84, brs	1.30, s[c]	4.02, brs
1‴	2.71, m	3.40, brd (7.4)	3.50, brd (7.5)	3.39, brd (7.4)	3.45, brd (7.1)
	2.80, m				
2‴	1.47, m	5.40, brt (7.4)	5.45, brt (7.5)	5.39, brt (7.4)	5.40, brt (7.1)
	1.81, m				
3‴	1.67, m				
4‴	3.43, m	1.75, brs	4.25, brd (4.5)	1.75, brs	1.75, brs
	3.50, m				
5‴	0.99, d (6.7)	1.76, brs	1.83, brs	1.76, brs	1.78, brs
OH-5	13.04, s	13.03, s	13.03, s	13.03, s	12.82, s
OH-7	9.12, brs	–[d]	9.10, brs	9.21, brs	–[d]
OH-4′	9.74, brs	–[d]	9.80, brs	9.71, brs	–[d]
OH-2″	5.89, brs	–[d]	5.70, brs	5.67, brs	
OH-5″					–[d]
OH-4‴	3.57, brt (5.0)		3.85, brt (4.5)		
OMe-3	3.86, s	3.86, s	3.87, s	3.86, s	3.86, s
OMe-6	3.88, s	3.87, s	3.88, s	3.87, s	

[a]Data were measured at 400 MHz in actone-d_6. [b]Data were measured at 500 MHz in actone-d_6. [c]Assignments are exchangeable. [d]Signal was not observed.

assigned to OH-5, OH-7, and OH-4′, respectively, as supported by the HMBC correlations from OH-5 to C-5, C-6, and C-10, from OH-7 to C-6, C-7, and C-8, and from OH-4′ to C-3′, C-4′, and C-5′. The HMBC correlations from one methoxy group at δ_H 3.86 to C-3 and from another one at δ_H 3.88 to C-6 revealed that they are located at C-3 and C-6, respectively. The 2-hydroxy-3-methyl-3-butenyl group was confirmed by the HMBC correlations from H_2-1″ to C-2″ and C-3″ and from H_3-5″ to C-2″, C-3″, and C-4″ and was placed at C-3′ by the HMBC correlations from H_2-1″ to C-2′, C-3′, and C-4′. The 4-hydroxy-3-methylbutyl group was confirmed by the HMBC correlations from H_2-1‴ to C-2‴ and C-3‴ and from H_3-5‴ to C-2‴, C-3‴, and C-4‴ and was located at C-5′ by the HMBC correlations from H_2-1‴ to C-4′, C-5′, and C-6′. In addition, the two *meta*-coupled aromatic protons at δ_H 7.78 and 7.86 and the aromatic singlet at δ_H 6.57 were assigned to H-2′, H-6′, and H-8, respectively, from the HMBC correlations shown in Figure 1. Thus, the structure of **1** (dodoviscin A) was elucidated as 2-[3-(2-hydroxy-3-methyl-3-buten-1-yl)-5-(4-hydroxy-3-methylbutyl)-4-hydroxyphenyl]-3,6-dimethoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compounds **2** and **3** were assigned the molecular formulas $C_{27}H_{30}O_8$ and $C_{27}H_{30}O_9$ by HREIMS, respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **2** and **3** were similar to those of **1**, except for those of the isoprenoid groups at C-5′ (Tables 1 and 3). The ^{1}H NMR spectrum of **2** showed signals for a 3-methyl-2-butenyl (prenyl) group at δ_H 3.40

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Table 2. ^{1}H NMR Spectroscopic Data of Compounds 6−10 (J in Hz)

position	6[b]	7[a]	8[a]	9[a]	10[b]
6			6.25, d (1.6)	6.16, d (2.4)	6.26, d (2.0)
8	6.58, s	6.54, s	6.48, d (1.6)	6.36, d (2.4)	6.49, d (2.0)
2′	7.96, d (1.5)	7.83, s	7.83, s	7.84, brs	7.64, brs
5′				6.85, d (8.6)	
6′	8.07, d (1.5)	7.83, s	7.83, s	7.78, brd (8.6)	7.63, brs
1″	3.41, brdd (8.0, 15.0)	3.50, brd (7.4)	2.75, m	2.63, m	3.42, brd (7.5)
	3.54, brdd (8.0, 15.0)		2.88, m	2.74, m	
2″	5.44, brt (8.0)	5.68, brt (7.4)	1.54, m	1.41, m	5.40, brt (7.5)
			1.81, m	1.75, m	
3″			1.72, m	1.65, m	
4″	4.20, brdd (6.0, 12.0)	1.77, brs	3.51, m	3.40, dd (6.7, 11.0)	1.76, brs
	4.34, brdd (6.0, 12.0)		3.56, m	3.48, dd (5.9, 11.0)	
5″	1.82, brs	4.01, brd (5.5)	0.96, d (6.7)	1.00, d (6.7)	1.76, brs
1‴	5.12, d (2.5)	3.45, brd (7.4)	3.43, brd (7.4)		
2‴	4.49, d (2.5)	5.40, brt (7.4)	5.40, brt (7.4)		
4‴	1.27, s[c]	1.75, brs	1.75, brs		
5‴	1.21, s[c]	1.78, brs	1.77, brs		
OH-5	12.99, s	13.02, s	12.84, s	−[d]	12.81, s
OH-7	9.11, brs	9.19, brs	9.70, brs	−[d]	9.56, brs
OH-4′		7.95, brs	8.31, brs	−[d]	8.19, brs
OH-4″	3.75, brt (6.0)		4.09, brt (5.0)	−[d]	
OH-5″		3.85, brt (5.5)			
OMe-3	3.88, s	3.87, s	3.86, s	3.74, s	3.89, s
OMe-6	3.89, s	3.87, s			
OMe-3′					3.95, s
OMe-1‴	3.48, s				

[a]Data were measured at 400 MHz (**7** and **8** in actone-d_6; **9** in methanol-d_4). [b]Data were measured at 500 MHz in actone-d_6. [c]Assignments are exchangeable. [d]Signal was not observed.

(2H, brd, J = 7.4 Hz, H_2-1‴), 5.40 (1H, brt, J = 7.4 Hz, H-2‴), 1.75 (3H, brs, H_3-4‴), and 1.76 (3H, brs, H_3-5‴), and this group was located at C-5′ by the HMBC correlations from H-6′ to C-1‴ and from H_2-1‴ to C-5′. Accordingly, the structure of **2** (dodoviscin B) was elucidated as 2-[3-(2-hydroxy-3-methyl-3-buten-1-yl)-5-(3-methyl-2-buten-1-yl)-4-hydroxyphenyl]-3,6-dimethoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compound **3** was found to possess a 4-hydroxy-3-methyl-2-butenyl group at C-5′ rather than the prenyl group in **2**, as established by the ^{1}H and ^{13}C NMR signals of a hydroxymethyl group at δ_H 4.25 (2H, brd, J = 4.5 Hz, H-4‴) and 3.85 (1H, brt, J = 4.5 Hz, OH-4‴) and at δ_C 61.5 (C-4‴). The *Z*-configuration of the double bond at C-2‴/C-3‴ was established by the ROESY correlations of H_2-1‴/H_2-4‴ and H-2‴/H_3-5‴. Thus, the structure of **3** (dodoviscin C) was elucidated as 2-{3-(2-hydroxy-3-methyl-3-buten-1-yl)-5-[(*Z*)-4-hydroxy-3-methyl-2-buten-1-yl]-4-hydroxyphenyl}-3,6-dimethoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compound **4** was assigned the molecular formula $C_{27}H_{32}O_9$ by HREIMS. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **4** and **2** indicated that their structural difference was the isoprenoid group at C-3′ (Tables 1 and 3). The presence of a 2,3-dihydroxy-3-methylbutyl group in **4** was deduced from the following ^{1}H and ^{13}C NMR signals: δ_H 2.87 (1H, dd, J = 9.0, 14.1 Hz, H-1″a), 3.00 (1H, brd, J = 14.1 Hz, H-1″b), 3.72 (1H, brd, J = 9.0 Hz, H-2″), 1.28 and 1.30 (each 3H, s, H_3-4″, H_3-5″), and 5.67 (1H, brs, OH-2″); δ_C 35.3 (C-1″), 81.9 (C-2″), 72.7 (C-3″), 25.5 (C-4″), and 25.1 (C-5″). The HMBC correlations from H_2-1″ to C-2′, C-3′, and C-4′ confirmed its location at C-3′. The absolute configuration of C-2″ in **4** was assigned using an in situ dimolybdenum CD method.[11−13] According to the empirical rule proposed by Snatzke, the observed induced CD (ICD) curve at around 300 nm showing the same sign with the O−C−C−O torsion angle in the favored conformation allows the assignment of the absolute configuration. The absolute configuration at C-2″ was assigned as *S* by the ICD spectrum of the metal complex of **4** in DMSO, which showed a positive Cotton effect at 298 nm (Figure 2). Therefore, the structure of **4** (dodoviscin D) was

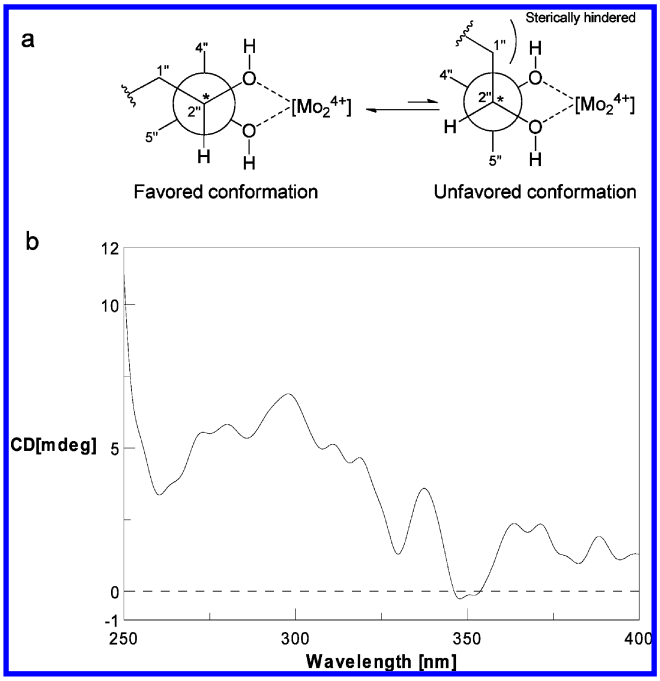

Figure 2. (a) Conformations of the Mo_2^{4+} complex of **4**. (b) ICD spectrum of the Mo_2^{4+} complex of **4** in DMSO.

elucidated as 2-{3-[(2*S*)-2,3-dihydroxy-3-methylbutyl]-5-(3-methyl-2-buten-1-yl)-4-hydroxyphenyl}-3,6-dimethoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compound **5** was assigned the molecular formula $C_{26}H_{28}O_7$ by HREIMS. The ^{1}H and ^{13}C NMR spectroscopic data of **5** indicated this compound to be a flavonol derivative with one methoxy and two isoprenoid groups (Tables 1 and 3). This compound was assigned a 5,7-dihydroxylated A ring and a 4-hydroxy-3-methyl-2-butenyl group in place of the 2,3-dihydroxy-3-methylbutyl group in **4**, as supported by the ^{1}H NMR signals at δ_H 6.24 (1H, d, J = 1.2 Hz, H-6), 6.46 (1H, d, J = 1.2 Hz, H-8), 3.50 (2H, brd, J = 7.4 Hz, H_2-1″), 5.68 (1H, brt, J = 7.4 Hz, H-2″), 1.77 (3H, brs, H_3-4″), and 4.02 (2H, brs, H_2-5″). The 4-hydroxy-3-methyl-2-butenyl group was located at C-3′ from the HMBC correlations from H-2′ to C-1″ and from H-1″ to C-3′ and C-4′. The *E*-configuration of the double bond

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Table 3. ^{13}C NMR Spectroscopic Data of Compounds 1−10

position	1[a]	2[a]	3[b]	4[a]	5[a]	6[b]	7[a]	8[a]	9[a]	10[b]
2	157.4	157.3	157.3	157.3	157.0	157.1	157.2	157.2	158.3	156.9
3	138.7	138.7	138.8	138.7	139.1	138.9	138.8	139.1	139.3	139.3
4	179.8	179.8	179.9	179.8	179.5	179.9	179.8	179.5	179.9	179.5
5	153.6	153.6	153.6	153.6	163.1	153.6	153.6	163.2	163.0	163.2
6	131.8	131.8	131.8	131.8	99.3	131.7	131.8	99.3	99.7	99.3
7	157.5	157.5	157.5	157.6	164.8	157.6	157.6	164.8	165.9	164.8
8	94.4	94.3	94.4	94.3	94.3	94.5	94.3	94.4	94.7	94.5
9	153.0	153.0	153.1	153.0	157.7	153.1	153.0	157.8	158.4	157.8
10	106.3	106.3	106.3	106.2	105.8	106.4	106.2	105.8	105.8	105.9
1′	122.3	122.3	122.4	122.4	122.8	123.7	122.8	122.5	122.4	122.0
2′	130.6	130.5	130.8	130.0	128.5	131.7	128.5	128.9	131.4	110.0
3′	127.0	126.9	127.1	128.1	128.9	124.5	128.9	129.1	130.8	147.9
4′	158.0	157.9	157.8	157.9	156.0	162.2	156.0	156.3	159.6	147.9
5′	131.4	130.1	129.5	130.1	128.7	128.3	128.7	129.8	115.9	128.7
6′	129.8	129.5	129.7	129.3	128.5	125.4	128.6	128.6	128.9	123.7
1″	39.5	39.6	39.4	35.3	28.7	28.4	28.7	28.5	28.7	28.8
2″	77.5	77.6	77.4	81.9	122.1	124.0	122.1	35.2	34.4	123.1
3″	147.7	147.7	147.9	72.7	138.2	138.0	138.2	35.5	36.7	133.2
4″	110.9	111.0	111.0	25.5[c]	13.8	61.1	13.8	68.2	68.4	17.9
5″	18.5	18.5	18.4	25.1[c]	68.0	21.6	68.0	17.4	17.1	25.9
1‴	28.6	29.2	29.3	29.2	29.2	82.1	29.2	29.2		
2‴	34.4	123.4	125.4	123.4	122.7	96.1	122.7	123.0		
3‴	36.4	133.0	137.2	133.0	133.9	71.2	133.9	133.6		
4‴	68.0	17.8	61.5	17.8	17.9	26.1[c]	17.9	17.8		
5‴	17.2	25.9	21.9	25.9	25.9	25.0[c]	25.9	25.9		
OMe-3	60.1	60.1	60.2	60.1	60.1	60.3	60.1	60.1	60.5	60.2
OMe-6	60.7	60.7	60.7	60.6		60.7	60.6			
OMe-3′										56.6
OMe-1‴						55.8				

[a]Data were measured at 100 MHz (**1**, **2**, **4**, **5**, 7, and **8** in actone-d_6; **9** in methanol-d_4). [b]Data were measured at 125 MHz in actone-d_6. [c]Assignments are exchangeable.

at C-2″/C-3″ was established by the ROESY correlations of H_2-1″/H_3-4″ and H-2″/H_2-5″. Thus, the structure of **5** (dodoviscin E) was established as 2-{3-[(*E*)-4-hydroxy-3-methyl-2-buten-1-yl]-5-(3-methyl-2-buten-1-yl)-4-hydroxyphenyl}-3-methoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compound **6** was assigned the molecular formula $C_{28}H_{32}O_{10}$ by HREIMS. It was found to be an isoprenylated flavonol derivative with the same ring A and C moieties as **1**−**4** (Tables 2 and 3). Furthermore, the ^{1}H NMR spectrum showed resonances for two *meta*-coupled aromatic protons at δ_H 7.96 (1H, d, J = 1.5 Hz, H-2′) and 8.07 (1H, d, J = 1.5 Hz, H-6′), a 4-hydroxy-3-methyl-2-butenyl group at δ_H 3.54 and 3.41 (each 1H, brdd, J = 8.0, 15.0 Hz, H-1″a, H-1″b), 5.44 (1H, brt, J = 8.0 Hz, H-2″), 4.34 and 4.20 (each 1H, brdd, J = 6.0, 12.0 Hz, H-4″a, H-4″b), 1.82 (3H, brs, H_3-5″), and 3.75 (1H, brt, J = 6.0 Hz, OH-4″), and a 2-(1-hydroxy-1-methylethyl)-3-methoxydihydrofuran ring[14] at δ_H 5.12 (1H, d, J = 2.5 Hz, H-1‴), 4.49 (1H, d, J = 2.5 Hz, H-2‴), 1.27 (3H, s, H_3-4‴), 1.21 (3H, s, H_3-5‴), and 3.48 (3H, s, OMe-1‴). The 4-hydroxy-3-methyl-2-butenyl group was located at C-3′ as a result of the HMBC correlations from H_2-1″ to C-2′, C-3′, and C-4′ (Figure 3). The *Z*-configuration of the double bond at C-2″/C-3″ was deduced from the NOESY correlations of H_2-1″/H_2-4″ and H-2″/H_3-5″ (Figure 3). The 2-(1-hydroxy-1-methylethyl)-3-methoxydihydrofuran ring was confirmed by the HMBC correlations from H-1‴ to OCH_3-1‴ and C-3‴ and from H_3-4‴ to C-2‴, C-3‴, and C-5‴ and was fused at C-4′ and C-5′ by the HMBC correlation from H-2‴ to C-4′. The relative configuration of the

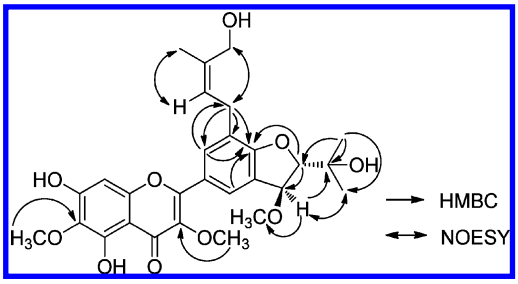

Figure 3. Key HMBC and NOESY correlations of **6**.

two oxygenated methine protons on the dihydrofuran ring was determined to be *trans* by the small coupling constant between H-1‴ and H-2‴ (J = 2.5 Hz).[14] The strong NOESY correlations of H-1‴/H_3-4‴ and H-1‴/H_3-5‴ and the absence of NOESY correlations of OMe-1‴/H_3-4‴ and OMe-1‴/H_3-5‴ further supported the *trans* relationship. The absolute configurations at C-1‴ and C-2‴ were both assigned as *S* on the basis of the CD spectrum, in which a positive Cotton effect at 274 nm was observed.[15] Thus, the structure of **6** (dodoviscin F) was identified as 2-{(2*S*,3*S*)-2,3-dihydro-3-methoxy-2-(1-hydroxy-1-methylethyl)-7-[(*Z*)-4-hydroxy-3-methyl-2-buten-1-yl]-5-benzofuranyl}-3,6-dimethoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compound **7** was assigned the molecular formula $C_{27}H_{30}O_8$ by HREIMS. Analysis of the ^{1}H and ^{13}C NMR, HSQC, and HMBC spectroscopic data implied this compound to be a 6-methoxy-substituted dodoviscin E derivative. Thus, the structure of **7**

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(dodoviscin G) was elucidated as 2-{3-[(*E*)-4-hydroxy-3-methyl-2-buten-1-yl]-5-(3-methyl-2-buten-1-yl)-4-hydroxyphenyl}-3,6-dimethoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compound **8** was assigned the molecular formula $C_{26}H_{30}O_7$ by HREIMS. The ^{1}H and ^{13}C NMR spectroscopic data of **8** indicated that a 4-hydroxy-3-methylbutyl group had replaced the 4-hydroxy-3-methyl-2-butenyl group of **5** (Tables 2 and 3). This group was located at C-3′ according to the HMBC correlations from H-2′ to C-1″ and from H_2-1″ to C-3′ and C-4′. Thus, the structure of **8** (dodoviscin H) was elucidated as 2-[3-(4-hydroxy-3-methylbutyl)-5-(3-methyl-2-buten-1-yl)-4-hydroxyphenyl]-3-methoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Compounds **9** and **10** were assigned the molecular formulas $C_{21}H_{22}O_7$ and $C_{22}H_{22}O_7$ by HREIMS, respectively. Their structures differed from those of **5** and **8** in the B ring substituents. The ^{1}H NMR spectrum of **9** showed an aromatic ABX spin system at δ_H 7.84 (1H, brs, H-2′), 6.85 (1H, d, J = 8.6 Hz, H-5′), and 7.78 (1H, brd, J = 8.6 Hz, H-6′) and a 4-hydroxy-3-methylbutyl group at δ_H 2.63 and 2.74 (each 1H, m, H-1″a, H-1″b), 1.41 and 1.75 (each 1H, m, H-2″a, H-2″b), 1.65 (1H, m, H-3″), 3.40 (1H, dd, J = 6.7, 11.0 Hz, H-4″a), 3.48 (1H, dd, J = 5.9, 11.0 Hz, H-4″b), and 1.00 (3H, d, J = 6.7 Hz, H_3-5″) (Table 2). On analysis of the HMBC correlations, the protons at δ_H 7.84, 6.85, and 7.78 were assigned to H-2′, H-5′, and H-6′, respectively. The isoprenoid group was located at C-3′ by the HMBC correlations from H-1″ to C-2′, C-3′, and C-4′. Thus, the structure of **9** (dodoviscin I) was elucidated as 2-[3-(4-hydroxy-3-methylbutyl)-4-hydroxyphenyl]-3-methoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

The ^{1}H NMR spectrum of **10** showed a hydroxy group signal at δ_H 8.19 (1H, brs, OH-4′), two *meta*-coupled protons at δ_H 7.64 (1H, brs, H-2′) and 7.63 (1H, brs, H-6′), a prenyl group at δ_H 3.42 (2H, brd, J = 7.5 Hz, H_2-1″), 5.40 (1H, brt, J = 7.5 Hz, H-2″), and 1.76 (6H, brs, H_3-4″, H_3-5″), and a methoxy group at δ_H 3.95 (3H, s, OMe-3′). The methoxy and the hydroxy groups were located at C-3′ and C-4′, respectively, as deduced from the HMBC correlations from the protons at δ_H 3.95 to C-3′ and from the signal at δ_H 8.19 to C-3′, C-4′, and C-5′. The prenyl group was located at C-5′ by the HMBC correlations from H-6′ to C-1″ and from H_2-1″ to C-4′, C-5′, and C-6′. Thus, the structure of **10** (dodoviscin J) was elucidated as 2-[3-methoxy-5-(3-methyl-2-buten-1-yl)-4-hydroxyphenyl]-3-methoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Seven known compounds were identified as 5,7,4′-trihydroxy-3′,5′-bis(3-methyl-2-buten-1-yl)-3-methoxyflavone (**11**),[16] 5,7,4′-trihydroxy-3′,5′-bis(3-methyl-2-buten-1-yl)-3,6-dimethoxyflavone (**12**),[2] 5,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methyl-2-buten-1-yl)-3,6-dimethyoxyflavone (**13**),[2] sakuranetin (**14**),[17] blumeatin (**15**),[18] 5,7,4′-trihydroxy-3,6-dimethoxyflavone (**16**),[19] and isokaempferide (**17**),[20] by comparison of their spectroscopic data with those reported.

Compounds **1**, **2**, **4**, **5**, **7**−**9**, and **11**−**17** were screened for their potential effects on adipogenesis in 3T3L1 mouse fibroblasts by measuring the triglyceride content in the cells. Compounds **1**, **2**, **4**, **5**, **7**−**9**, and **11**−**15** were shown to increase the triglyceride content (Table 4), whereas **16** and **17** were inactive. Compounds **1**, **13**, and **15** were evaluated further for their effects on adipogenesis, wherein 3T3L1 cells in the presence of these three compounds were stained with Oil Red O and showed that they enhanced the accumulation of intracellular lipid droplets significantly (Figure 4). Adipogenesis is accompanied by increased expression of adipocyte-specific genes. Compounds **1**, **13**, and **15** induced upregulation of the gene expression of *GLUT4* and *aP2* by RT-PCR analysis (Figure 5). The results of Western blotting demonstrated that **1**, **13**, and **15** increased the levels of *GLUT4* and *aP2* (Figure 6). Compounds **1**, **13**, and **15** may stimulate glucose uptake into adipocytes accompanied by increased expression of *GLUT4*. Compound **14** has been reported previously to induce adipogenesis of 3T3L1 cells through enhanced expression of *PPARγ2*.[21]

Table 4. Effects of Compounds Isolated from *D. viscosa* on Triglyceride Content in 3T3L1 Cells

compound	concentration (μM)	triglyceride content (%)
control	0	100.0 ± 9.8
1	3	138.5 ± 8.5[a]
	10	248.3 ± 29.4[a]
2	3	328.1 ± 3.7[a]
4	3	103.2 ± 6.0
	10	117.8 ± 8.0
	30	198.2 ± 18.9[a]
5	3	280.2 ± 11.1[a]
7	3	355.7 ± 22.9[a]
8	3	263.1 ± 36.9[a]
9	3	189.6 ± 25.7[a]
11	3	169.9 ± 8.4[a]
12	3	256.2 ± 4.7[a]
13	3	233.0 ± 9.2[a]
	10	289.8 ± 14.4[a]
14	3	278.8 ± 23.0[a]
	10	419.9 ± 5.0[a]
	30	459.4 ± 16.7[a]
15	3	121.2 ± 4.3
	10	161.3 ± 20.5[a]
	30	219.2 ± 10.6[a]
rosiglitazone	1	335.6 ± 20.9[a]

[a] $p < 0.01$, compared with control.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 polarimeter. UV spectra were recorded on a Hitachi U-2900 spectrophotometer. CD spectra were obtained on JASCO J-715 and JASCO 810 spectrometers. IR spectra were measured on a Nicolet Avatar-360 spectrometer with KBr pellets. NMR spectra were obtained on Bruker DRX-500 and Varian Mercucy Plus 400 instruments. Chemical shifts were reported with TMS as internal standard or with respect to acetone-d_6 (δ_H 2.04, δ_C 206.0 ppm) and methanol-d_4 (δ_H 3.31, δ_C 49.0 ppm). EIMS (70 eV) were recorded on an Agilent 5973N mass spectrometer. HREIMS was carried out on a Waters Micromass GCT mass spectrometer. Semipreparative HPLC was performed on an Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA) and a Sepax Amethyst C_{18} column (150 × 10 mm, 5 μm, Sepax Techologies, Inc., Newark, DE, USA), using a UV detector set at 210 nm. Column chromatography (CC) was performed on silica gel (200−300 mesh, Yantai Institute of Chemical Technology, Yantai, People's Republic of China) and Sephadex LH-20 gel (GE Healthcare Amersham Biosciences, Uppsala, Sweden). TLC analysis was run on precoated silica gel GF254 plates (10−40 μm, Yantai Institute of Chemical Technology).

Plant Material. The aerial parts of *Dodonaea viscosa* were collected in Gejiu County, Yunnan Province, People's Republic of China, in July 2009. The plant material was identified by one of the authors (Q.-S.Z.), and a voucher specimen (TCM 09-07-10 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

Extraction and Isolation. The milled, air-dried aerial parts of *D. viscosa* (15.0 kg) were percolated with 95% EtOH at room temperature (160 L). The filtrate was evaporated in vacuo to give a

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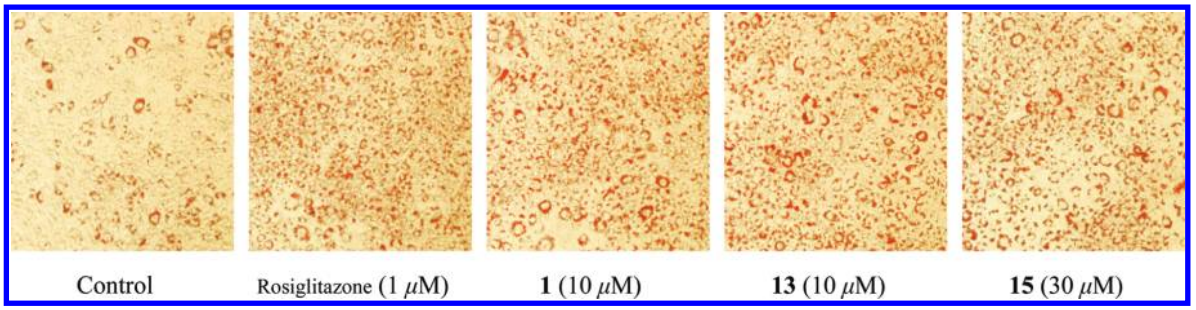

Figure 4. Effects of compounds **1**, **13**, and **15** on adipocyte differentiation (Oil Red O staining).

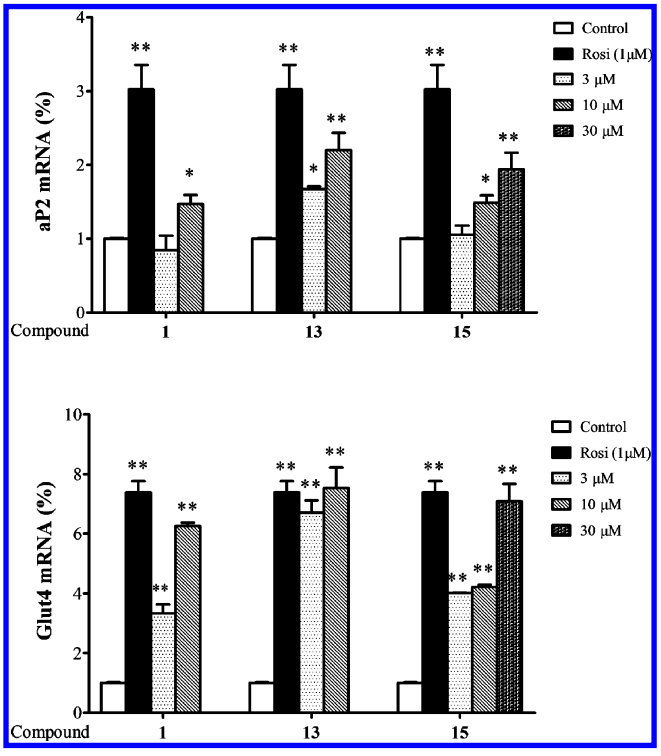

Figure 5. Effects of compounds **1**, **13**, and **15** on *aP2* and *GLUT4* gene expression in 3T3L1 cells by RT-PCR. The differences were statistically significant from control: $*p < 0.05$, $**p < 0.01$. Rosi, rosiglitazone.

residue (3.0 kg), which was suspended in H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract (300 g) was subjected to CC on silica gel eluted with a gradient of petroleum ether−EtOAc (1:0, 10:1, 5:1, 1:1, 1:3) to give fractions A−J. Fraction B was subjected to CC on Sephadex LH-20 eluted with MeOH to yield fractions B1−B6. Fraction B2 was separated by semipreparative HPLC (CH_3OH−H_2O, 85:15, flow rate 1 mL/min) to provide **11** (5 mg). Fraction B3 was chromatographed over silica gel eluted with a gradient of petroleum ether−EtOAc (25:1, 10:1, 5:1) to afford **12** (158 mg). Fraction B4 was isolated by CC on silica gel eluted with a gradient of CH_2Cl_2−Me_2CO (30:1, 10:1, 2:1) to yield **2** (32 mg). Fraction B5 was separated by semipreparative HPLC (CH_3OH−H_2O, 78:22, flow rate 1 mL/min) to provide **14** (42 mg). Fraction E was subjected to CC on silica gel eluted with a gradient of CH_2Cl_2−Me_2CO (30:1, 15:1, 5:1) to afford fractions E1−E7. Fraction E1 was isolated by semipreparative HPLC (CH_3OH−H_2O, 80:20, flow rate 1 mL/min) to yield **10** (3 mg). Fraction E3 was separated by CC on Sephadex LH-20 eluted with MeOH to afford **3** (4 mg) and **6** (3 mg). Fraction E4 was separated by semipreparative HPLC (CH_3OH−H_2O, 78:22, flow rate 1 mL/min) to produce **1** (12 mg). Fraction E5 was separated by CC on Sephadex LH-20 eluted with MeOH to afford **15** (8 mg). Fraction E6 was separated by CC on Sephadex LH-20 eluted with MeOH to yield **16**

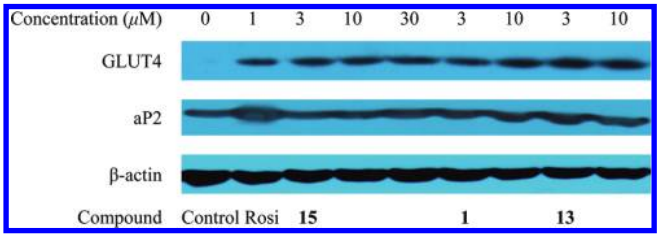

Figure 6. Effects of compounds **1**, **13**, and **15** on *aP2* and *GLUT4* gene expression in 3T3L1 cells by Western blotting. Rosi, rosiglitazone.

(84 mg) and **17** (36 mg). Fraction G was separated by CC on silica gel eluted with a gradient of CH_2Cl_2−Me_2CO (30:1, 10:1, 5:1, 2:1) to yield fractions G1−G5. Fraction G2 was chromatographed on Sephadex LH-20 eluted with $CHCl_3$−MeOH (1:1) to give fractions G2.1−G2.4. Fraction G2.2 was separated by semipreparative HPLC (CH_3OH−H_2O, 77:23, flow rate 1 mL/min) to yield **7** (18 mg) and **8** (6 mg). Fraction G2.3 was chromatographed by semipreparative HPLC (CH_3OH−H_2O, 81:19, flow rate 1 mL/min) to afford **13** (26 mg). Fraction G4 was separated by CC on silica gel eluted with a gradient of CH_2Cl_2−Me_2CO (10:1, 5:1) to yield **5** (12 mg). Fraction G5 was separated by semipreparative HPLC (CH_3OH−H_2O, 81:19, flow rate 1 mL/min) to provide **4** (7 mg). Fraction H was separated by CC on silica gel eluted with a gradient of CH_2Cl_2−Me_2CO (20:1, 10:1, 5:1) to give fractions H1−H4. Fraction H3 was separated by CC on Sephadex LH-20 eluted with MeOH to afford **9** (73 mg).

Dodoviscin A (1): yellow gum; $[\alpha]^{25}_D$ −88.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.39), 249 (3.97), 270 (3.93), 349 (4.13) nm; IR (KBr) ν_{max} 3386, 2953, 2904, 1653, 1611, 1465, 1370, 1209, 1052, 800, 668 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 500 $[M]^+$ (40), 482 (25), 467 (24), 429 (31), 358 (41), 281 (16), 208 (15), 70 (100), 59 (4); HREIMS *m*/*z* 500.2050 $[M]^+$ (calcd for $C_{27}H_{32}O_9$, 500.2046).

Dodoviscin B (2): yellow gum; $[\alpha]^{25}_D$ −68.1 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.33), 249 (394), 271 (3.91), 349 (4.08) nm; IR (KBr) ν_{max} 3298, 2970, 2904, 1651, 1634, 1455, 1429, 1210, 1006, 831, 703 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 482 $[M]^+$ (26), 411 (21), 369 (58), 355 (100), 341 (37), 281 (52), 238 (38), 207 (34), 191 (73), 133 (65), 71 (54); HREIMS *m*/*z* 482.1943 $[M]^+$ (calcd for $C_{27}H_{30}O_8$, 482.1941).

Dodoviscin C (3): yellow gum; $[\alpha]^{25}_D$ −48.9 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.10), 250 (3.74), 271 (3.71), 346 (3.82) nm; IR (KBr) ν_{max} 3377, 2975, 2926, 1653, 1611, 1463, 1210, 847, 670 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 498 $[M]^+$ (41), 480 (85), 410 (100), 395 (41), 199 (77), 185 (43), 165 (34), 136 (51), 64 (19); HREIMS *m*/*z* 498.1893 $[M]^+$ (calcd for $C_{27}H_{30}O_9$, 498.1890).

Dodoviscin D (4): yellow, amorphous powder; $[\alpha]^{25}_D$ −56.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.35), 249 (3.97), 270 (3.80), 348 (4.10) nm; IR (KBr) ν_{max} 3423, 2971, 2932, 1653, 1622, 1460, 1412, 1205, 1008, 832, 704 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 500 $[M]^+$ (100), 482 (41), 467 (26), 411 (45), 395 (32), 369 (27), 183 (16), 84 (18), 59 (48); HREIMS *m*/*z* 500.2045 $[M]^+$ (calcd for $C_{27}H_{32}O_9$, 500.2046).

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*Dodoviscin E (**5**):* yellow gum; UV (MeOH) λ_{max} (log ε) 202 (4.18), 250 (3.76), 267 (3.73), 300 (sh) (3.49), 355 (3.81) nm; IR (KBr) ν_{max} 3387, 2970, 2926, 1652, 1622, 1461, 1429, 1202, 846, 670 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 452 $[M]^+$ (48), 434 (100), 419 (31), 391 (28), 365 (40), 153 (51), 69 (28), 43 (23); HREIMS *m/z* 452.1837 $[M]^+$ (calcd for $C_{26}H_{28}O_7$, 452.1835).

*Dodoviscin F (**6**):* yellow, amorphous powder; $[\alpha]^{25}_D$ +28.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.31), 250 (392), 271 (3.92), 345 (4.08) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 244 (+0.48), 259 (−0.16), 274 (+0.36), 345 (+2.79); IR (KBr) ν_{max} 3418, 2969, 2926, 1652, 1627, 1468, 1406, 1211, 1014, 827, 701 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 528 $[M]^+$ (28), 412 (19), 369 (17), 281 (28), 109 (32), 99 (27), 83 (33), 71 (23), 59 (41), 52 (100); HREIMS *m/z* 528.1996 $[M]^+$ (calcd for $C_{28}H_{32}O_{10}$, 528.1995).

*Dodoviscin G (**7**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 206 (4.32), 248 (3.93), 271 (3.91), 349 (4.04) nm; IR (KBr) ν_{max} 3416, 2970, 2915, 1652, 1627, 1463, 1413, 1205, 1003, 827, 701 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 482 $[M]^+$ (32), 464 (100), 449 (55), 421 (18), 395 (14), 149 (52), 69 (41), 44 (21); HREIMS *m/z* 482.1940 $[M]^+$ (calcd for $C_{27}H_{30}O_8$, 482.1941).

*Dodoviscin H (**8**):* yellow, amorphous powder; $[\alpha]^{25}_D$ −64.7 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.35), 249 (3.97), 267 (3.95), 299 (sh) (3.71), 353 (4.01) nm; IR (KBr) ν_{max} 3415, 2958, 2926, 1652, 1627, 1451, 1418, 1173, 1008, 827, 701 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 454 $[M]^+$ (89), 436 (99), 381 (69), 367 (100), 325 (68), 153 (61), 115 (33), 64 (43), 41 (40); HREIMS *m/z* 454.1989 $[M]^+$ (calcd for $C_{26}H_{30}O_7$, 454.1992).

*Dodoviscin I (**9**):* yellow, amorphous powder; $[\alpha]^{25}_D$ +12.3 (*c* 0.90, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.46), 248 (4.06), 267 (4.11), 298 (sh) (3.87), 353 (4.17) nm; IR (KBr) ν_{max} 3351, 2964, 2940, 1653, 1627, 1448, 1405, 1176, 1006, 831, 704 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 386 $[M]^+$ (100), 369 (20), 313 (26), 299 (52), 269 (26), 241 (22), 153 (24), 91 (10), 69 (14), 41 (6); HREIMS *m/z* 386.1369 $[M]^+$ (calcd for $C_{21}H_{22}O_7$, 386.1366).

*Dodoviscin J (**10**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 208 (4.37), 254 (4.04), 267 (sh) (3.96), 300 (sh) (3.67), 359 (4.06) nm; IR (KBr) ν_{max} 3385, 2970, 2926, 1651, 1633, 1460, 1428, 1209, 1163, 832, 701 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 398 $[M]^+$ (100), 383 (29), 343 (24), 327 (29), 299 (34), 91 (60), 69 (27), 41 (21); HREIMS *m/z* 398.1364 $[M]^+$ (calcd for $C_{22}H_{22}O_7$, 398.1366).

Cell Culture and Differentiation. 3T3L1 mouse fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco), containing 10% newborn calf serum (NCS; Hyclone) at 37 °C in 5% CO_2. Two days after confluence, differentiation was induced by treating cells with a mixture of 2 μg/mL insulin (Sigma), 1 μM dexamethasone (Sigma), and 125 μM 3-isobutyl-1-methylxanthine (Sigma) in DMEM with 10% fetal bovine serum (FBS; Hyclone). The medium was changed with 10% FBS/DMEM supplemented with 2 μg/mL insulin every two days. The test compounds were administered at the initiation of differentiation at different concentrations and incubated with cells for 8 days.

Triglyceride Measurement. Cells were lysed by freezing/thawing three times, and then the triglyceride content was measured with a commercial enzyme assay kit (Rongsheng, Shanghai, People's Republic of China).

Oil Red O Staining. After the induction of differentiation for eight days, cells were stained with Oil Red O (Sakura Finetek USA Inc., Torrance, CA, USA) and photographed.

Quantitative Real-Time PCR (RT-PCR). Total RNA was extracted from 3T3L1 cells using TRIzol (Invitrogen). Reverse transcription of RNA and quantitative PCR amplification were carried out using a procedure described previously.[22] The designed primers were as follows: *aP2* (forward, 5′-GCGTAAATGGGGATTTGGTC-3′, and reverse, 5′-TCCTGTCGTCTGCGGTGATT-3′), *GLUT4* (forward, 5′-TCCTTCTATTTGCCGTCCTC-3′, and reverse, 5′-TGTTT-TGCCCCTCAGTCATT-3′), and *β-actin* (forward, 5′-CACGATG-GAGGGGCCGGACTCATC-3′, and reverse, 5′-TAAAGACCTC-TATGCCAACACAGT-3′).

Western Blotting. Cells were harvested in RIPA lysis buffer with protease inhibitor cocktail (Sigma). Proteins were separated by SDS-PAGE and transferred to PVDF membranes (Bio-Rad Laboratories). Then, membranes were incubated with primary antibodies for *aP2* (Santa Cruz), *GLUT4* (Santa Cruz), and *β-actin* (Sigma), followed by HRP-conjugated secondary antibodies (Jackson Immuno Research Laboratories). Immunoblots were visualized with ECL substrate (Pierce).

Statistical Analysis. Data are expressed as means ± SD. The comparison of different groups was assessed by one-way analysis of variance (ANOVA). Statistical analyses were performed using MS Office Excel 2007, and the plotting images were processed by Tanon Gel Image System 4.0 and analyzed by Gel-Pro analyzer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR and MS spectra of compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel and Fax: +86-21-51980005. E-mail: ajhou@shmu.edu.cn.

Notes

The authors declare no competing financial interest.

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