Lanostane Triterpenes from the Fruiting Bodies of *Ganoderma lucidum* and Their Inhibitory Effects on Adipocyte Differentiation in 3T3-L1 Cells

IkSoo Lee,[†] JungJu Seo,[‡] JinPyo Kim,[†] HongJin Kim,[†] UiJung Youn,[†] JoonSeok Lee,[§] HyunJu Jung,[⊥] MinKyun Na,[∥] Masao Hattori,[▽] ByungSun Min,*[,§] and KiHwan Bae*[,†]

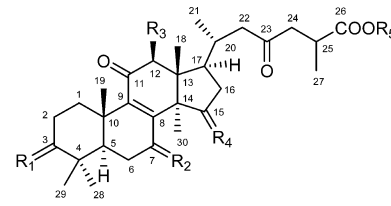
College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea, Department of Molecular Medicine, Kyungpook National University, Daegu 700-422, Korea, College of Pharmacy, Catholic University of Daegu, Kyungbook 712-702, Korea, Department of Oriental Pharmacy, Wonkwang University of Iksan, Jeonbuk 570-749, Korea, College of Pharmacy, Yeungnam University, Gyeongbuk 712-749, Korea, and Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

Received September 17, 2009

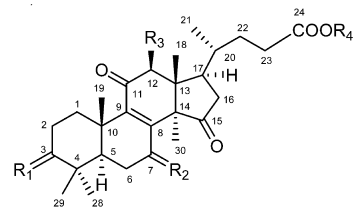
Four new lanostane triterpenes, butyl ganoderate A (**1**), butyl ganoderate B (**2**), butyl lucidenate N (**3**), and butyl lucidenate A (**4**), were isolated from the fruiting bodies of *Ganoderma lucidum* together with 14 known compounds (**5**−**18**). The structures of the new triterpenes were established by extensive spectroscopic studies and chemical evidence. In addition, the inhibitory effect of isolated compounds on adipocyte differentiation in 3T3-L1 cells was examined.

Ganoderma lucidum (Fr.) Karst, a species of basidiomycetes that belongs to the family Polyporaceae, has been widely used as a medical remedy to promote health and longevity in China, Korea, and Japan for centuries.[1] In addition, it has been used for the treatment of a wide range of ailments and chronic diseases, including migraine, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, hemorrhoids, diabetes, hypercholesterolemia, nephritis, dysmenorrhea, constipation, lupus erythematosis, hepatitis, and cardiovascular problems.[2−4] Because of the potential medicinal value and wide acceptability of *G. lucidum*, much attention has been paid to search for the pharmacologically significant compounds from this mushroom. Numerous triterpenes including highly oxygenated lanostane derivatives have been isolated from the fruiting bodies, cultured mycelia, and spores of *G. lucidum*. Some of these were linked to possible therapeutic effects, such as cytotoxic activity against hepatoma HTC (ganoderic acids U−Y),[5] hepatoma PLC/PRF/5 and KB cells (ganoderic aldehyde A),[6] histamine-releasing inhibitory activity in rat mast cells (ganoderic acids C and D),[7] inhibitory activity against angiotensin-converting enzyme (ganoderic acid F),[8] hepatoprotective activity (ganoderic acid A),[9] inhibitory effect on cholesterol synthesis (ganoderic acid B and C derivatives),[10,11] inhibitory effect on farnesyl protein transferase (ganoderic acid A and methyl ganoderate A),[12] and antihuman immunodeficiency virus type 1 (anti-HIV-1) activity (ganoderiol F and ganodermanontriol).[13]

In our continuing study to find new antiobesity agents from natural sources, we have found that the $CHCl_3$-soluble fraction of the MeOH extract of the fruiting bodies of *G. lucidum* had an inhibitory effect on adipocyte differentiation in 3T3-L1 cells (56% inhibition at 100 μg/mL). Further phytochemical study on this fraction resulted in the isolation of four new lanostane-type triterpenes (**1**−**4**), together with 14 known triterpenes (**5**−**18**). The present paper describes the isolation and structure elucidation of these triterpenes and their in vitro inhibitory effect on adipocyte differentiation in 3T3-L1 cells.



	R_1	R_2	R_3	R_4	R_5
1	O	βOH	H	αOH	Bu
2	βOH	βOH	H	O	Bu
5	O	βOH	H	αOH	H
6	O	βOH	H	αOH	Me
7	βOH	βOH	H	O	H
8	βOH	βOH	H	O	Me
9	O	βOH	H	O	Me
10	O	O	H	O	H
11	O	O	H	O	H
12	O	H_2	H	αOH	H
13	βOH	O	OAc	O	Me



	R_1	R_2	R_3	R_4
3	βOH	βOH	H	Bu
4	O	βOH	H	Bu
14	βOH	βOH	H	H
15	O	βOH	H	Me
16	βOH	βOH	OAc	Me
17	βOH	O	OAc	Me
18	O	O	H	Me

Results and Discussion

The MeOH extract of the fruiting bodies of *G. lucidum* was suspended in H_2O and successively partitioned with *n*-hexane and $CHCl_3$. The $CHCl_3$-soluble fraction, with inhibitory effect on

* To whom correspondence should be addressed. (B.K.) Tel: +82-42-821-5925. Fax: +82-42-823-6566. E-mail: baekh@cnu.ac.kr. (M.B.) Tel: +82-53-850-3613. Fax: +82-53-850-3602. E-mail: bsmin@cu.ac.kr.
[†] Chungnam National University.
[‡] Kyungpook National University.
[§] Catholic University of Daegu.
[⊥] Wonkwang University of Iksan.
[∥] Yeungnam University.
[▽] University of Toyama.

10.1021/np900578h
Published on Web 12/29/2009

Table 1. ^{1}H NMR Data of Compounds **1**−**4** (J in Hz) in $CDCl_3$

H	**1**[a]	**2**[a]	**3**[b]	**4**[b]
1-H(α)	1.46, m	0.98, m	0.98, m	1.48, m
1-H(β)	2.84, m	2.84, m	2.85, m	2.94, m
2-H(α)	2.46, m	1.62, m	1.62, m	2.46, m
2-H(β)	2.52, m	1.66, m	1.65, m	2.52, m
3-H(α)		3.21, dd (10.2, 5.6)	3.21, dd (11.0, 5.5)	
5-H(α)	1.68, m	0.88, m	0.88, m	1.58, m
6-H(α)	1.70, m	2.18, m	2.19, m	1.68, m
6-H(β)	2.06, m	1.60, m	1.61, m	2.12, m
7-H(α)	4.62, dd (10.0, 6.2)	4.80, ddd (9.2, 8.5, 4.5)	4.80, ddd (9.3, 8.5, 4.5)	4.86, ddd (9.3, 8.5, 4.5)
12-H(α)	2.76, bd (16.2)	2.70, bd (16.5)	2.78, bd (16.5)	2.78, bd (16.5)
12-H(β)	2.50, bd (16.2)	2.75, bd (16.5)	2.74, bd (16.5)	2.74, bd (16.5)
15-H(β)	4.80, br t (6.6)			
16-H(α)	1.84, m	2.67, m	2.80, m	2.81, m
16-H(β)	1.80, m	2.04, m	2.12, m	2.17, m
17-H(α)	1.82, m	2.12, m	2.00, m	2.00, m
18-H_3	0.99, s	1.01, s	0.98, s	1.01, s
19-H_3	1.28, s	1.22, s	1.22, s	1.26, s
20-H	2.03, m	2.12, m	1.60, m	1.60, m
21-H_3	0.90, d (6.3)	0.99, d (6.3)	0.97, d (6.6)	0.98, d (6.6)
22-H	2.40, m	1.79, m	1.80, m	1.37, m
22-H	2.40, m	1.79, m	1.80, m	1.37, m
23-H			2.28, m	2.30, m
23-H			2.40, m	2.42, m
24-H	2.46, dd (17.0, 5.5)	2.42, dd (17.0, 5.6)		
24-H	2.84, dd (17.0, 8.5)	2.84, dd (17.0, 8.4)		
25-H	2.94, dd (8.5, 5.5)	2.94, dd (8.4, 5.6)		
27-H_3	1.18, d (7.2)	1.18, d (7.2)		
28-H_3	1.12, s	1.04, s	1.04, s	1.13, s
29-H_3	1.10, s	0.86, s	0.86, s	1.11, s
30-H_3	1.26, s	1.34, s	1.34, s	1.34, s
butyl 1′	4.08, t (6.6)	4.06, t (6.6)	4.08, t (6.6)	4.08, t (6.6)
2′	1.60, m	1.60, m	1.60, m	1.60, m
3′	1.38, m	1.39, m	1.39, m	1.39, m
4′	0.94, t (7.2)	0.93, t (7.2)	0.94, t (7.2)	0.94, t (7.2)

[a] 300 MHz. [b] 500 MHz.

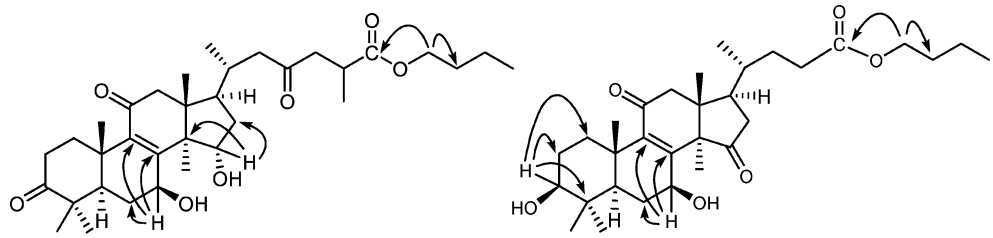
adipocyte differentiation in 3T3-L1 cells, was subjected to a series of chromatographic techniques and led to the isolation of 18 triterpenes (**1**−**18**). Of these, 14 compounds were identified as ganoderic acid A (**5**),[14] methyl ganoderate A (**6**),[15] ganoderic acid B (**7**),[14] methyl ganoderate B (**8**),[14] methyl ganoderate D (**9**),[7] ganoderic acid E (**10**),[16] methyl ganoderate E (**11**),[16] ganolucidic acid A (**12**),[17] methyl ganoderate H (**13**),[18] lucidenic acid N (**14**),[19] methyl lucidenate A (**15**),[19] methyl lucidenate P (**16**),[20] methyl lucidenate E_2 (**17**),[18] and methyl lucidenate F (**18**)[21] by comparing their observed and published physicochemical data.

Compound **1** was obtained as a white, amorphous powder with a positive specific rotation, $[\alpha]^{25}_D$ +18.0 (c 0.1, $CHCl_3$). The HREIMS spectrum of **1** exhibited a molecular ion peak at m/z 572.3727 $[M]^+$ corresponding to the molecular formula $C_{34}H_{52}O_7$. The IR spectrum exhibited absorption bands for hydroxy (3376 cm^{-1}), ester carbonyl (1728 cm^{-1}), and α,β-unsaturated carbonyl (1709 cm^{-1}) groups. Its UV absorption maximum at 253 nm was characteristic of an α,β-unsaturated carbonyl group. The ^{1}H and ^{13}C NMR spectra suggested **1** to be an oxygenated lanostane-type triterpene with a conjugated enone moiety.[13] In addition, these data were almost superimposable with those of ganoderic acid A (**5**) except for the *O*-butyl signals [δ_H 0.94 (t, J = 7.2 Hz), 1.38 (m), 1.60 (m), and 4.08 (t, J = 6.6 Hz); δ_C 13.9 (q), 19.3 (t), 30.9 (t), and 64.8 (t)] (Tables 1 and 2), suggesting that **1** is a butyl ester derivative of ganoderic acid A. A significant fragment ion peak observed at m/z 499 $[M - OCH_2CH_2CH_2CH_3]^+$ in the EIMS spectrum further supported the presence of an *O*-butyl group in **1**. The location of the *O*-butyl moiety at C-26 was confirmed by the HMBC cross-peak between CH_2 (δ_H 4.08, H-1′) of the butyl group and C-26 (δ_C 176.0) (Figure 1). The orientation of the C-7 and C-15 hydroxy groups was assigned as β and α, respectively, from the coupling constants of H-7 (dd, J = 10.0, 6.2 Hz) and H-15 (br t, J = 6.5 Hz), which was further confirmed by the NOE correlations

Table 2. ^{13}C NMR Data of Compounds **1**−**4** in $CDCl_3$

C	**1**[a]	**2**[a]	**3**[b]	**4**[b]
1	35.7	35.0	35.0	35.8
2	34.5	27.9	27.9	34.5
3	217.1	78.5	78.5	216.8
4	46.8	39.0	38.8	47.0
5	49.0	49.3	49.3	49.1
6	29.3	26.7	26.8	27.8
7	69.1	67.1	67.0	66.5
8	159.1	157.1	157.2	158.1
9	140.5	142.9	142.9	141.4
10	38.2	38.8	38.8	38.4
11	199.6	198.0	198.2	197.9
12	51.9	50.5	50.6	50.4
13	47.0	45.5	45.5	45.1
14	54.1	59.6	59.6	59.5
15	72.7	217.7	218.1	218.2
16	36.7	41.1	41.2	41.3
17	48.4	45.8	46.4	46.5
18	17.5	17.6	17.6	17.9
19	19.5	18.7	18.6	18.3
20	32.8	32.1	35.4	35.4
21	19.7	19.8	18.3	18.2
22	49.9	49.4	30.8	30.9
23	208.6	207.9	31.3	31.3
24	46.9	46.8	173.8	173.8
25	35.0	35.1		
26	176.0	175.9		
27	17.3	17.3		
28	27.5	28.4	28.4	27.2
29	20.9	15.6	15.6	20.9
30	19.8	24.6	24.6	24.9
butyl 1′	64.8	64.8	64.6	64.6
2′	30.9	30.9	30.9	30.9
3′	19.3	19.3	19.3	19.3
4′	13.9	13.9	13.9	13.9

[a] 75 MHz. [b] 125 MHz.



1 3

Figure 1. Key HMBC correlations (H→C) of compounds **1** and **3**.

between H-7 and H-5_{ax}/H_3-30 and between H-15 and H_3-18. Accordingly, the structure of **1** was established as butyl ganoderate A.

Compound **2** was obtained as a white, amorphous powder with $[\alpha]^{25}_D$ +20.0 (*c* 0.1, $CHCl_3$). It gave a molecular ion peak at *m*/*z* 572.3718 $[M]^+$ in the HREIMS spectrum, consistent with the molecular formula $C_{34}H_{52}O_7$. The UV absorption maxima at 252 nm and the IR band at 1708 cm^{-1} suggested the presence of an α,β-unsaturated carbonyl system similar to that in compound **1**. The ^1H and ^{13}C NMR spectra of **2** were superimposable with those of ganoderic acid B[14] except for the resonances of an *O*-butyl group [δ_H 0.93 (t, J = 7.2 Hz), 1.39 (m), 1.60 (m), and 4.06 (t, J = 6.6 Hz); δ_C 13.9 (q), 19.3 (t), 30.9 (t), and 64.8 (t)] (Tables 1 and 2), suggesting the presence of an ester-linked butyl group in **2**. This was supported by the observation of a fragment ion peak at *m*/*z* 499 $[M - OCH_2CH_2CH_2CH_3]^+$ in the EIMS spectrum. The β-orientation of the hydroxy groups at C-3 and C-7 was deduced from the coupling constants of H-3 (dd, J = 10.2, 5.6 Hz) and H-7 (ddd, J = 9.2, 8.5, 4.5 Hz) as well as the NOESY experiment, showing NOE correlations between H-3 and H-1_{ax}/H-5_{ax} and between H-7 and H-5_{ax}/H_3-30, respectively. Hence, the structure of **2** was determined as butyl ganoderate B.

Compound **3** was obtained as a white, amorphous powder with $[\alpha]^{25}_D$ +16.0 (*c* 0.1, $CHCl_3$). The molecular formula of **3** was found to be $C_{31}H_{48}O_6$ on the basis of a molecular ion peak at *m*/*z* 516.3456 $[M]^+$ in the HREIMS spectrum. The UV absorption maxima at 252 nm and the IR band at 1654 cm^{-1} suggested the presence of a conjugated carbonyl group. The ^1H and ^{13}C NMR spectra of **3** were similar to those of lucidenic acid N (**14**) except that **3** showed additional signals consistent with the presence of an *O*-butyl unit [δ_H 0.93 (t, J = 7.2 Hz), 1.39 (m), 1.60 (m), and 4.06 (t, J = 6.6 Hz); δ_C 13.9 (q), 19.3 (t), 30.9 (t), and 64.8 (t)] (Tables 1 and 2), suggesting that the carboxylic group of **3** is esterified with BuOH. The presence of an *O*-butyl unit was supported by the EIMS spectrum showing a fragment ion peak at *m*/*z* 443 $[M - OCH_2CH_2CH_2CH_3]^+$, and the HMBC coupling observed between H-1′ (δ_H 4.08) and C-24 (δ_C 173.8) confirmed the location of the *O*-butyl group at C-24 (Figure 1). In addition, the strong NOE correlations between H-3 and H-1_{ax}/H-5_{ax}, and between H-7 and H-5_{ax}/H_3-30 verified the β-orientation for the C-3 and C-7 hydroxy groups. Consequently, the structure of **3** was assigned as butyl lucidenate N.

Compound **4** was obtained as a white, amorphous powder with $[\alpha]^{25}_D$ +25.0 (*c* 0.1, $CHCl_3$) and gave a molecular ion at *m*/*z* 514.3295 $[M]^+$ in the HREIMS spectrum corresponding to the molecular formula $C_{34}H_{52}O_7$. Its UV and IR spectra exhibited absorptions at 253 nm and 1672 cm^{-1}, respectively, ascribable to a conjugated carbonyl system. When compared with lucidenic acid A,[19] the presence of an additional *O*-butyl group in **4** was indicated by a signal at δ_H 4.08 in the ^1H NMR spectrum as well as a signal at δ_C 64.6 in the ^{13}C NMR spectrum (Tables 1 and 2), which was further confirmed from the fragment ion peak at *m*/*z* 441 $[M - OCH_2CH_2CH_2CH_3]^+$ in the EIMS spectrum of **4**, suggesting that **4** is a butyl ester derivative of lucidenic acid A. In addition, the position of the *O*-butyl group at C-24 was confirmed from the HMBC correlation between H-1′ (δ_H 4.08) and C-24 (δ_C 173.8), and the β-orientation of the C-7 hydroxy group was inferred from the NOE correlations between H-7 and H-5_{ax}/H_3-30. Thus, the structure of **4** was determined as butyl lucidenate A.

These four lanostane triterpene butyl esters (**1**−**4**) are considered to be genuine natural products and not artifacts. In a separate experiment in which ganoderic acid A (**5**) was treated with a slightly acidic (0.001% (w/v) H_2SO_4) BuOH solution (70 °C for 2 h), only unreacted **5** was detected without evidence of the formation of esterified **1**.

To determine whether the isolated compounds have a cytotoxic effect in 3T3-L1 preadipocytes, cells were treated with or without various concentrations (10, 20, 40, and 80 μg/mL) of compounds **1**−**18**. Following 2 days of incubation, cell viability was measured, and no cytotoxic effect was detectable for all compounds in concentrations up to 40 μg/mL. However, a slightly decreased cell viability of some compounds (**1**, **4**, **6**, **12**, and **15**) compared to the control was observed at 80 μg/mL (data not shown). Therefore, to test the effect of isolated compounds (**1**−**18**) on differentiation of 3T3-L1 preadipocytes, cells were treated as a control or with 40 μg/mL of **1**−**18** during differentiation, and on day 8, cells were stained with oil red O. As illustrated in Figure 2, most of the compounds exhibited inhibition of lipid deposit (22−56% inhibitions at 40 μg/mL), indicative of 3T3-L1 cell differentiation, compared to the nontreated control. Butyl lucidenate N (**3**) exhibited the highest inhibition of lipid droplet formation (56%). As shown in Figure 3A, accumulation of lipid droplets within the cells was largely reduced by the treatment of **3** in a dose-dependent manner compared to the nontreated control. The cytosolic enzyme GPDH plays a central role in the triglyceride synthesis pathway. Thus, the effect of **3** on GPDH activity in 3T3-L1 cells was evaluated. As shown in Figure 3B, butyl lucidenate N (**3**) suppressed GPDH activity effectively in a similar way to lipid accumulation. This compound, at the highest concentration used (40 μg/mL), reduced the GPDH activity by more than 80%. Accordingly, our results indicate that these lanostane triterpenes contributed to the inhibitory effect of the $CHCl_3$-soluble fraction of the MeOH extract of the fruiting bodies of *G. lucidum* on adipocyte differentiation in 3T3-L1 cells.

A previous study revealed that *G. lucidum* has the ability to reduce the intracellular triglyceride accumulation level during differentiation of 3T3-L1 preadipocytes.[22] However, the literature regarding the inhibitory effect of triterpenes isolated from this mushroom on adipocyte differentiation in 3T3-L1 cells remains unclear. Therefore, this is the first study to report the inhibitory effect of triterpenes isolated from *G. lucidum* on adipocyte differentiation in 3T3-L1 cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV and IR spectra were recorded on JASCO V-550 UV/vis and JASCO 100 IR spectrometers, respectively. HREIMS and EIMS were recorded on a Hewlett-Packard

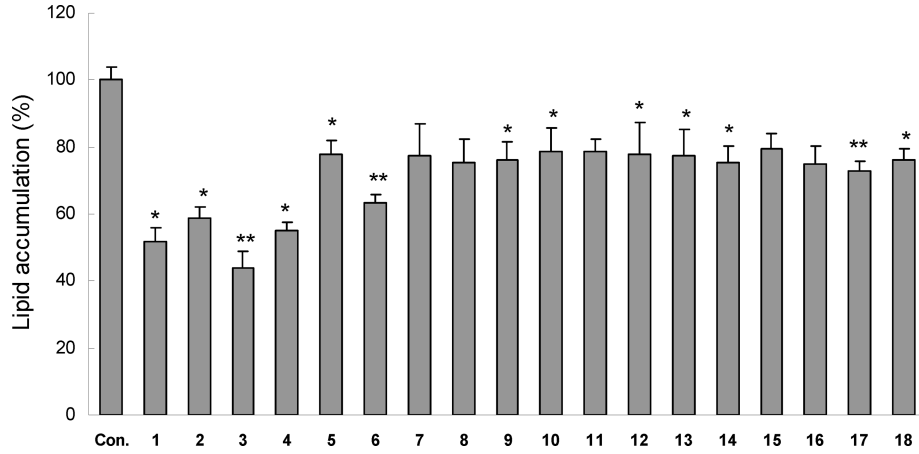


Figure 2. Effect of compounds **1**−**18** on adipocyte differentiation in 3T3-L1 cells. 3T3-L1 preadipocytes were treated as a control or with **1**−**18** (each 40 μg/mL) during differentiation. On day 8, cells were stained with oil red O. Stained oil red O was quantified by measuring the optical absorbance at 510 nm. Results are expressed as mean ± SD of three individual experiments. *$p < 0.05$, **$p < 0.001$ vs control.

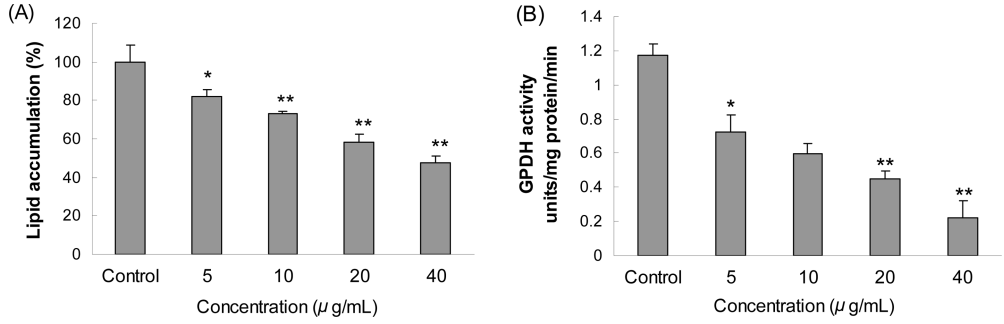


Figure 3. Effect of butyl lucidenate N (**3**) on adipocyte differentiation in 3T3-L1 cells. 3T3-L1 preadipocytes were treated as a control or with different concentrations of butyl lucidenate N (**3**) (5−40 μg/mL) during differentiation. On day 8, the lipid accumulation (A) and GPDH activity (B) of the cells were measured. Results are expressed as mean ± SD of three individual experiments. *$p < 0.05$, **$p < 0.001$ vs control.

5989B mass spectrometer. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on Bruker DRX-300 spectrometer with TMS as internal standard. 2D NMR experiments (HMQC, HMBC, and NOESY) were run on a Bruker Avance 500 spectrometer. Column chromatography was performed using silica gel (70−230 mesh and 230−400 mesh, Merck) and YMC-GEL ODS-A (12 nm, S-75 μm, YMC). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254s} plates (0.25 mm, Merck). Spots were detected by UV light (254 nm) and spraying 10% H_2SO_4 followed by heating. Preparative HPLC was performed on a Gilson TRILUTION system with a 321 pump and a UV/vis 151 detector, and a GX-271 liquid handler (Gilson, Inc.), using a YMC-pack Pro C_{18} (250 × 10 mm, i.d.) column.

Plant Material. The dried fruiting bodies of *G. lucidum* (cultured at Nagano, Japan, and kept at room temperature) were supplied by one of the authors (M.H.) in May 2008 and identified by B.-S.M. A voucher specimen (CUD 3170) was deposited at the herbarium of the college of Pharmacy, Catholic University of Daegu, Korea.

Extraction and Isolation. The dried fruiting bodies of *G. lucidum* (10 kg) were extracted with MeOH (3 × 50 L) at room temperature for 7 days, filtered, and concentrated to give a MeOH extract (390 g). The MeOH extract was suspended in H_2O (2 L) and then partitioned successively with *n*-hexane (2 × 2 L) and $CHCl_3$ (3 × 2.5 L) to afford *n*-hexane- (40 g) and $CHCl_3$-soluble fractions (200 g), respectively. The $CHCl_3$-soluble fraction (200 g), exhibiting the most inhibitory effect on adipocyte differentiation in 3T3-L1 cells, was subjected to silica gel column chromatography (80 × 12 cm) eluting with a gradient solvent system of $CHCl_3$−MeOH (200:1 → 1:1) to afford 10 fractions (A−J). Since fractions H−J exhibited considerable activity, these fractions were investigated extensively.

Fraction H (12 g) was chromatographed on a silica gel column (60 × 6.5 cm) using a gradient solvent system of *n*-hexane−EtOAc (50:1 → 10:1) to give **3** (15 mg), **4** (4 mg), **5** (4 mg), **6** (9 mg), and **7** (40 mg). Fraction I (8 g) was subjected to silica gel column chromatography (60 × 6.5 cm) eluting with a gradient solvent system of $CHCl_3$−MeOH (100:1 → 50:1) to yield six subfractions (I1−I6), one of which, fraction I2 (2 g), was further chromatographed on a YMC RP-18 column (50 × 3.5 cm) with MeOH−H_2O (85:15) as an eluent to yield **8** (9 mg), **9** (5 mg), **10** (17 mg), and **11** (2 mg). Fraction I4 (1 g) was further purified over a YMC RP-18 column (50 × 3.5 cm) using a gradient solvent system of MeOH−H_2O (85:15 → 90:10) to afford **1** (30 mg), **2** (10 mg), and **12** (22 mg). Compounds **13** (8 mg) and **14** (7 mg) were isolated from fraction I6 (1 g) using a YMC RP-18 column (50 × 3.5 cm) eluting with a gradient solvent system of MeOH−H_2O (80:20 → 85:15). Fraction J (10 g) was chromatographed on a silica gel column (60 × 6.5 cm) eluting with a gradient solvent system of $CHCl_3$−MeOH (50:1 → 10:1) to yield four subfractions (J1−I4). Fraction J3 (1.5 g) was further purified by extensive preparative RP-HPLC [Gilson TRILUTION system; YMC-pack Pro C_{18} (250 × 10 mm, i.d.) column; MeOH−H_2O (70:30, v/v); UV detection, 254 nm; flow rate, 2.5 mL/min] to afford **15** (10 mg), **16** (2 mg), **17** (6 mg), and **18** (5 mg).

Butyl ganoderate A (1): white, amorphous powder; $[\alpha]^{25}_D$ +18.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 253 (3.42) nm; IR (KBr) ν_{max} 3376, 2970, 1728, 1709, 1630 cm^{-1}; EIMS *m/z* (rel int) 572 $[M]^+$ (32), 554 $[M - H_2O]^+$ (22), 499 $[M - OCH_2CH_2CH_2CH_3]^+$ (12), 459 (21), 406 (25), 368 (27), 366 (26), 313 (32), 285 (45), 259 (32), 230 (54), 187 (47), 173 (43), 139 (85), 115 (100); HREIMS *m/z* 572.3727 $[M]^+$ (calcd for $C_{34}H_{52}O_7$, 572.3713); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Butyl ganoderate B (2): white, amorphous powder; $[\alpha]^{25}_{D}$ +20.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 252 (3.22) nm; IR (KBr) ν_{max} 3538, 2973, 1740, 1708, 1649 cm^{-1}; EIMS *m/z* (rel int) 572 $[M]^+$ (18), 554 $[M - H_2O]^+$ (12), 544 (18), 499 $[M - OCH_2CH_2CH_2CH_3]^+$ (12), 417 (12), 358 (65), 331 (45), 306 (28), 246 (30), 213 (25), 175 (33), 173 (28), 115 (100); HREIMS *m/z* 572.3718 $[M]^+$ (calcd for $C_{34}H_{52}O_7$, 572.3713); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Butyl lucidenate N (3): white, amorphous powder; $[\alpha]^{25}_{D}$ +16.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 252 (3.40) nm; IR (KBr) ν_{max} 3440, 2960, 1720, 1654 cm^{-1}; EIMS *m/z* (rel int) 516 $[M]^+$ (42), 498 $[M - H_2O]^+$ (15), 488 (30), 443 $[M - OCH_2CH_2CH_2CH_3]^+$ (12), 376 (45), 331 (100), 277 (18), 275 (18), 175 (19), 121 (15), 57 (27); HREIMS *m/z* 516.3456 $[M]^+$ (calcd for $C_{31}H_{48}O_6$, 516.3451); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Butyl lucidenate A (4): white, amorphous powder; $[\alpha]^{25}_{D}$ +25.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 253 (3.45) nm; IR (KBr) ν_{max} 3440, 2964, 1735, 1702, 1672 cm^{-1}; EIMS *m/z* (rel int) 514 $[M]^+$ (30), 498 $[M - H_2O]^+$ (15), 486 (22), 441 $[M - OCH_2CH_2CH_2CH_3]^+$ (8), 377 (12), 376 (40), 329 (100), 273 (15), 175 (14), 173 (12), 57 (31); HREIMS *m/z* 514.3295 $[M]^+$ (calcd for $C_{31}H_{46}O_6$, 514.3294); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Cell Culture and Differentiation. 3T3-L1 preadipocytes (ATCC, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with 10% bovine calf serum (BCS) (GIBCO) at 37 °C in a 5% CO_2-humidified incubator. Two days after confluence, cells were induced to differentiate with DMI mixture, containing 1 mM dexamethasone (DEX) (Sigma), 0.5 mM 1-methyl-3-isobutylxanthine (IBMX) (Sigma), and 1 μg/mL insulin (Sigma), in DMEM with 10% FBS for 2 days. The cells were maintained in DMEM supplemented with 10% FBS and 1 mg/mL insulin for 2 days, which was refreshed every 2 days. During differentiation, the cells were treated with samples every 2 days, for 8 days.

Cytotoxicity Assay. 3T3-L1 preadipocytes were seeded in DMEM supplemented with 10% bovine calf serum into 96-well microtiter plates (1×10^4 cells per each well) and incubated at 37 °C in 5% CO_2. After 24 h, vehicle or five concentrations of sample (from 10 to 80 μg/mL) in serum-free DMEM medium were added to each well. After 24 h, 20 μL of MTT solution (5 mg/mL) was added to each well. Two hours later, the plate was centrifuged for 5 min at 1500 rpm, the medium was removed, and the resulting formazan crystals were dissolved with 150 μL of DMSO. The optical density (OD) was measured at 570 nm using a Titertek microplate reader (Multiskan MCC/340, Flow).

Oil Red O Staining. Adipocyte differentiation was estimated by staining the cultured cells with oil red O. Briefly, the cells were washed with ice-cold PBS twice, fixed with 4% formaldehyde at room temperature for 20 min, and stained with oil red O (Sigma) for 30 min. Cells were then washed with 60% 2-propanol three times to remove unbound dye and photographed. For quantification analysis, stained oil red O was eluted with 2-propanol and quantified by measuring the optical absorbance at 510 nm.

Measurement of GPDH Activity. The glycerol-3-phosphate dehydrogenase (GPDH) activity was measured with a GPDH activity assay kit (Takara, Kyoto, Japan). Briefly, the cells were washed with ice-cold PBS twice, enzyme extraction buffer was added, and cells were collected by scraping with a cell scraper. After centrifugation at 12 000 rpm for 5 min at 4 °C, the absorbance reduction of supernatant at 340 nm for 5 min was measured to estimate the rate of NADH oxidation during the GPDH-catalyzed reduction of dihydroxyacetone phosphate. One unit of enzyme activity corresponded to the oxidation of 1 nmol NADH/min, and the results are expressed as units per mg protein. The protein concentration was determined by using a Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis. Data are expressed as mean ± SD of three individual experiments. The significance of differences was analyzed using one-way ANOVA using SPSS software, and differences were considered statistically significant when $p < 0.05$.

Acknowledgment. This research was supported by Korea Research Foundation Grant (KRF-2007-331-E00331) and by Linzhi General Institute Co. Ltd. (Tokyo, Japan).

Note Added after ASAP Publication: This paper was published on the Web on Dec 29, 2009, with an error in the graphic on the first page. The corrected version was reposted on Jan 8, 2010.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kim, S. S.; Kim Y. S. *Korean Mushrooms*; Yupoong: Seoul, 1990; p 298.
(2) Jong, S. C.; Birmingham, J. M. *Adv. Appl. Microbiol.* **1992**, *37*, 101–134.
(3) Hobbs, C. *Medicinal Mushrooms: An Exploration of Tradition, Healing, and Culture*, 2nd ed.; Botanica Press, Inc.: Santa Cruz, CA, 1995.
(4) McKenna, D. J.; Jones, K.; Hughes, K. *Reishi Botanical Medicines: The Desk Reference for Major Herbal Supplements*, 2nd ed.; The Haworth Herbal Press: New York, 2002; p 825.
(5) Toth, J. O.; Luu, B.; Ourision, G. *Tetrahedron Lett.* **1983**, *24*, 1081–1084.
(6) Lin, C. N.; Tome, W. P.; Won, S. J. *J. Nat. Prod.* **1991**, *54*, 998–1002.
(7) Kohda, H.; Tokumoto, W.; Sakamoto, K.; Fujii, M.; Hirai, Y.; Yamasaki, K.; Komoda, Y.; Nakamura, H.; Ishihara, S.; Uchida, M. *Chem. Pharm. Bull.* **1985**, *33*, 1367–1374.
(8) Morigiwa, A.; Kitabatake, K.; Fujimoto, Y.; Ikekawa, N. *Chem. Pharm. Bull.* **1986**, *34*, 3025–3028.
(9) Kim, D. H.; Shin, S. B.; Kim, N. J.; Jang, I. S. *Biol. Pharm. Bull.* **1999**, *22*, 162–164.
(10) Sonoda, Y.; Sekigawa, Y.; Sato, Y. *Chem. Pharm. Bull.* **1988**, *36*, 966–973.
(11) Komoda, Y.; Shimizu, M.; Sonoda, Y.; Sato, Y. *Chem. Pharm. Bull.* **1989**, *37*, 531–533.
(12) Lee, S. K.; Park, S. Y.; Oh, J. W.; Yang, C. H. *Planta Med.* **1998**, *64*, 303–308.
(13) Min, B. S.; Nakamura, N.; Miyashiro, H.; Bae, K. W.; Hattori, M. *Chem. Pharm. Bull.* **1998**, *46*, 1607–1612.
(14) Kubota, T.; Asaka, Y.; Miura, I.; Mori, H. *Helv. Chim. Acta* **1982**, *65*, 611–619.
(15) Hirotani, M.; Furuya, T.; Shiro, M. *Phytochemistry* **1985**, *24*, 2055–2061.
(16) Komoda, Y.; Nakamura, H.; Ishihara, S.; Uchida, M.; Kohda, H.; Yamasaki, K. *Chem. Pharm. Bull.* **1985**, *33*, 4829–4835.
(17) Kikuchi, T.; Kanomi, S.; Murai, Y.; Kadota, S.; Tsubono, K; Ogita, Z. I. *Chem. Pharm. Bull.* **1986**, *34*, 4030–4036.
(18) Kikuchi, T.; Kanomi, S.; Murai, Y.; Kadota, S.; Tsubono, K; Ogita, Z. I. *Chem. Pharm. Bull.* **1986**, *34*, 4018–4029.
(19) Nishitoba, T.; Sato, H.; Kasai, T.; Kawagishi, H.; Sakamura, S. *Agric. Biol. Chem.* **1985**, *49*, 1793–1798.
(20) Iwatsuki, K.; Akihisa, T.; Tokuda, H.; Ukiya, M.; Oshikubo, M.; Kimura, Y.; Asano, T.; Nomura, A.; Nishino, H. *J. Nat. Prod.* **2003**, *66*, 1582–1585.
(21) Wu, T. S.; Shi, L. S.; Kuo, S. C. *J. Nat. Prod.* **2001**, *64*, 1121–1122.
(22) Lee, C. W.; Yoon, H. M.; Kang, K. H. *J. Kor. Inst. Herbal Acu.* **2009**, *12*, 213–220.

NP900578H