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Isoprenylated Flavonoids and Adipogenesis-Promoting Constituents from *Morus nigra*

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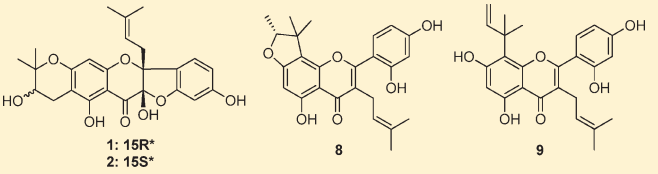
Supporting Information

ABSTRACT: Ten new isoprenylated flavonoids, nigrasins A–J (**1**–**10**), and three known compounds were isolated from the twigs of *Morus nigra*. Compounds **8** and **9** promoted adipogenesis, characterized by increased lipid droplet and triglyceride content in 3T3L1 cells, and induced up-regulation of the expression of adipocyte-specific genes, aP2 and GLUT4.



The genus *Morus* (Moraceae), consisting of around 16 species, is of important economic and medicinal value. Some *Morus* plants are widely cultivated in China, and their leaves are indispensable food for silk-worms. Sericulture plays an important role in the splendid oriental silk civilization. On the other hand, the root bark of some *Morus* species has been used as the traditional Chinese medicine "Mori Cortex" for the treatment of diabetes, arthritis, and rheumatism. This genus is a rich source of isoprenylated flavonoids with structural and biological interest.[1,2] Sanggenon-type flavanones and Diels–Alder adducts are characteristic constituents with biological activities including antioxidant and anti-inflammatory effects and inhibition of COXs and HIF-1.[3−6]

Morus nigra Linn., known as "Yao-Sang" in Xinjiang, China, has been used in the Uygur medicine as antihypertensive, anti-aging, and anti-inflammatory agents. Some flavonoids, coumarins, stilbenes, and 2-arylbenzofurans have been isolated from this plant previously.[7,8] However, sanggenon-type flavanones from *M. nigra* were reported only by our group.[9] As part of our program on the discovery of antimetabolic disease agents from natural products, reinvestigations of the chemical constituents from *M. nigra* and their effects on adipogenesis were carried out.

Adipose tissue, being composed of adipocytes that store energy in the form of triglycerides, is important for the regulation of energy balance.[10] Adipocytes have been regarded as a potential target for obesity and type 2 diabetes. Adipocyte differentiation follows a defined program. 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.[11] The increased expression of GLUT4 may promote insulin-stimulated glucose uptake in the adipose tissue and skeletal muscles and reduce the peripheral glucose level.[12]

In this study, fractionation of an ethanol extract of the twigs of *M. nigra* afforded 10 new isoprenylated flavonoids, nigrasins A–J (**1**–**10**), and three known compounds, sanggenol H (**11**), cudraflavone C (**12**), and macaranone B (**13**). Compounds **1**–**7** are new sanggenon-type flavanones. Compounds **8** and **9** promoted adipocyte differentiation and increased the gene expression of aP2 and GLUT4 in 3T3L1 cells. We herein present the structure elucidation of compounds **1**–**10** and their biological evaluation.

RESULTS AND DISCUSSION

Nigrasins A (**1**) and B (**2**), yellowish oils, gave a positive reaction with ferric chloride reagent, indicating their phenolic nature. Their molecular formulas were both established as $C_{25}H_{26}O_8$ by HREIMS. The IR spectrum of **1** showed absorptions for hydroxy moieties (3384 cm^{-1}), a carbonyl group (1632 cm^{-1}), and benzene rings (1605 and 1459 cm^{-1}). The UV spectrum resembled those of sanggenon-type flavanone (3-hydroxy-2-prenylflavanones with a benzofuran moiety between the B and C rings).[13] The ^{1}H NMR spectrum displayed signals of a hydrogen-bonded hydroxy group at δ_H 11.98 (1H, s, OH-5), two hydroxy groups at δ_H 7.05 (1H, s, OH-3) and 8.66 (1H, s, OH-4′), an aromatic ABX spin system at δ_H 7.35 (1H, d, J = 8.2 Hz, H-6′), 6.52 (1H, dd, J = 2.0, 8.2 Hz, H-5′), and 6.39 (1H, d, J = 2.0 Hz, H-3′), an aromatic singlet at δ_H 5.75 (1H, s, H-8), a 3,3-dimethylallyl (prenyl) group containing an olefinic methine proton at δ_H 5.26 (1H, br t, J = 7.0 Hz, H-10), two

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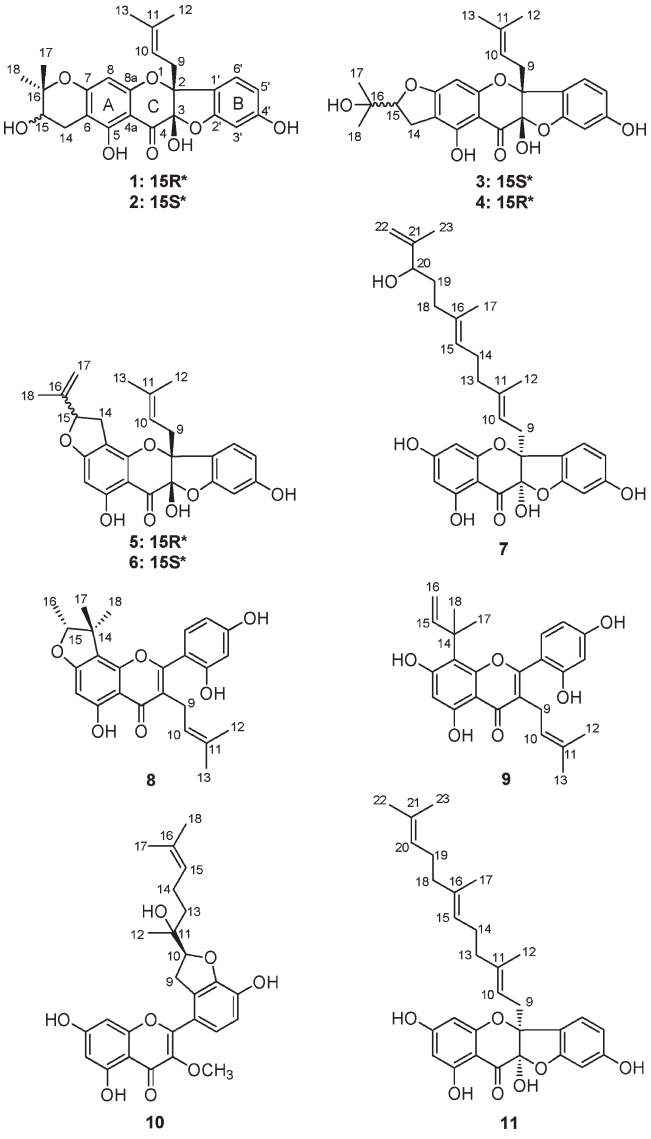

coupled methylene protons at δ_H 3.12 (1H, br dd, J = 9.0, 14.8 Hz, H-9a) and 2.77 (1H, br dd, J = 6.0, 14.8 Hz, H-9b), and two methyl groups at δ_H 1.61 and 1.53 (each 3H, br s, H_3-12, H_3-13) (Table 1). The ^{13}C NMR spectrum exhibited 25 carbon signals, including two quaternary carbons at δ_C 91.82 (C-2) and 102.58 (C-3) (Table 2). These NMR signals were consistent with the presence of a sanggenon-type flavanone.[13] Furthermore, the ^{1}H NMR spectrum showed signals of a 3-hydroxy-2,2-dimethyldihydropyran moiety at δ_H 4.29 (1H, d, J = 5.0 Hz, OH-15), 3.82 (1H, m, H-15), 2.83 (1H, dd, J = 5.2, 16.8 Hz, H-14α), 2.52 (1H, dd, J = 6.9, 16.8 Hz, H-14β), 1.35 (3H, s, H_3-18), and 1.28 (3H, s, H_3-17). The HMBC correlations from OH-3 to C-2, C-3, and C-4 and from H_2-9 to C-2, C-3, and C-1′ confirmed the sanggenon-type flavanone skeleton of **1** (Figure 1a). The 4′-hydroxylated B ring was deduced by the HMBC correlations from H-6′ to C-2, C-2′, and C-4′ and from OH-4′ to C-3′, C-4′, and C-5′. The 3-hydroxy-2,2-dimethyldihydropyran ring was attached at C-6 and C-7 according to the HMBC correlations from H_2-14 to C-6 and C-7 and from H-15 to C-6. The NOESY correlation of H_3-17 with H-14β suggested their synperiplanar relationship, randomly shown in a β-orientation (Figure 1b). The NOESY correlations of OH-15 with H_3-17 and H-14β indicated the β-orientation of OH-15. H-15 was assigned an α-orientation, confirmed by the NOESY correlation of H-15 with H_3-18. The absolute configuration at C-15 remains to be determined. The absolute configurations at C-2 and C-3 were assigned as 2*R* and 3*S* by the CD spectrum of **1**, which showed positive Cotton effects at 218, 252, 292, and 313 nm and negative Cotton effects at 238 and 274 nm.[14] As for **2**, its UV, IR, ^{1}H NMR, ^{13}C NMR, EIMS, and CD data were similar to those of **1**. It could be inferred that **2** was an epimer of **1** at C-15. In the NOESY spectrum of **2**, the correlations of H_3-17 (δ_H 1.33) with H-14β (δ_H 2.82) and H-15 (δ_H 3.80) suggested the β-orientation of H-15 (Figure 1c). Thus, the structure of nigrasin A (**1**) was elucidated as (3*R**,6a*S*,11b*R*)-6a,11b-dihydro-3,5,6a,9-tetrahydroxy-2,2-dimethyl-11b-(3-methyl-2-buten-1-yl)-2*H*,4*H*,6*H*-benzofuro[3,2-*b*]pyrano[3,2-*g*][1]benzopyran-6-one, and nigrasin B (**2**) was elucidated as its 3*S**-diastereomer.

Nigrasins C (**3**) and D (**4**) were isolated as yellowish oils. Similar to **1** and **2**, **3** and **4** were separated by preparative HPLC and assigned the same molecular formula of $C_{25}H_{26}O_8$ by HRESIMS and HREIMS, respectively. As for **3**, it was also regarded as a sanggenon-type flavanone derivative by the UV, IR, and NMR spectroscopic data. Compound **3** contains a 2-(1-hydroxy-1-methylethyl) dihydrofuran ring rather than the 3-hydroxy-2,2-dimethyldihydropyran ring in **1**, as established by the ^{1}H NMR signals of an ABX spin system at δ_H 4.79 (1H, dd, J = 7.8, 9.0 Hz, H-15), 3.06 (1H, dd, J = 7.8, 15.4 Hz, H-14a), and 3.01 (1H, dd, J = 9.0, 15.4 Hz, H-14b), a hydroxy group at δ_H 3.74 (1H, s, OH-16), and two methyl groups at δ_H 1.22 and 1.18 (each 3H, s, H_3-17, H_3-18). The HMBC correlations from H_2-14 and H-15 to C-6 and C-7 indicated that the 2-(1-hydroxy-1-methylethyl)-dihydrofuran moiety was fused at C-6 and C-7. The absolute configurations at C-2 and C-3 were assigned as 2*R* and 3*S* according to the CD spectrum of **3**. The configuration at C-15 could not be determined and is randomly shown as *S**. Spectroscopic characteristics of **3** and **4** showed that the structure difference between **3** and **4** was the absolute configuration at C-15, which was indicated as *R** in **4**. Thus, the structure of nigrasin C (**3**) was elucidated as (2*S**,5a*S*,10b*R*)-5a,10b-dihydro-4,5a,8-trihydroxy-2-(1-hydroxy-1-methylethyl)-10b-(3-methyl-2-buten-1-yl)-2*H*,3*H*,6*H*-benzofuro[3,2-*b*]pyrano[3,2-*f*]-[1]benzofuro-5-one, and nigrasin D (**4**) was elucidated as the 2*R**-diastereomer of nigrasin C.

Similarly, nigrasins E (**5**) and F (**6**), yellowish oils, were separated by preparative HPLC and had the same molecular formula of $C_{25}H_{24}O_7$ as determined by HREIMS. Comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of **5** and **3** indicated that **5** had a sanggenon-type flavanone skeleton with the same substitution patterns of the B and C rings as **3** (Tables 1 and 2). The presence of a 2-(1-methylethenyl)dihydrofuran ring in **5** was deduced by the following ^{1}H and ^{13}C NMR signals: δ_H 4.89 (1H, br s, H-17a), 4.74 (1H, br s, H-17b), 4.38 (1H, dd, J = 3.9, 7.4 Hz, H-15), 2.94 (1H, dd, J = 3.9, 14.5 Hz, H-14a), 2.83 (1H, dd, J = 7.4, 14.5 Hz, H-14b), and 1.80 (3H, br s, H_3-18); δ_C 25.90 (C-14), 76.25 (C-15), 148.06 (C-16), 110.42 (C-17), and 18.22 (C-18). This dihydrofuranoid moiety was located at C-7 and C-8, established by the HMBC correlations from H_2-14 to C-7, C-8, and C-8a and from H-15 to C-8. The absolute configurations at C-2 and C-3 were assigned as 2*R* and 3*S* by the CD spectrum. The absolute configuration of C-15 was uncertain and is randomly shown as *R**. The spectroscopic data of **6** showed that it was an epimer of **5**. Both compounds had the same absolute configurations at C-2 and C-3, except for the absolute

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

position	1[a]	2[b]	3[b]	4[b]	5[a]	6[a]
3	7.05 s (OH)	7.05 s (OH)	7.06 br s (OH)	7.05 br s (OH)	7.04 br s (OH)	7.01 br s (OH)
5	11.98 s (OH)	11.98 br s (OH)	11.74 s (OH)	11.76 br s (OH)	12.12 br s (OH)	12.12 br s (OH)
6					5.85 s	5.83 s
8	5.75 s	5.74 s	5.78 s	5.79 s		
3′	6.39 d (2.0)	6.38 d (2.1)	6.38 d (2.1)	6.39 d (2.1)	6.40 d (2.1)	6.39 d (2.1)
4′	8.66 s (OH)	8.67 br s (OH)	8.70 br s (OH)	8.70 br s (OH)	8.53 br s (OH)	8.69 br s (OH)
5′	6.52 dd (2.0, 8.2)	6.51 dd (2.1, 8.2)	6.51 dd (2.1, 8.2)	6.52 dd (2.1, 8.2)	6.53 dd (2.1, 8.2)	6.52 dd (2.1, 8.2)
6′	7.35 d (8.2)	7.35 d (8.2)	7.36 d (8.2)	7.36 d (8.2)	7.37 d (8.2)	7.36 d (8.2)
9	3.12 br dd (9.0, 14.8)	3.11 br dd (9.0, 14.8)	3.12 br dd (8.6, 14.6)	3.13 br dd (9.0, 14.6)	3.14 br dd (9.0, 14.6)	3.13 br dd (8.6, 14.6)
	2.77 br dd (6.0, 14.8)	2.75 br dd (6.0, 14.8)	2.77 br dd (6.2, 14.6)	2.75 br dd (6.2, 14.6)	2.78 br dd (6.3, 14.6)	2.78 br dd (6.3, 14.6)
10	5.26 br t (7.0)	5.25 br t (7.0)	5.25 br t (7.0)	5.24 br t (7.0)	5.26 br t (7.4)	5.23 br t (7.0)
12	1.61 br s	1.60 br s	1.61 br s	1.60 br s	1.63 br s	1.61 br s
13	1.53 br s	1.51 br s	1.53 br s	1.50 br s	1.53 br s	1.51 br s
14	2.52 dd (6.9, 16.8, H-β)	2.49 dd (7.4, 16.6, H-α)	3.06 dd (7.8, 15.4)	3.06 dd, (7.6, 15.4)	2.94 dd (3.9, 14.5)	2.92 dd (3.8, 14.6)
	2.83 dd (5.2, 16.8, H-α)	2.82 dd (5.5, 16.6, H-β)	3.01 dd (9.0, 15.4)	3.01 dd (9.4, 15.4)	2.83 dd (7.4, 14.5)	2.85 dd (7.3, 14.6)
15	3.82 m 4.29 d (5.0, OH)	3.80 m 4.35 d (5.0, OH)	4.79 dd (7.8, 9.0)	4.77 dd (7.6, 9.4)	4.38 dd (3.9, 7.4)	4.38 dd (3.8, 7.3)
16			3.74 s (OH)	3.78 s (OH)		
17	1.28 s	1.33 s	1.22 s[c]	1.23 s[c]	4.89, 4.74 br s	4.91, 4.75 br s
18	1.35 s	1.28 s	1.18 s[c]	1.21 s[c]	1.80 br s	1.79 br s

[a] Data were measured at 500 MHz. [b] Data were measured at 400 MHz. [c] The assignments are exchangeable.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−6

position	1[a]		2[a]		3[a]		4[a]		5[b]		6[b]	
2	91.82	qC	91.78	qC	92.14	qC	92.18	qC	91.79	qC	91.90	qC
3	102.58	qC	102.58	qC	102.51	qC	102.49	qC	102.51	qC	102.54	qC
4	188.65	qC	188.63	qC	188.31	qC	188.37	qC	188.17	qC	188.31	qC
4a	100.35	qC	100.37	qC	100.64	qC	100.76	qC	99.92	qC	99.97	qC
5	162.99	qC	162.95	qC	159.48	qC	159.52	qC	162.01	qC	162.08	qC
6	101.59	qC	101.79	qC	106.89	qC	106.91	qC	96.27	CH	96.24	CH
7	164.48	qC	164.44	qC	171.56	qC	171.59	qC	168.59	qC	168.48	qC
8	96.36	CH	96.40	CH	90.67	CH	90.69	CH	106.74	qC	106.67	qC
8a	161.08	qC	161.08	qC	164.28	qC	164.33	qC	163.21	qC	163.21	qC
1′	121.31	qC	121.34	qC	121.25	qC	121.24	qC	121.39	qC	121.40	qC
2′	161.19	qC	161.18	qC	161.24	qC	161.24	qC	161.16	qC	161.22	qC
3′	99.42	CH	99.42	CH	99.43	CH	99.45	CH	99.45	CH	99.46	CH
4′	161.17	qC	161.18	qC	161.21	qC	161.24	qC	161.16	qC	161.22	qC
5′	109.75	CH	109.73	CH	109.77	CH	109.75	CH	109.72	CH	109.74	CH
6′	125.73	CH	125.75	CH	125.71	CH	125.72	CH	125.67	CH	125.63	CH
9	32.08	CH_2	32.07	CH_2	32.04	CH_2	32.03	CH_2	32.04	CH_2	32.09	CH_2
10	118.67	CH	118.67	CH	118.57	CH	118.62	CH	118.63	CH	118.60	CH
11	136.46	qC	136.49	qC	136.58	qC	136.58	qC	136.55	qC	136.68	qC
12	18.07	CH_3	18.07	CH_3	18.08	CH_3	18.06	CH_3	18.07	CH_3	18.06	CH_3
13	25.89	CH_3	25.88	CH_3	25.92	CH_3	25.87	CH_3	25.90	CH_3	25.90	CH_3
14	25.48	CH_2	25.52	CH_2	26.44	CH_2	26.49	CH_2	25.90	CH_2	25.90	CH_2
15	68.70	CH	68.79	CH	93.25	CH	93.18	CH	76.25	CH	76.16	CH
16	80.25	qC	80.23	qC	71.38	qC	71.46	qC	148.06	qC	147.97	qC
17	21.46	CH_3	25.78	CH_3	25.43[c]	CH_3	25.15[c]	CH_3	110.42	CH_2	110.41	CH_2
18	25.60	CH_3	20.99	CH_3	25.82[c]	CH_3	25.85[c]	CH_3	18.22	CH_3	18.31	CH_3

[a] Data were measured at 100 MHz. [b] Data were measured at 125 MHz. [c] The assignments are exchangeable.

configuration at C-15. Thus, the structure of nigrasin E (**5**) was elucidated as (2*R**,6a*S*,11b*R*)-6a,11b-dihydro-5,6a,9-trihydroxy-2-(1-methylethenyl)-11b-(3-methyl-2-buten-1-yl)-1*H*,2*H*,6*H*-benzofuro[3,2-*b*]pyrano[2,3-*g*][1]benzofuro-6-one, and nigrasin F (**6**) was elucidated as the 2*S**-diastereomer of nigrasin E.

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Nigrasin G (**7**), a yellowish oil, was assigned a molecular formula of $C_{30}H_{34}O_8$ by HRESIMS. The UV and IR spectra showed that **7** should have a sanggenon-type flavanone skeleton. The ^{1}H NMR spectrum showed signals of two hydroxy groups at δ_H 11.62 (1H, br s, OH-5) and 7.14 (1H, br s, OH-3), two *meta*-coupled aromatic protons (A ring) at δ_H 5.92 (1H, d, J = 2.0 Hz, H-8) and 5.82 (1H, d, J = 2.0 Hz, H-6), a 2′,4′-dioxygenated B ring at δ_H 7.39 (1H, d, J = 8.2 Hz, H-6′), 6.53 (1H, dd, J = 2.1, 8.2 Hz, H-5′), and 6.40 (1H, d, J = 2.1 Hz, H-3′), and a farnesyl-derived side chain with a hydroxy group and a terminal double bond at δ_H 5.24 (1H, br t, J = 7.0 Hz, H-10), 5.06 (1H, m, H-15), 4.89 (1H, br s, H-22a), 4.73 (1H, br s, H-22b), 3.97 (1H, br t, J = 6.5 Hz, H-20), 3.19 (1H, br dd, J = 8.8, 14.6 Hz, H-9a), 2.79 (1H, br dd, J = 6.5, 14.6 Hz, H-9b), 2.05 (1H, overlap, H-18a), 1.96 (1H, m, H-18b), 1.82 (2H, m, H_2-13), 1.81 (2H, m, H_2-14), 1.68 (3H, br s, H_3-23), 1.65 (3H, br s, H_3-12), 1.57 (2H, m, H_2-19), and 1.57 (3H, br s, H_3-17) (Table 3). The ^{13}C NMR spectrum showed 30 carbon signals, corresponding to a sanggenon-type flavanone with a C_{15} isoprenoid group (Table 4). This isoprenoid group was further confirmed by the HMBC correlations from H-10 to C-12 and C-13, from H_2-13 to C-10, C-12, C-14, and C-15, from H_2-18 to C-15, C-16, C-17, C-19, and C-20, from H-20 to C-18, C-19, C-21, C-22, and C-23, and from H_2-22 to C-20 and C-23, which was located at C-2 by HMBC correlations from H_2-9 to C-2, C-3, and C-1′ (Figure 2). The *E*-configuration of the double bonds at C-10/11 and C-15/16 was established by the ROESY correlations of H-10 with H_2-13 and H-15 with H_2-18. The configuration at C-20 needs to be solved. Different from compounds **1**−**6**, the absolute configurations at C-2 and C-3 were assigned as 2*S* and 3*R* by the CD spectrum.[14] Thus, the structure of nigrasin G (**7**) was elucidated as (5a*S*,10a*R*)-5a,10a-dihydro-1,3,8,10a-tetrahydroxy-5a-[(2*E*,6*E*)-10-hydroxy-3,7,11-trimethyl-2,6,11-dodecatrien-1-yl]-11*H*-benzofuro[3,2-*b*]-[1]benzopyran-11-one.

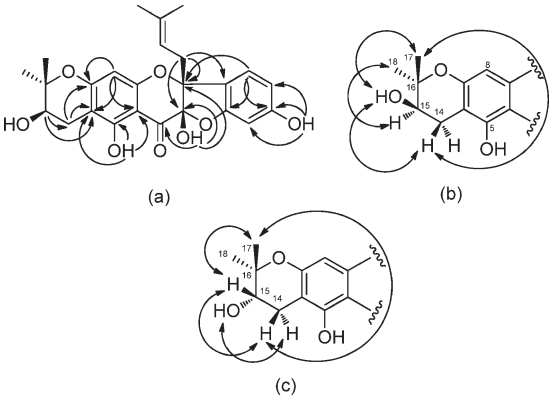

Figure 1. (a) Key HMBC correlations (H→C) of **1**. (b) Key NOESY correlations (H↔H) of **1**. (c) Key NOESY correlations (H↔H) of **2**.

Nigrasin H (**8**), a yellow, amorphous powder, was assigned a molecular formula of $C_{25}H_{26}O_6$ by HREIMS. The UV and IR spectroscopic data were similar to those of flavone derivatives.[15] The ^{1}H NMR spectrum exhibited signals of three hydroxy groups at δ_H 13.40 (1H, s, OH-5), 8.84 (1H, s, OH-2′), and 8.80

Table 3. ^{1}H NMR Spectroscopic Data of Compounds 7−10

position	7[a]	8[a]	9[a]	10[a]
3	7.14 br s (OH)			
5	11.62 br s (OH)	13.40 s (OH)	13.48 s (OH)	12.78 br s (OH)
6	5.82 d (2.0)	6.14 s	6.28 s	6.26 br s
7			9.22 br s (OH)	
8	5.92 d (2.0)			6.43 br s
2′		8.84 s (OH)	8.75 br s (OH)	
3′	6.40 d (2.1)	6.58 d (2.2)	6.56 d (2.2)	
4′		8.80 s (OH)	8.75 br s (OH)	
5′	6.53 dd (2.1, 8.2)	6.52 dd (2.2, 8.4)	6.50 dd (2.2, 8.3)	6.81 d (8.4)
6′	7.39 d (8.2)	7.25 d (8.4)	7.19 d (8.3)	7.30 d (8.4)
9	3.19 br dd (8.8, 14.6)	3.13 br d (7.0)	3.10 br d (7.0)	3.56 dd (8.4, 16.7)
	2.79 br dd (6.5, 14.6)			3.43 dd (9.6, 16.7)
10	5.24 br t (7.0)	5.13 br t (7.0)	5.11 br t (7.0)	4.74 t (9.0)
12	1.65 br s	1.44 br s	1.42 br s	1.25 s
13	1.82 m	1.57 br s	1.56 br s	1.57 t (8.5)
14	1.81 m			2.17 m
15	5.06 m	4.50 q (6.6)	6.24 dd (10.6, 17.4)	5.14 br t (7.0)
16		1.35 d (6.6)	4.82 dd (1.2, 17.4)	
			4.72 dd (1.2, 10.6)	
17	1.57 br s	1.41 s	1.54 s	1.60 br s
18	1.96 m 2.05 overlap	1.18 s	1.54 s	1.64 br s
19	1.57 m			
20	3.97 br t (6.5)			
22	4.89, 4.73 br s			
23	1.68 br s			
3-OMe				3.76 s

[a] Data were measured at 400 MHz.

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Table 4. ^{13}C NMR Spectroscopic Data of Compounds 7−10

position	7[a]		8[a]		9[b]		10[a]	
2	92.2	qC	161.8	qC	162.1	qC	158.0	qC
3	102.6	qC	121.3	qC	121.1	qC	139.6	qC
4	188.1	qC	183.2	qC	183.6	qC	179.4	qC
4a	100.0	qC	105.7	qC	105.8	qC	106.0	qC
5	164.0	qC	163.8	qC	161.3	qC	162.9	qC
6	95.6	CH	94.2	CH	100.3	CH	99.3	CH
7	169.2	qC	165.6	qC	163.1	qC	164.9	qC
8	96.8	CH	113.1	qC	111.7	qC	94.4	CH
8a	161.2	qC	154.2	qC	157.3	qC	157.9	qC
1′	121.4	qC	112.9	qC	112.9	qC	119.5	qC
2′	161.2	qC	157.4	qC	157.1	qC	129.8	qC
3′	99.5	CH	104.0	CH	103.8	CH	148.5	qC
4′	161.2	qC	161.6	qC	161.2	qC	144.4	qC
5′	109.7	CH	108.2	CH	107.9	CH	115.9	CH
6′	125.6	CH	132.4	CH	132.3	CH	123.4	CH
9	32.0	CH_2	24.7	CH_2	24.3	CH_2	32.3	CH_2
10	118.2	CH	122.7	CH	122.7	CH	89.9	CH
11	140.7	qC	132.1	qC	132.0	qC	73.3	qC
12	16.6	CH_3	17.7	CH_3	17.6	CH_3	22.1	CH_3
13	40.6	CH_2	25.8	CH_3	25.8	CH_3	39.2	CH_2
14	27.0	CH_2	44.3	qC	41.5	qC	22.6	CH_2
15	124.8	CH	91.4	CH	150.6	CH	125.7	CH
16	135.8	qC	14.4	CH_3	108.8	CH_2	131.6	qC
17	16.1	CH_3	25.9	CH_3	29.5	CH_3	17.7	CH_3
18	36.4	CH_2	21.5	CH_3	29.5	CH_3	25.8	CH_3
19	34.6	CH_2						
20	75.3	CH						
21	149.3	qC						
22	110.3	CH_2						
23	17.8	CH_3						
3-OMe							60.6	CH_3

[a] Data were measured at 100 MHz. [b] Data were measured at 125 MHz.

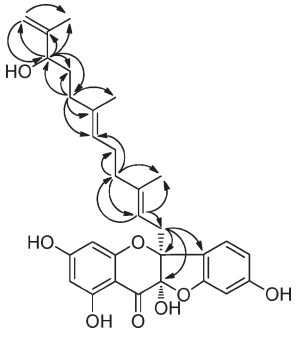

Figure 2. Key HMBC correlations (H→C) of 7.

(1H, s, OH-4′), an aromatic ABX spin system at δ_H 7.25 (1H, d, J = 8.4 Hz, H-6′), 6.58 (1H, d, J = 2.2 Hz, H-3′), and 6.52 (1H, dd, J = 2.2, 8.4 Hz, H-5′), an aromatic singlet at δ_H 6.14 (1H, s, H-6), and a prenyl group at δ_H 5.13 (1H, br t, J = 7.0 Hz, H-10), 3.13 (2H, br d, J = 7.0 Hz, H_2-9), 1.57 (3H, br s, H_3-13), and 1.44 (3H, br s, H_3-12) (Table 3). In addition, the presence of a 2,3,3-trimethyldihydrofuran ring was suggested by the ^{1}H NMR signals at

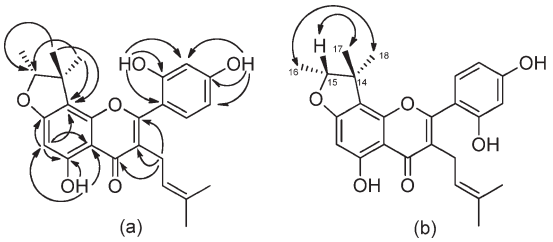

Figure 3. (a) Key HMBC correlations (H→C) of 8. (b) Key NOESY correlations (H↔H) of 8.

δ_H 4.50 (1H, q, J = 6.6 Hz, H-15), 1.41 (3H, s, H_3-17), 1.35 (3H, d, J = 6.6 Hz, H_3-16), and 1.18 (3H, s, H_3-18), which were consistent with the ^{13}C NMR signals at δ_C 44.3 (C-14), 91.4 (C-15), 14.4 (C-16), 25.9 (C-17), and 21.5 (C-18) (Table 4). The HMBC correlations from H_2-9 to C-2, C-3, and C-4 assigned the prenyl group at C-3 (Figure 3a). The 2,3,3-trimethyldihyrofuran ring was fused at C-7 and C-8, as supported by the HMBC correlations from both H_3-17 and H_3-18 to C-8. The 2′,4′-dihydroxylated B ring was determined by the HMBC correlations from OH-2′ to C-1′, C-2′, and C-3′ and from OH-4′ to C-3′, C-4′, and C-5′. In the NOESY experiment, the correlation between H-15 and H_3-17 suggested their *cis*-relationship and *β*-orientation. The α-orientation of H_3-16 and H_3-18 was indicated by their mutual NOESY correlation (Figure 3b). Thus, the structure of nigrasin H (**8**) was elucidated as (8*R**)-2-(2,4-dihydroxyphenyl)-8,9-dihydro-5-hydroxy-8,9,9-trimethyl-3-(3-methyl-2-buten-1-yl)-4*H*-furo[2,3-*h*]-1-benzopyran-4-one.

Nigrasin I (**9**), a yellow, amorphous powder, was assigned a molecular formula of $C_{25}H_{26}O_6$ by HRESIMS. The ^{1}H NMR and ^{13}C NMR spectroscopic data indicated that **9** was an isoprenylated flavone with the same B and C rings as **8** (Tables 3 and 4). Furthermore, the ^{1}H NMR spectrum showed signals of a 1,1-dimethyallyl group at δ_H 6.24 (1H, dd, J = 10.6, 17.4 Hz, H-15), 4.82 (1H, dd, J = 1.2, 17.4 Hz, H-16a), 4.72 (1H, dd, J = 1.2, 10.6 Hz, H-16b), and 1.54 (6H, s, H_3-17, H_3-18). The location of this isoprenoid group was determined at C-8 by the HMBC correlations from H_3-17 and H_3-18 to C-8. Thus, the structure of nigrasin I (**9**) was elucidated as 2-(2,4-dihydroxyphenyl)-3-(3-methyl-2-buten-1-yl)-5,7-dihydroxy-8-(1,1-dimethyl-2-propen-1-yl)-4*H*-1-benzopyran-4-one.

Nigrasin J (**10**), a yellow, amorphous powder, was assigned a molecular formula of $C_{26}H_{28}O_8$ by HREIMS. The ^{1}H NMR spectrum showed signals of a hydrogen-bonded hydroxy group at δ_H 12.78 (1H, br s, OH-5), two *ortho*-coupled aromatic protons at δ_H 7.30 (1H, d, J = 8.4 Hz, H-6′) and 6.81 (1H, d, J = 8.4 Hz, H-5′), two *meta*-coupled aromatic protons at δ_H 6.43 (1H, br s, H-8) and 6.26 (1H, br s, H-6), and a methoxy group at δ_H 3.76 (3H, s, OMe-3) (Table 3). The following ^{1}H NMR signals were observed: δ_H 5.14 (1H, br t, J = 7.0 Hz, H-15), 4.74 (1H, t, J = 9.0 Hz, H-10), 3.56 (1H, dd, J = 8.4, 16.7 Hz, H-9a), 3.43 (1H, dd, J = 9.6, 16.7 Hz, H-9b), 2.17 (2H, m, H_2-14), 1.64 (3H, br s, H_3-18), 1.60 (3H, br s, H_3-17), 1.57 (2H, t, J = 8.5 Hz, H_2-13), and 1.25 (3H, s, H_3-12), which were assigned to a 2-(1-hydroxy-1,5-dimethyl-4-hexenyl)dihydrofuran.[16] The ^{13}C NMR spectrum revealed signals of a flavonol skeleton, a methoxy group, and a C_{10} dihydrofuranoid moiety (Table 4). The HMBC correlation from the *O*-methyl protons to C-3 assigned the methoxy group to be at C-3 (Figure 4). The 2-(1-hydroxy-1,5-dimethyl-4-hexenyl)-dihydrofuran moiety was confirmed by the HMBC correlations from H_2-9 to C-10 and C-11, from H-10 to C-12 and C-13, and

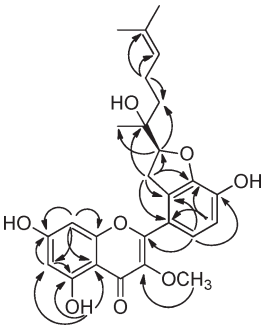

Figure 4. Key HMBC correlations (H→C) of **10**.

from H_2-14 to C-13, C-15, and C-16, which was located at C-2′ and C-3′ on the basis of the HMBC correlations from H_2-9 to C-1′, C-2′, and C-3′. The two *ortho*-coupled aromatic protons at δ_H 7.30 and 6.81 and the two *meta*-coupled aromatic protons at δ_H 6.43 and 6.26 were assigned to H-6′, H-5′, H-8, and H-6, respectively, as supported by the HMBC correlations shown in Figure 4. Due to the free rotation of the C-10 side chain link, it was impossible to determine the relative configuration of C-10 and C-11 with a ROESY experiment. The CD spectrum showed a positive Cotton effect at 278 nm, assigning the absolute configuration at C-10 as *S*.[17] Thus, the structure of nigrasin J (**10**) was elucidated as 2-[(2*S*)-2,3-dihydro-7-hydroxy-2-(1-hydroxy-1,5-dimethyl-4-hexen-1-yl)-4-benzofuranyl]-3-methoxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

The three known compounds were identified as sanggenol H (**11**),[13] cudraflavone C (**12**),[18] and macaranone B (**13**)[19] by comparison of their spectroscopic data with those reported. The absolute configurations at C-2 and C-3 of **11** were assigned as 2*S* and 3*R* by the CD spectrum for the first time.[14]

It is interesting that compounds **1**−**6** represent three pairs of epimers, as deduced from the similar spectroscopic data and the pairwise chromatographic separation. Moreover, although the isoprenoid groups in the form of 3-hydroxy-2,2-dimethyldihydropyran, 2-(1-hydroxy-1-methylethyl)dihydrofuran, and 2-(1-methylethenyl)dihydrofuran appear quite commonly in xanthones,[20−22] **1**−**6** are the first sanggenon-type flavanones with these substituents. The farnesyl-derived isoprenoid group in **7** in this class of compounds is reported for the first time.

Compounds **1**−**9**, **11**, and **12** were screened for the potential effects on adipogenesis. 3T3L1 mouse fibroblasts were treated with compounds for 8 days, and triglyceride content measurement and Oil Red O staining were carried out to assess adipocyte differentiation. Compared with control, **8** and **9** increased the triglyceride content (Table 5) and enhanced the accumulation of intracellular lipid droplet (Figure 5), whereas **1**−**7**, **11**, and **12** were inactive. Moreover, **8** and **9** were examined for their effects on the expression of adipocyte-specific genes, aP2 and GLUT4. As shown in Figure 6, treatment with **8** and **9** of 10 μM induced a significant up-regulation of the gene expression of aP2 and GLUT4 by RT-PCR analysis. The results by Western blotting showed that **8** and **9** increased the protein levels of aP2 and GLUT4 (Figure 7). These results indicated that **8** and **9** could promote adipogenesis in 3T3L1 cells. Compounds **8** and **9** may augment glucose uptake into adipocytes along with the increased expression of GLUT4, which will be of significance for exploring new agents for glucose homeostasis and finally for the treatment of type 2 diabetes. Compounds **8** and **9** are 3-isoprenylated flavones, which are mainly isolated from *Artocarpus* plants (Moraceae). Some of the

Table 5. Effects of Compounds 8 and 9 on Triglyceride Content in 3T3L1 Cells

compound	concentration (μM)	triglyceride content (%)
control	0	100.01 ± 15.35
8	12.5	102.16 ± 15.75
	25	143.94 ± 9.97[a]
	50	203.39 ± 9.57[b]
9	12.5	179.84 ± 31.27[a]
	25	317.18 ± 11.50[b]
	50	250.63 ± 29.59[b]
rosiglitazone	1	450.32 ± 54.32[b]

[a] $p < 0.05$ and [b] $p < 0.01$, compared with control.

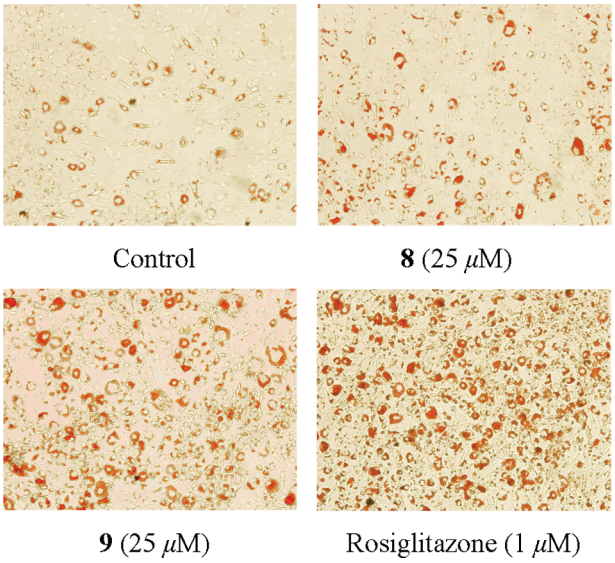

Figure 5. Effects of compounds **8** and **9** on adipocyte differentiation (Oil Red O staining).

3-isoprenylated flavones were found to show cytotoxicity,[23] inhibit melanin biosynthesis[24] and mushroom tyrosinase,[25] and exert anti-inflammatory,[26] antioxidant,[27] and hepatoprotective activities.[28] The effects of 3-isoprenylated flavones on adipogenesis have not yet been reported.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were recorded on a Hitachi U-2900 spectrophotometer. CD spectra were obtained on a JASCO J-715 spectropolarimeter. IR spectra were measured on a Nicolet Avatar-360 spectrometer with KBr pellets. NMR spectra were obtained on Bruker DRX-400 and -500 and Varian Mercucy Plus 400 instruments using acetone-d_6 as solvent. 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). EIMS (70 eV) were recorded on Agilent 5973N, VG Autospec-3000, and Finnigan MAT 95 mass spectrometers. HREIMS and HRESIMS were carried out on Waters Micromass GCT, API Qstar Pulsar, and Bruker Daltonics ApexIII mass spectrometers, respectively. Analytical and semipreparative HPLC was performed on an Agilent 1200 (Agilent Technologies, USA), a YMC C_{18} column (250 × 4.6 mm, 5 μm, Agilent Technologies, USA), and a Sepax Amethyst C_{18} column (150 × 10 mm, 5 μm, Sepax Techologies, Inc., USA), respectively.

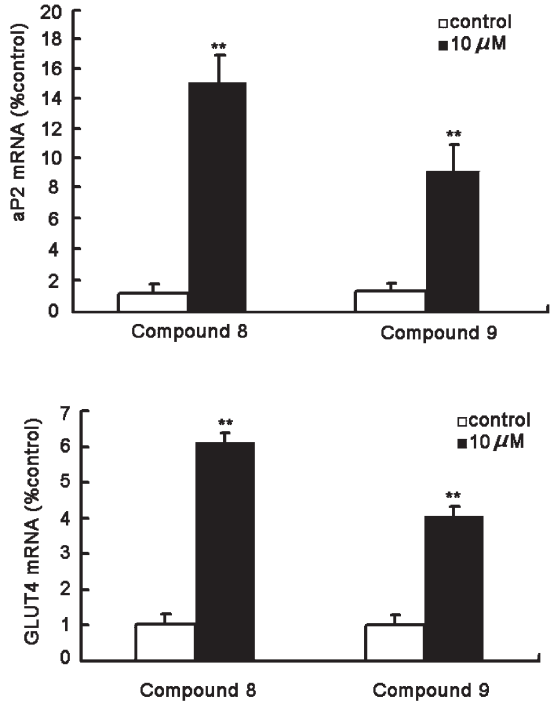

Figure 6. Effects of compounds **8** and **9** on the gene expression of aP2 and GLUT4 in 3T3L1 cells by RT-PCR. Differences statistically significant from control: $^{**}p < 0.01$.

Column chromatography was performed on silica gel (200−300 mesh, Yantai Institute of Chemical Technology, Yantai, P. R. China), ODS (50 μm, YMC Co., Kyoto, Japan), Sephadex LH-20 gel (GE Healthcare Amersham Biosciences, Uppsala, Sweden), and MCI gel CHP-20P (75−150 μm, Mitsubishi Chemical Co., Tokyo, Japan). TLC analysis was run on precoated silica gel GF254 plates (10−40 μm, Yantai Institute of Chemical Technology, Yantai, P. R. China).

Plant Material. The twigs of *Morus nigra* Linn. were collected in Xinjiang Uygur Autonomous Region, P. R. China, in May 2006. The plant material was identified by Dr. Yun Kang, Fudan University, and a voucher specimen (TCM 06-05-10 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

Extraction and Isolation. The milled, air-dried twigs of *M. nigra* (8.0 kg) were percolated with 95% EtOH (90 L) at room temperature. The filtrate was evaporated in vacuo to give a residue (800 g), which was suspended in H_2O and extracted successively with petroleum ether, $CHCl_3$, and EtOAc. A portion of the $CHCl_3$ extract (50 g) was subjected to column chromatography (CC) on silica gel eluting with a gradient of petroleum ether−EtOAc (6:1, 4:1, 2:1, 1:1) to give fractions A−H. Fraction D was separated by CC on silica gel eluted with petroleum ether−Me_2CO (5:1, 5:2, 1:1) to yield fractions D1−D9. Fraction D6 was chromatographed over ODS eluted with CH_3OH−H_2O (8.5:1.5) to give **11** (20 mg). Fraction D8 was subjected to CC on silica gel eluted with $CHCl_3$−MeOH (40:1, 20:1, 10:1) to afford fractions D8a−D8e. Fraction D8b was separated by semipreparative HPLC (CH_3CN−H_2O, 5.5:4.5, flow rate 1 mL/min, UV detector 210 nm) to provide **5** (15 mg) and **6** (10 mg). Fraction D8c was purified by CC on ODS eluted with CH_3OH−H_2O (7:3) to yield **8** (12 mg) and **9** (8 mg). Fraction D8d was separated by CC on MCI gel CHP-20P eluted with CH_3OH−H_2O (9:1), followed by semipreparative HPLC (CH_3OH−H_2O, 7.5:2.5, flow rate 1 mL/min, UV detector 210 nm), to provide **1** (13 mg) and **2** (10 mg). Fraction D8e was purified by semipreparative HPLC (CH_3OH−H_2O, 7.5:2.5, flow rate 1 mL/min, UV detector 210 nm) to yield **3** (15 mg) and **4** (12 mg). Fraction F was subjected to CC on silica gel eluted with a gradient of petroleum ether−EtOAc (7:3, 1:1) to give fractions F1−F8. Fraction F4 was chromatographed over silica gel eluted with $CHCl_3$−Me_2CO (15:1) to afford fractions F4a−F4c. Fraction F4b was purified by CC on ODS eluted with CH_3OH−H_2O (6.5:3.5) to yield **13** (12 mg). Fraction F5 was separated by CC on silica gel with $CHCl_3$−MeOH (20:1) to give fractions F5a−F5d. Fraction F5a was chromatographed over ODS with MeOH−H_2O (6.5:3.5) to yield **12** (8 mg). Fraction F5c was purified by semipreparative HPLC (CH_3OH−H_2O, 8.8:1.2, flow rate 1 mL/min, UV detector 210 nm) to give **7** (5 mg). Fraction F6 was subjected to CC on Sephadex LH-20 gel with $CHCl_3$−MeOH (1:1) to give fractions F6a and F6b. Fraction F6b was separated by semipreparative HPLC (CH_3OH−H_2O, 7:3, flow rate 1 mL/min, UV detector 210 nm) to yield **10** (5 mg).

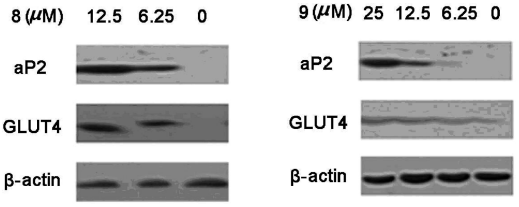

Figure 7. Effects of compounds **8** and **9** on the gene expression of aP2 and GLUT4 in 3T3L1 cells by Western blotting.

Nigrasin A (***1***): yellowish oil; $[\alpha]^{25}_D$ +137 (*c* 0.3, acetone); UV (MeOH) λ_{max} (log ε) 205 (4.30), 232 (sh) (3.78), 289 (sh) (3.61), 307 (3.65) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 218 (+2.61), 238 (−0.64), 252 (+0.48), 274 (−0.53), 292 (+0.63), 313 (+0.14); IR (KBr) ν_{max} 3384, 2950, 2925, 1632, 1605, 1459, 1342, 1199, 1147, 1097, 960 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 454 $[M]^+$ (9), 386 (13), 357 (7), 315 (8), 237 (100), 219 (24), 165 (21), 162 (20), 139 (17), 134 (10); HREIMS *m*/*z* 454.1624 $[M]^+$ (calcd for $C_{25}H_{26}O_8$, 454.1628).

Nigrasin B (***2***): yellowish oil; $[\alpha]^{25}_D$ +129 (*c* 0.3, acetone); UV (MeOH) λ_{max} (log ε) 206 (3.75), 234 (sh) (3.25), 289 (sh) (3.10), 307 (3.22) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 218 (+2.09), 238 (−1.09), 254 (+0.49), 274 (−0.60), 292 (+0.61), 313 (+0.25); IR (KBr) ν_{max} 3417, 2950, 2925, 1635, 1605, 1460, 1343, 1200, 1147, 1097, 960 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 454 $[M]^+$ (13), 386 (19), 357 (20), 315 (6), 237 (100), 219 (48), 165 (34), 162 (32), 134 (18); HREIMS *m*/*z* 454.1640 $[M]^+$ (calcd for $C_{25}H_{26}O_8$, 454.1628).

Nigrasin C (***3***): yellowish oil; $[\alpha]^{25}_D$ +171 (*c* 0.3, acetone); UV (MeOH) λ_{max} (log ε) 205 (4.15), 232 (sh) (3.62), 289 (sh) (3.47), 307 (3.52) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 215 (+3.89), 237 (−0.30), 251 (+0.89), 275 (−1.56), 304 (+0.48); IR (KBr) ν_{max} 3444, 2978, 2930, 1659, 1609, 1463, 1381, 1343, 1206, 1150, 1125, 1097, 959, 737 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 454 $[M]^+$ (11), 386 (20), 357 (18), 327 (4), 237 (100), 219 (24), 177 (18), 165 (45), 162 (38), 134 (21); HRESIMS *m*/*z* 477.1514 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_8Na$, 477.1525).

Nigrasin D (***4***): yellowish oil; $[\alpha]^{25}_D$ +131 (*c* 0.5, acetone); UV (MeOH) λ_{max} (log ε) 205 (3.70), 233 (sh) (3.21), 289 (sh) (3.05), 307 (3.50) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 217 (+5.87), 238 (−0.52), 252 (+2.42), 275 (−2.26), 305 (+2.92); IR (KBr) ν_{max} 3417, 2978, 2930, 1659, 1609, 1463, 1380, 1344, 1205, 1150, 1126, 1098, 959, 737 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 454 $[M]^+$ (11), 386 (14), 357 (5), 327 (7), 237 (100), 219 (11), 177 (16), 165 (22), 162 (20), 134 (10); HREIMS *m*/*z* 454.1626 $[M]^+$ (calcd for $C_{25}H_{26}O_8$, 454.1628).

Nigrasin E (***5***): yellowish oil; $[\alpha]^{25}_D$ +86 (*c* 0.6, acetone); UV (MeOH) λ_{max} (log ε) 205 (4.25), 233 (sh) (3.70), 289 (sh) (3.61), 307 (3.65) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 209 (+3.79), 240 (+0.15,

observed as valley), 250 (+1.64), 273 (−2.94), 292 (+3.33); IR (KBr) ν_{max} 3417, 2924, 1633, 1600, 1456, 1383, 1131, 1095 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 436 $[M]^+$ (1), 383 (4), 315 (10), 219 (11), 165 (8), 150 (30), 67 (100), 53 (50); HREIMS *m/z* 436.1521 $[M]^+$ (calcd for $C_{25}H_{24}O_7$, 436.1522).

Nigrasin F (**6**): yellowish oil; $[\alpha]^{25}_D$ +66 (*c* 0.2, acetone); UV (MeOH) λ_{max} (log ε) 206 (4.15), 233 (sh) (3.65), 289 (sh) (3.54), 307 (3.58) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 212 (+3.31), 243 (+0.55, observed as valley), 251 (+1.07), 273 (−1.68), 291 (+2.36); IR (KBr) ν_{max} 3417, 2924, 1626, 1600, 1460, 1344, 1147, 1096 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 436 $[M]^+$ (2), 383 (5), 315 (10), 219 (18), 165 (20), 150 (60), 67 (100), 53 (58); HREIMS *m/z* 436.1524 $[M]^+$ (calcd for $C_{25}H_{24}O_7$, 436.1522).

Nigrasin G (**7**): yellowish oil; $[\alpha]^{25}_D$ −99 (*c* 0.6, acetone); UV (MeOH) λ_{max} (log ε) 206 (4.70), 232 (sh) (4.16), 290 (sh) (4.03), 304 (4.08) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 219 (−7.93), 238 (+4.13), 249 (−0.68), 273 (+1.08), 292 (−2.77); IR (KBr) ν_{max} 3417, 2359, 2341, 1635, 1605, 1440, 1350, 1130 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m/z* 545.7 $[M + Na]^+$; HRESIMS *m/z* 545.21376 $[M + Na]^+$ (calcd for $C_{30}H_{34}O_8Na$, 545.21459).

Nigrasin H (**8**): yellow, amorphous powder; $[\alpha]^{25}_D$ +13 (*c* 0.6, acetone); UV (MeOH) λ_{max} (log ε) 202 (4.37), 224 (sh) (4.16), 241 (4.00), 254 (sh) (4.15), 263 (4.21), 333 (3.77) nm; IR (KBr) ν_{max} 3375, 2975, 2920, 1652, 1614, 1477, 1434, 1403, 1264, 1157 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 3 and 4; EIMS *m/z* 422 $[M]^+$ (58), 407 (48), 389 (20), 379 (100), 205 (24); HREIMS *m/z* 422.1729 $[M]^+$ (calcd for $C_{25}H_{26}O_6$, 422.1729).

Nigrasin I (**9**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 201 (4.44), 223 (sh) (4.20), 242 (3.99), 255 (sh) (3.89), 264 (4.19), 337 (3.72) nm; IR (KBr) ν_{max} 3433, 2925, 2848, 2360, 2342, 1634, 1610, 1410, 668 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 3 and 4; EIMS *m/z* 422 $[M]^+$ (53), 407 (29), 379 (100), 205 (38), 137 (14); HRESIMS *m/z* 445.1631 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_6Na$, 445.1627).

Nigrasin J (**10**): yellow, amorphous powder; $[\alpha]^{25}_D$ +57 (*c* 0.3, acetone); UV (MeOH) λ_{max} (log ε) 205 (4.50), 224 (sh) (4.22), 260 (4.20), 306 (sh) (3.88), 352 (4.02) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 235 (−1.21), 259 (+5.71), 270 (−0.59), 278 (+0.23), 352 (+1.73); IR (KBr) ν_{max} 3440, 2926, 1632, 1614, 1500, 1436, 1386 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Tables 3 and 4; EIMS *m/z* 468 $[M]^+$ (27), 341 (100), 326 (18), 310 (13), 297 (7), 153 (14), 109 (23), 69 (29); HREIMS *m/z* 468.1787 $[M]^+$ (calcd for $C_{26}H_{28}O_8$, 468.1784).

Sanggenol H (**11**): yellowish oil; $[\alpha]^{25}_D$ −118 (*c* 0.9, acetone); CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 220 (−4.12), 237 (+1.97), 249 (−0.68), 272 (+0.61), 294 (−1.52). ^1H NMR, ^{13}C NMR, and MS data were in agreement with those reported.[13]

Cell Culture and Differentiation. 3T3L1 mouse fibroblasts were cultured in growth medium consisting of Dulbecco's modified Eagle's medium (DMEM; Hyclone) supplemented with 10% newborn calf serum (NCS; Hyclone) at 37 °C under 5% CO_2. Two days after confluence, adipocyte differentiation was induced in a differentiation mixture containing 2 μg/mL insulin (Sigma), 1 μM dexamethasone (Sigma), and 0.125 mM 3-isobutyl-1-methylxanthine (Sigma) in DMEM with 10% fetal bovine serum (FBS; Hyclone). Then, cells were maintained in 10% FBS/DMEM supplemented with 2 μg/mL insulin. The tested compounds were administered at the initiation of differentiation at different concentrations and incubated with cells for 8 days.

Triglyceride Assay and Oil Red O Staining. Triglyceride content was determined using a commercial enzyme assay kit (Rongsheng, Shanghai, China). After the induction of differentiation, cells were stained with Oil Red O (Sakura Finetek USA Inc.) and photographed.

Quantitative Real-Time PCR. Total RNA was extracted from 3T3L1 cells using TRIzol (Invitrogen). Reverse transcription of RNA and quantitative PCR amplification were carried out using procedures reported previously.[22] The designed primers were as follows: aP2 (forward, 5′-GCGTAAATGGGGATTTGGTC-3′, and reverse, 5′-CTCCTGTCGTCTGCGGTGATT-3′), GLUT4 (forward, 5′-TCCTTCTATTTGCCGTCCTC-3′, and reverse, 5′-TGTTTTGCCCCTCAGTCATT-3′), and β-actin (forward, 5′-CACGATGGAGGGGCCGGACTCATC-3′, and reverse, 5′-CTAAAGACCTCTATGCCAACACAGT-3′). Values were normalized according to the amount of β-actin mRNA and represented the average of four independent experiments.

Western Blotting. Cells were harvested by a RIPA lysis buffer. Proteins were detected using a method reported previously.[29] The primary antibodies were aP2 (1:200, Santa Cruz Biotechnology) and GLUT4 (1:1000, Cell Signaling Technology).

Statistical Analysis. All data are expressed as mean ± SD. The comparison of different groups was assessed by two-tailed unpaired Student's *t* test. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using MS Office Excel 2007, and the plotting images were processed by Tanon Gel Image System 4.0.

■ ASSOCIATED CONTENT

Supporting Information. MS, ^1H, ^{13}C NMR, and 2D NMR spectra of compounds **1**−**10** and HPLC chromatograms of compounds **1**−**6** are available free of charge via the Internet at http://pubs.acs.org.

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