Terpenylated Coumarins As SIRT1 Activators Isolated from *Ailanthus altissima*

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Ⓢ *Supporting Information*

ABSTRACT: Four new terpenylated coumarins (**1−4**) were isolated from the stem bark of *Ailanthus altissima* by bioactivity-guided fractionation using an in vitro SIRT1 deacetylation assay. Their structures were identified as (2′R,3′R)-7-(2′,3′-dihydroxy-3′,7′-dimethylocta-6′-enyloxy)-6,8-dimethoxycoumarin (**1**), 6,8-dimethoxy-7-(3′,7′-dimethylocta-2′,6′-dienyloxy)coumarin (**2**), (2′R,3′R,6′R)-7-(2′,3′-dihydroxy-6′,7′-epoxy-3′,7′-dimethyloctaoxy)-6,8-dimethoxycoumarin (**3**), and (2′R,3′R,4′S,5′S)-6,8-dimethoxy-7-(3′,7′-dimethyl-4′,5′-epoxy-2′-hydroxyocta-6′-enyloxy)coumarin (**4**). Compounds **1−4** strongly enhanced SIRT1 activity in an in vitro SIRT1-NAD/NADH assay and an in vivo SIRT1-p53 luciferase assay. These compounds also increased the NAD-to-NADH ratio in HEK293 cells. The present results suggest that terpenylated coumarins from *A. altissima* have a direct stimulatory effect on SIRT1 deacetylation activity and may serve as lead molecules for the treatment of some age-related disorders.
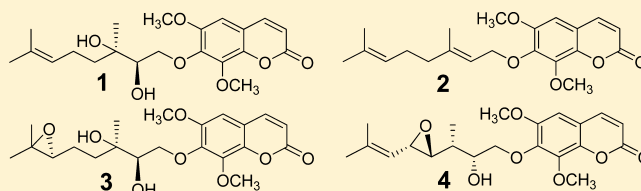
Silent information regulator two homologue 1 (SIRT1) is a member of the sirtuin family that possesses NAD⁺-dependent deacetylase activity.[1] SIRT1 regulates a variety of cellular processes such as energy metabolism, cell-cycle progression, muscle differentiation, fat mobilization, and aging, through the deacetylation of its substrates, including histones H1, H3, and H4, p53, p300, FOXOs 1, 3a, and 4, p65, HIVTat, PGC-1α, PCAF, MyoD, peroxisome proliferation-activated receptor γ, Ku70, and others.[2−4] SIRT1 has attracted considerable attention as a target for new therapies in a broad range of age-related diseases and in metabolic dysfunction. A major effort is thus focused on developing SIRT1 activators.[5−8] Recently, resveratrol has been identified as the most potent natural SIRT1 activator.[9,10] However, it has a low bioavailability and a rapid metabolism, and its direct effect on the SIRT1 enzyme is controversial.[11−13] Thus, a search for new potent SIRT1 activators from natural product sources seems worthwhile.

During the course of a SIRT1 activator screening program on natural products, a methanol extract of *Ailanthus altissima* was found to activate potential SIRT1 deacetylation activity. *A. altissima* (Mill.) Swingle (Simaroubaceae), commonly known as the "tree of heaven", is a deciduous tree (6−20 m in height). It is native to mainland China and is now naturalized in many temperate regions of the world.[14] Its leaves have been used in traditional medicine to treat gastric ailments, while the root bark and stem bark have been used as folk remedies for colds, fever, and breast tumors in China and Korea.[15,16] Previous phytochemical studies have led to the characterization of

quassinoids, alkaloids, lipids, coumarins, and other phenolic derivatives, of which quassinoids are the major components, with antitumor, antimalarial, antifeedant, anti-inflammatory, and other activities.[17,18] However, there have been no studies on the effects of *A. altissima* on SIRT1 activity. Thus, we report herein on the isolation and structure elucidation of four terpenylated coumarins (**1−4**) and their effects on SIRT1 deacetylase activity.
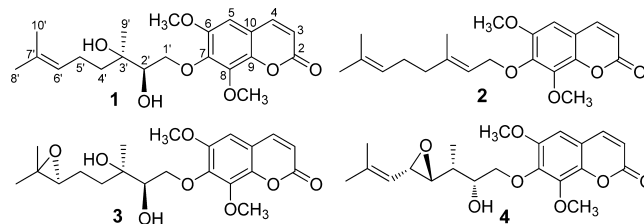
■ RESULTS AND DISCUSSION

Four new terpenylated coumarins (**1−4**) were isolated from a methanol extract of the stem bark of *A. altissima* by successive chromatographic procedures (silica gel, Sephadex LH-20, RP-18, and HPLC).

Compound **1** was obtained as a colorless oil. Its molecular formula of $C_{21}H_{28}O_7$ was established by HREIMS, *m/z*

Table 1. NMR Spectroscopic Data for Compounds 1−4[a]

position	1 δ_H mult. (J in Hz)	1 δ_C	2 δ_H mult. (J in Hz)	2 δ_C	3 δ_H mult. (J in Hz)	3 δ_C	4 δ_H mult. (J in Hz)	4 δ_C
2		160.2		160.6		160.2		160.4
3	6.36 d (9.5)	115.5	6.34 d (10.0)	115.1	6.37 d (9.5)	115.6	6.35 d (9.5)	115.2
4	7.62 d (9.5)	143.4	7.61 d (10.0)	143.5	7.62 d (9.5)	143.4	7.61 d (9.5)	143.4
5	6.69 s	103.8	6.66 s	103.5	6.69 s	103.9	6.68 s	103.9
6		149.7		150.7		149.9		149.6
7		144.6		144.9		144.5		144.7
8		141.0		141.8		141.2		140.6
9		142.9		142.9		142.9		143.1
10		114.8		114.4		114.9		114.3
1′	4.55 dd (10.5, 2.0) 4.02 dd (10.5, 8.0)	76.1	4.68 d (7.0)	70.3	4.48 dd (10.5, 2.0) 3.91 dd (10.5, 8.5)	76.4	4.42 dd (10.0, 3.5) 4.11 dd (10.0, 8.0)	73.1
2′	3.70 dd (8.0, 2.0)	75.1	5.56 t (7.0)	119.6	3.88 dd (8.5, 2.0)	75.2	3.99 dd (8.0, 3.5)	79.4
3′		73.4		142.5		83.8	2.20 m	47.4
4′	1.62 m 1.40 m	37.9	2.05 m	39.6	2.23 m 1.55 m	33.2	4.03 m	84.6
5′	2.13 m 2.05 m	22.1	2.08 m	26.3	1.99 m 1.92 m	26.4	4.86 dd (15.5, 8.5)	74.3
6′	5.11 t (7.0)	124.2	5.06 t (7.0)	123.8	3.85 m	85.3	5.19 d (8.5)	125.3
7′		131.9		131.7		71.6		136.9
8′	1.67 s	25.6	1.67 s	25.6	1.26 s	27.7	1.72 s	25.8
9′	1.22 s	23.2	1.69 s	16.4	1.19 s	23.4	1.47 s	23.5
10′	1.61 s	17.6	1.59 s	17.6	1.10 s	24.9	1.71 s	18.2
OCH₃-6	3.90 s	56.3	3.89 s	56.3	3.91 s	56.4	3.88 s	56.2
OCH₃-8	4.06 s	61.9	4.03 s	61.7	4.06 s	62.0	4.02 s	61.9
OH-2′					3.75 s			

[a] In CDCl₃, 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR.

392.1837 [M]⁺ (calcd for $C_{21}H_{28}O_7$, 392.1835). Strong UV peaks at λ_{max} 225, 293, and 335 nm and an IR band at ν_{max} 1727 cm⁻¹ suggested a 7-oxygenated coumarin skeleton.[18] In the ^{1}H NMR spectrum (Table 1), the characteristic signals of a 6,7,8-trisubstituted coumarin, with doublets at δ_H 7.62 (1H, d, J = 9.5 Hz, H-4) and 6.36 (1H, d, J = 9.5 Hz, H-3), a singlet at δ_H 6.69 (1H, s, H-5), and two methoxy signals at δ_H 4.06 (3H, s, OCH₃-8) and 3.90 (3H, s, OCH₃-6), were observed. The positions of the methoxy groups were inferred as C-6 and C-8 by the HMBC correlations from the methoxy protons to C-6 (δ_C 149.7) and C-8 (δ_C 141.0), respectively (Figure 1), and a
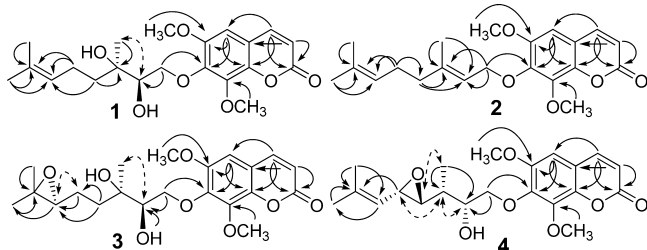

Figure 1. Selected HMBC [H (solid arrow) C] and NOESY [H (dotted arrow) H] correlations for compounds 1−4.

diagnostic fragment peak at m/z 222 ([M − 170]⁺) in the EIMS.[18,19] In addition, its ^{1}H NMR spectrum showed eight other signals due to three methyl groups [δ_H 1.67 (3H, s, H-8′), 1.61 (3H, s, H-10′) and 1.22 (3H, s, H-9′)], two methylenes [δ_H 2.13 (1H, m, H-5′a), 2.05 (1H, m, H-5′b), and 1.62 (1H, m, H-4′a), 1.40 (1H, m, H-4′b)], an oxymethine [δ_H 3.70 (1H, dd, J = 8.0, 2.0 Hz, H-2′)], an oxymethylene [δ_H 4.55 (1H, dd, J = 10.5, 2.0 Hz, H-1′a), 4.02 (1H, dd, J = 10.5, 8.0 Hz, H-1′b)],

and an olefinic proton [δ_H 5.11 (1H, t, J = 7.0 Hz, H-6′)]. On the basis of the observed HMQC correlations, these signals correspond to the ^{13}C NMR signals for an oxygenated C_{10} terpenyl side-chain [−OCH₂CHOHC(CH₃)-OHCH₂CH₂CH=C(CH₃)₂].[18,20] This terpenyl group was attached to the C-7 position because the H-1′ proton at δ_H 4.02 displayed a HMBC connectivity with C-7 (δ_C 144.6). Furthermore, the HMBC correlations between oxymethylene at H-1′/C-2′ and C-3′ and between H-9′/C-2′, C-3′, and C-4′ indicated that two hydroxy groups are attached at C-2′ and C-3′, respectively. The planar structure of 1 was confirmed by additional HMBC correlations of H-4′/C-2′, C-3′, C-5′, C-6′, and C-9′; H-5′/C-3′, C-4′, C-6′, and C-7′; H-6′/C-4′, C-7′, C-8′, and C-10′; and H-8′/C-6′, C-7′, and C-10′. The *syn* relative configuration of the 2′,3′-diol of compound 1 was determined by 2D-NOESY experiments, which showed a strong NOE cross-peak between H-2′ and H-9′ (Figure 1).[18,21] Assignment of the absolute configuration of the 2′,3′-diol of 1 was also confirmed by the Mosher ester method.[22,23] Compound 1 was treated individually with (R)- and (S)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in dry pyridine to yield the (S)- or (R)- MTPA ester derivatives 1a and 1b, respectively. Analysis of the ^{1}H NMR chemical shift differences ($\Delta\delta_{S-R}$) (Figure 2) of these derivatives allowed the assignment of the absolute configuration of C-2′ of 1 as R. The absolute configuration of C-3′ was thus assigned as R. Therefore, compound 1, named altissimacoumarin C, was assigned as (2′R,3′R)-7-(2′,3′-dihydroxy-3′,7′-dimethylocta-6′-enyloxy)-6,8-dimethoxycoumarin.

Compound 2 was obtained as a colorless oil. The presence of 6,8-dimethoxy-7-substituted coumarin was indicated from the UV absorptions at λ_{max} 230, 295, and 340 nm, an IR band at
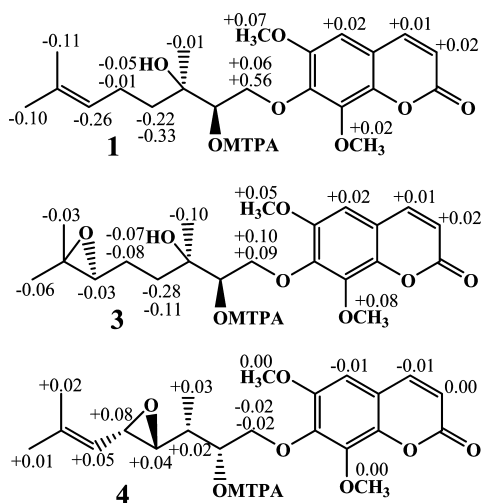
Figure 2. $\Delta\delta_{S-R}$ values (in ppm) for the MTPA esters of **1**, **3**, and **4**.

ν_{max} 1727 cm^{-1}, the typical AB-type signals at δ_H 7.61 (1H, d, J = 10.0 Hz, H-4), 6.34 (1H, d, J = 10.0 Hz, H-3), a singlet at δ_H 6.66 (1H, s, H-5), and two methoxy signals at δ_H 4.03 (3H, s, OCH$_3$-8) and 3.89 (3H, s, OCH$_3$-6) in the ^{1}H NMR spectrum.[18] In addition, the ^{1}H and ^{13}C NMR spectra (Table 1) revealed signals for a geranyl group [δ_H 4.68 (2H, d, J = 7.0 Hz, H-1′), 5.56 (1H, t, J = 7.0 Hz, H-2′), 2.05 (2H, m, H-4′), 2.08 (2H, m, H-5′), 5.06 (1H, t, J = 6.5 Hz, H-6′), 1.67 (3H, s, H-8′), 1.69 (3H, s, H-9′), 1.59 (3H, s, H-10′); δ_C 70.3, 119.6, 142.5, 39.6, 26.3, 123.8, 131.7, 25.6, 16.4, and 17.6, respectively]. This geranyl group was placed at the C-7 position because the H-1′ proton displayed a HMBC connectivity with C-7 (δ_C 144.9). These NMR data were similar to those of 6-methoxyaurapten, except for an additional methoxy group at C-8.[24] This was supported further by the molecular ion peak at m/z 359.1848 [M + H]$^+$ in the HRFABMS, which indicated a molecular formula of C$_{21}$H$_{26}$O$_5$ (calcd for C$_{21}$H$_{27}$O$_5$, 359.1858). The location of this methoxy group was established through a HMBC correlation from the methoxy protons to C-8 (δ_C 141.8) (Figure 1). From the above data, the structure of **2**, named altissimacoumarin D, was proposed as 6,8-dimethoxy-7-(3′,7′-dimethylocta-2′,6′-dienyloxy)coumarin.

Compound **3** was obtained as a colorless oil with the molecular formula of C$_{21}$H$_{28}$O$_8$, which was determined by HREIMS, m/z 408.1786 [M]$^+$ (calcd for C$_{21}$H$_{28}$O$_8$, 408.1784). The UV and IR spectra of **3** were similar to those of **1**. The ^{1}H and ^{13}C NMR spectral data (Table 1) suggested features of a 6,8-dimethoxy-7-terpenylated coumarin, which were almost identical to those of **1**, except for some chemical shift differences due to the position of the epoxy group in the terpenoid chain. Extensive NMR experiments (HMQC, HMBC, and NOESY) indicated the epoxy ring to be at C-6′ and C-7′ with two hydroxy groups occurring at C-2′ and C-3′. These assignments were deduced from the HMBC correlations between H-1′/C-2′ and C-3′; H-2′/C-1′, C-3′, C-4′, and C-9′; H-5′/C-3′, C-4′, C-6′, and C-7′; H-6′/C-4′, C-7′, C-8′, and C-10′; and OH-2′/C-2′ (Figure 1). The relative configuration of **3** was then established on the basis of the specific rotation ($[\alpha]_D^{25}$ +13) and NOESY data, which showed NOE correlations between H-2′/H-9′ and H-5′/H-6′, whereas no NOE was observed between H-5′/H-9′ (Figure 1).[18,21] After the reaction with compound **3** and the Mosher ester reagents, (S)-MTPA

(**3a**) and (R)-MTPA (**3b**), respectively, analysis of the ^{1}H NMR data (Figure 2) indicated the R configuration at the C-2′ stereocenter in **3**. Thus, the structure of altissimacoumarin E (**3**) was assigned as (2′R,3′R,6′R)-7-(2′,3′-dihydroxy-6′,7′-epoxy-3′,7′-dimethyloctaoxy)-6,8-dimethoxycoumarin.

Compound **4** was obtained as a colorless oil, and its molecular formula, C$_{21}$H$_{26}$O$_7$, was established by HREIMS, m/z 390.1679 [M]$^+$ (calcd for C$_{21}$H$_{26}$O$_7$, 390.1679). The UV and IR absorptions resembled those of **1**−**3**, and the characteristic ^{1}H NMR signals suggested a skeleton of a 6,8-dimethoxy-7-terpenylated coumarin.[18] The positions of two methoxy groups were confirmed by the HMBC correlations from the methoxy protons to C-6 (δ_C 149.6) and C-8 (δ_C 140.6), respectively (Figure 1). In addition, analysis of the ^{1}H and ^{13}C NMR spectra, including the HMQC and HMBC data, suggested the presence of a C$_{10}$ terpenyl side-chain at C-7, consisting of three methyl [δ_H 1.72 (3H, s, H-8′), 1.71 (3H, s, H-10′), 1.47 (3H, s, H-9′)], a methine [δ_H 2.20 (1H, m, H-3′)], three oxymethine [δ_H 4.86 (1H, dd, J = 15.5, 8.5 Hz, H-5′), 4.03 (1H, m, H-4′), and 3.99 (1H, dd, J = 8.5, 3.5 Hz, H-2′)], an oxymethylene [δ_H 4.42 (1H, dd, J = 10.0, 3.5 Hz, H-1′a), 4.11 (1H, dd, J = 10.0, 8.0 Hz, H-1′b)], and two olefinic signals [δ_H 5.19 (1H, d, J = 8.5 Hz, H-6′), δ_C 125.3 (C-6′), and δ_C 136.9 (C-7′)]. The observation of mass fragment ions (EIMS) at m/z 390 ([M]$^+$), 309, and 222 resulting from cleavage at C-4′/C-5′ and 7-O/C-1′ suggested the presence of an epoxy and a hydroxy group in the terpenoid side-chain. The HMBC correlations of H-2′/C-1′, C-3′, and C-9′; H-3′/C-2′, C-4′, and C-9′; H-4′/C-3′ and C-5′; H-5′/C-3′, C-6′, and C-7′; and H-9′/C-2′, C-3′, and C-4′ confirmed the epoxy ring at C-4′ and C-5′ and the hydroxy group at the C-2′ position. The cis configuration of this epoxy ring was established by the large coupling constant (15.5 Hz) between H-4′ and H-5′ and NOE correlations between H-3′/H-5′ and H-4′/H-9′ in the ^{1}H NMR and 2D-NOESY spectra, respectively (Figure 1). Furthermore, the relative configurations of C-2′ and C-3′ in **4** were deduced from the NOE cross-peaks between H-2′/H-3′ and H-1′/H-9′ (Figure 1).[18,21] Finally, the absolute configuration of the stereogenic center at C-2′ was addressed by the Mosher ester method (Figure 2). The R configuration of C-2′ in **4** was determined by the interpretation of the data obtained.[22,23] Therefore, the structure of compound **4**, named altissimacoumarin F, was proposed as (2′R,3′R,4′S,5′S)-6,8-dimethoxy-7-(3′,7′-dimethyl-4′,5′-epoxy-2′-hydroxyocta-6′-enyloxy)coumarin.

SIRT1, a mammalian ortholog of yeast Sir2, binds to and deacetylates p53 at the K382 residue, thereby negatively regulating p53-mediated transcriptional activation, which prevents the cellular senescence and apoptosis caused by DNA damage and stress.[25,26] To evaluate the effects of compounds **1**−**4** on SIRT1, the in vitro enzymatic deacetylation and the p53 transcription activities were determined. In a bioluminescence assay that measures deacetylated peptide signals via the consumption of NAD$^+$,[27] these compounds increased the observation of deacetylated substrate (Figure 3A). Moreover, after co-transfection with p53-luc and SIRT1 into HEK293 cells, treatment with compounds **1**−**4** or resveratrol (10 μM) strongly decreased p53 transcriptional activity compared to the control (Figure 3B).

Since compounds **1**−**4** induced an increase in SIRT1 enzymatic activity and a decrease in p53 transcriptional activity, their direct effects on SIRT1 deacetylation activity were confirmed by determining the actual level of substrate used in
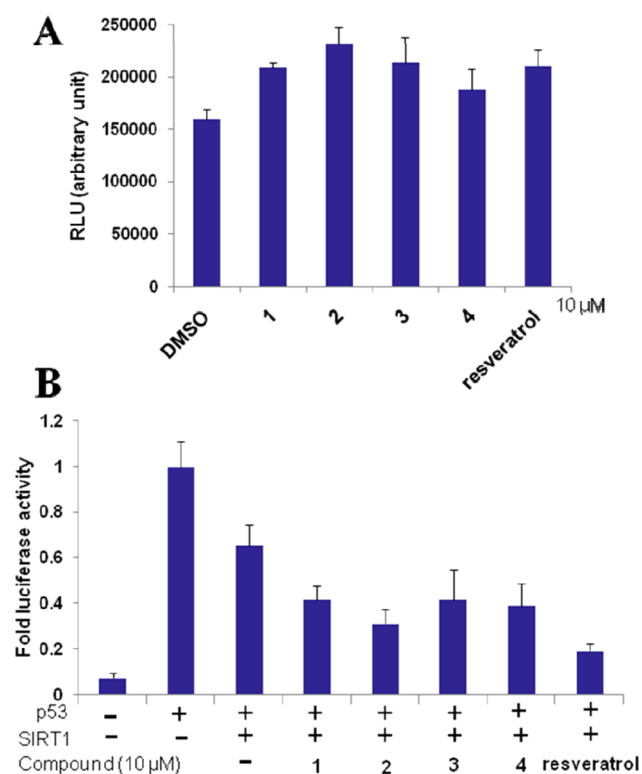
Figure 3. Compounds **1**−**4** enhance SIRT1 deacetylation activity in vitro and in vivo. (A) Effects of **1**−**4** on SIRT1 enzymatic activity with p53 peptide substrate determined by a bioluminescence assay. SIRT1 reactions showed an increase in the deacetylated peptide product and a decrease in the acetylated peptide substrate. (B) Effects of **1**−**4** on the transcription activity of p53 in HEK293 cells. The cells were transfected with p53-luc, myc-p53, flag-SIRT1, and β-gal vectors. After 1 day, cells were treated with the compounds and resveratrol for 12 h. Data are presented as means ± standard deviation of triplicate experiments.

the SIRT1 reaction using a NAD/NADH assay. As shown in Figure 4A and B, the SIRT1 deacetylation activity was increased in a dose-dependent manner in both in vitro and in vivo assays. Interestingly, compounds **1**−**4** also increased the NAD-to-NADH ratio by more than 50% (Figure 4C), further supporting the catalytic activity of SIRT1 in vivo.[28,29]

In recent years, SIRT1 has become an interesting and promising target in terms of its effects on longevity, metabolism, and other aging-related disorders. While several small-molecule activators of SIRT1 have been identified by utilizing a commercially available deacetylase activity assay, almost all of them are synthetic compounds.[5,6,30−32] Howitz and colleagues have identified some compounds of natural origin, such as resveratrol, fisetin, and butein, but these compounds have a low bioavailability.[33] Moreover, this particular method for the determination of SIRT1 activity is controversial due to its dependence on the use of a specific fluorophore substrate.[12,13] Other previous researchers have reported synthesized compounds with SIRT1 stimulatory activity, including quinoxalines, SRT derivatives, oxazolo[4,5-b]pyridines, dihydropyridines, and imidazol[1,2-b]-thiazoles.[5,6,30−32] The present study shows that the terpenylated coumarins **1**−**4** stimulate SIRT1 deacetylating activity, accompanied by SIRT1 binding to enhance SIRT1-mediated deacetylation of p53 in vitro and in vivo. This is the first report showing that terpenylated coumarins increase SIRT1 deacety-

lation. Thus, further investigation and optimization of these derivatives may enable the design of new agents in the treatment of metabolic and age-related diseases.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined on a Rudolph Autopol IV polarimeter using a 100 mm glass microcell. UV spectra were recorded in MeOH on a JASCO V-550 UV/vis spectrometer with a 0.5 nm resolution, and IR spectra (KBr) were obtained using a Nicolet 6700 FT-IR (Thermo Electron Corp.). NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer with TMS as the internal standard at the Korea Basic Science Institute (KBSI, Gwangju Center, Korea). EIMS and HREIMS data were recorded on a Micromass QTOF2 (Micromass, Wythenshawe, UK) mass spectrometer. Silica gel (Merck, 63−200 μm particle size), RP-18 (Merck, 40−63 μm particle size), and Sephadex LH-20 were used for column chromatography. TLC was carried out with silica gel 60 F_{254} and RP-18 F_{254} plates. HPLC was carried out using a Gilson system with a UV detector and Optima Pak C_{18} column (10 × 250 mm, 10 μm particle size, RS Tech, Korea). All solvents used for extraction and isolation were of analytical grade.

Plant Material. The stem bark of *A. altissima* was purchased from Kangwon Herbal Medicine Company (Kangwon-do, Republic of Korea) in November 2009 and botanically identified by Prof. Y. H. Moon. A voucher specimen (CU2009-18) has been deposited at the Herbarium of Chosun University, Gwangju, Republic of Korea.

Extraction and Isolation. The dried stem bark of *A. altissima* (3.0 kg) was extracted with MeOH (10 L × 2 times) at room temperature for one week. The combined MeOH extracts were concentrated to yield a dry residue (190.0 g). This crude extract was then suspended in H_2O (2 L) and partitioned successively with *n*-hexane (3 × 1.5 L), EtOAc (3 × 1.5 L), and *n*-BuOH (3 × 1.5 L). The EtOAc fraction (83.0 g), which exhibited strong SIRT1 activity, was chromatographed over a silica gel column (10 × 30 cm; 63−200 μm particle size) and eluted with gradient mixtures of *n*-hexane−acetone (9:1, 8:2, ..., 1:9, each 2.5 L) to yield seven pooled fractions (F1: 10.8 g; F2: 6.5 g; F3: 7.5 g; F4: 4.3 g; F5: 5.8 g; F6: 11.6 g; F7: 14.5 g). Fraction F4 was applied to an RP-18 column (7 × 30 cm; 40−63 μm particle size) and eluted with a stepwise gradient of MeOH−H_2O (2:1 to 10:1) to afford five subfractions (F4.1−F4.5). F4.4 (110 mg) was purified by HPLC [Optima Pak C_{18} column (10 × 250 mm, 10 μm particle size, RS Tech, Korea); mobile phase MeOH in H_2O containing 0.1% HCO_2H (0−70 min: 74% MeOH, 70−75 min: 74−100% MeOH, 75−85 min: 100% MeOH); flow rate 2 mL/min; UV detection at 205 and 254 nm] to yield compound **1** (t_R = 55 min, 22.0 mg). Fraction F5 was chromatographed over a Sephadex LH-20 column (7 × 30 cm) using MeOH as the eluting solvent to give four subfractions (F5.1−F5.4). F5.2 (2.6 g) was further fractionated into five subfractions (F5.2.1−F5.2.5), using a RP-18 column (5 × 30 cm; 40−63 μm particle size), with a stepwise gradient of MeOH−H_2O (1:1 to 10:1). Subfraction F5.2.1 (130 mg) was further purified by HPLC (0−45 min: 50% MeOH, 45−50 min: 50−100% MeOH, 50−60 min: 100% MeOH) to yield compound **2** (t_R = 42 min, 6.5 mg). Finally, compounds **3** (t_R = 54 min, 6.0 mg) and **4** (t_R = 60 min, 8.0 mg) were purified by HPLC (0−65 min: 57% MeOH, 65−70 min: 57−100% MeOH, 70−80 min: 100% MeOH) from subfraction F5.2.3 (220 mg).

Altissimacoumarin C (1): colorless oil; $[\alpha]_D^{25}$ +17 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.32), 225 (4.13), 293 (3.59), 335 (3.18) nm; IR (KBr) ν_{max} 3430, 2928, 1727, 1564, 1460, 1269, 1086 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; EIMS m/z 392 ([M]$^+$, 9), 374 (7), 265 (71), 222 (100), 207 (69), 176 (38), 109 (50); HREIMS *m/z* 392.1837 [M]$^+$ (calcd for $C_{21}H_{28}O_7$, 392.1835).

Altissimacoumarin D (2): colorless oil; $[\alpha]_D^{25}$ 0.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.27), 230 (3.74), 295 (3.09), 340 (3.14) nm; IR (KBr) ν_{max} 1727, 1563, 1456, 1290, 1122 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; EIMS *m/z* 358 ([M]$^+$, 1), 222 (100), 207 (20), 179 (16), 123 (15); HRFABMS *m/z* 359.1848 [M + H]$^+$ (calcd for $C_{21}H_{27}O_5$, 359.1858).
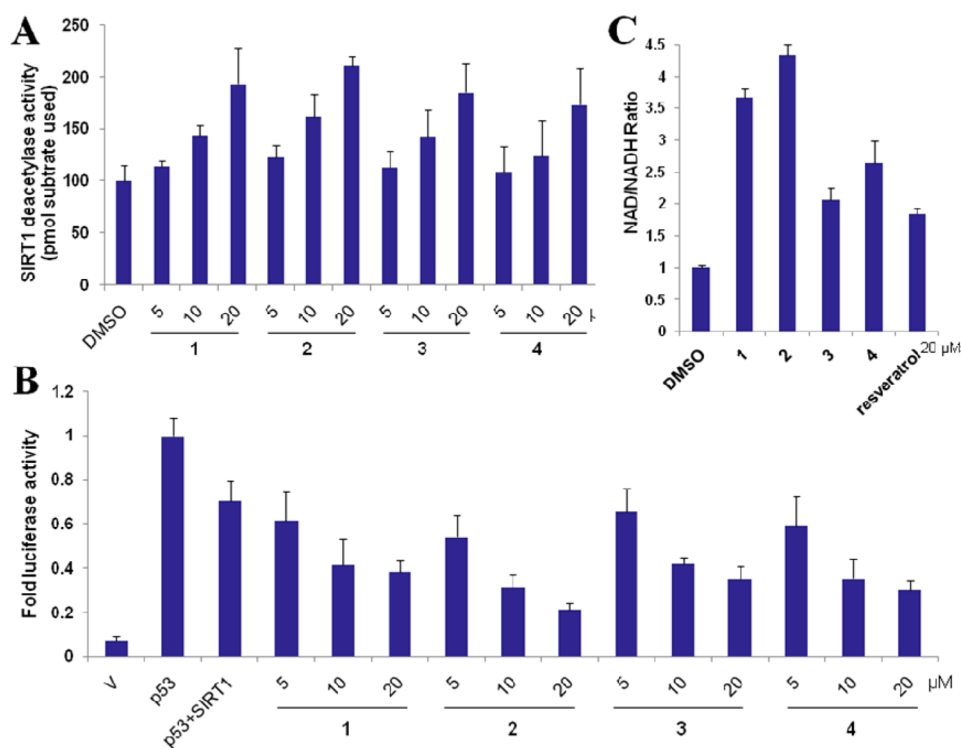
Figure 4. Compounds **1**−**4** increased the intracellular NAD-to-NADH ratio in HEK293 cells and the SIRT1 deacetylation activity in a dose-dependent manner. (A, B) Compounds **1**−**4** stimulate SIRT1 enzymatic activity with p53 peptide substrate, as determined by a NAD$^+$/NADH assay, and decrease the transcriptional activity of p53 in a dose-dependent manner. The cells were transfected with p53-luc, myc-p53, and flag-SIRT1 plasmid and then treated with compounds **1**−**4** (5, 10, 20 μM). A luciferase reporter assay was performed 12 h later. Each bar shows three independent experiments. (C) Intracellular NAD-to-NADH ratio in HEK293 cells treated with compounds **1**−**4** and resveratrol (20 μM) for 12 h. Results are means $\pm$ standard deviation of triplicate experiments.

Altissimacoumarin E (3): colorless oil; $[\alpha]_D^{25}$ +13 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.42), 225 (4.16), 295 (3.23), 335 (3.05) nm; IR (KBr) ν_{max} 3490, 2937, 1727, 1564, 1488, 1291, 1044 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; EIMS m/z 408.1786 ([M]$^+$, 4), 350 (4), 307 (9), 267 (3), 222 (100), 169 (17), 143 (92), 125 (32), 107 (23); HREIMS m/z 408.1786 [M]$^+$ (calcd for C$_{21}$H$_{28}$O$_8$, 408.1784).

Altissimacoumarin F (4): colorless oil; $[\alpha]_D^{25}$ +12 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.26), 225 (4.19), 291 (3.62), 335 (3.38) nm; IR (KBr) ν_{max} 3490, 2938, 1727, 1563, 1457, 1291, 1042 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; EIMS m/z 390 ([M]$^+$, 47), 375 (26), 309 (19), 265 (32), 222 (100), 207 (29), 194 (21), 151 (52); HREIMS m/z 390.1679 [M]$^+$ (calcd for C$_{21}$H$_{26}$O$_7$, 390.1679).

Preparation of (S)- and (R)-MTPA Ester Derivatives of 1, 3, and 4. Compound **1** (1.0 mg) was dissolved in 100 μL of pyridine and stirred at room temperature (rt) for 10 min. For the preparation of the (S)-MTPA ester (**1a**) of **1**, 10 μL of (R)-MTPA-Cl was added to the reaction vial. After being stirred at rt for 3 h, the mixture was evaporated to dryness and purified by RP-HPLC with 87% MeOH to give **1a** (0.8 mg). Using a similar procedure, treatment of **1** (1.0 mg) with (S)-MTPA-Cl afforded an (R)-MTPA ester (**1b**) (1.0 mg). The (S)-MTPA ester **3a** (0.6 mg) and (R)-MTPA ester **3b** (0.7 mg) were obtained from **3** (each 0.8 mg), while the (S)-MTPA ester **4a** (0.6 mg) and (R)-MTPA ester **4b** (0.9 mg) were obtained from **4** (each 1.0 mg).

Cell Culture and Transfection. HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Welgene) supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic at 37 °C and 5% CO$_2$. The cells were counted and plated in 24-well plates at a density of 10^5 cells/well in 1 mL of medium. After 24 h, the plasmid DNA was introduced into each well by using PEI transfection reagent (Polyscience, Inc.), according to the manufacturer's instructions. Briefly, the cells were incubated with 0.6 μg of DNA

and 3 μg of PEI reagent for 12 h at 37 °C. After transfection, the cultures were maintained in 10% FBS−DMEM medium. At 24 h post-transfection, the cells were treated with the test compounds or the positive control.

In Vitro SIRT1 Deacetylation in a Bioluminescence Assay. The SIRT1 enzyme assay was performed as previously reported.[26] Briefly, this was performed using acetylated p53 (Ac-p53) [HLKSKKGQSTSRHKK(Ac)LMFK] peptide, NAD$^+$, and SIRT1 in the absence or presence of the compound in the SIRT1 reaction buffer (100 mM Tris/Cl pH 8.0, 20 mM NaCl, 4 mM DTT, 100 μg/mL BSA, 0.003% Brij-35). The assay buffer and the enzyme−compound mixture initially were incubated for 10 min. The enzyme reaction was initiated by the addition of the Ac-p53 peptide and NAD$^+$ and incubated for 2 h. The reaction was quenched by the addition of 10 μL of LDH reagent (5 mM nicotinamide, 10 mM lactic acid, 62.5 U/mL LDH, 600 μM glycine, 500 μM hydrazine). After 1 h of incubation, 4 μL of the SIRT1/LDH mixture was added to 50 μL of a luminescence reaction mixture [50 mM Hepes, pH 7.5, 1 mM DTT, 1.5 mM EDTA, 100 μg/mL BSA, 10 μg/mL luciferase, 0.05 U/mL NAD(P)H:FMN-oxidoreductase, 500 μM decanal, 5 μM FMN]. Finally, luminescence in the reaction was measured within 15 min using a SpectraMax luminescence system (Molecular Devices, Inc.). The positive control for each plate was a SIRT1 reaction in the presence of DMSO, and the negative control was a reaction mixture without enzyme.

In Vitro SIRT1 Deacetylation in a NAD/NADH Assay. The SIRT1 enzyme reaction was performed in a final volume of 25 μL per well in a 384-well microplate. A standard SIRT1 reaction solution contained 0.1 U/mL enzyme, 10 μM NAD$^+$, and 20 μM Ac-p53 peptide in the absence or presence of the compound in the SIRT1 assay buffer (25 mM Tris/Cl pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl$_2$, 1 mg/mL BSA). The assay buffer and the enzyme−compound mixture (10 μL) were incubated for 10 min at room temperature. The reaction was then initiated by the addition of 15 μL of a solution containing Ac-p53 peptide and NAD$^+$. After incubation

for 6 h at ambient temperature, the reaction was quenched by the addition of 25 μL of a NAD/NADH recycling enzyme mixture containing 1 mM nicotinamide. After 2 h of incubation, the absorbance increase in the reaction was measured using a VersaMax absorbance microplate reader (Molecular Devices, Inc.). The positive control for each plate was a SIRT1 reaction in the presence of DMSO, and the negative control was a reaction mixture without enzyme.

In Vivo SIRT1 Deacetylation in a Luciferase Reporter Assay. Cells were transiently transfected with the reporter plasmid, PG13-luc (wt p53 binding sites), in combination with the plasmid encoding myc-tagged p53 (myc-p53) and the plasmid encoding flag-tagged SIRT1 (flag SIRT1) with RSV-β-gal plasmid as an internal control. The total amount of transfected DNA was the same in each well. The luciferase activity was measured by adding 40 μL of luciferin into 60 μL of lysate using an analytical luminescence luminometer. Promoter activity was measured using a Dual-Luciferase assay kit (Promega, Madison, WI), measuring both luciferase and renilla luciferase with a luminometer (Promega). Cells were lysed and assayed for p53, myc-p53, and flag SIRT1 reporter activities, which were corrected for constitutive β-galactosidase luciferase expression. Normalized values were calculated by dividing the luciferase activity by the renila luciferase activity.

NAD⁺-to-NADH Ratio Assay. The NAD^+-to-NADH ratio was measured from whole-cell extracts of HEK293 cells using the Biovision NAD/NADH quantitation kit, performed according to the manufacturer's instructions.

Statistical Analysis. A statistical calculation was carried out using Microsoft Excel 2003. The results are expressed as the means ± SD of three to five independent experiments.

■ ASSOCIATED CONTENT

ⓢ Supporting Information
^1H and ^{13}C NMR, HSQC, and HMBC spectra of compounds **1−4**, along with the NMR data of (S)- and (R)-MTPA ester derivatives of compounds **1**, **3**, and **4**, are available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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■ REFERENCES

(1) Shaday, M.; Sinclair, D. *Biochem. J.* **2007**, *404*, 1−13.
(2) Sauve, A. A.; Wolberger, C.; Schramm, V. L.; Boeke, J. D. *Annu. Rev. Biochem.* **2006**, *75*, 435−465.
(3) Vaziri, H.; Dessain, S. K.; Ng-Eaton, E.; Imai, S. I.; Frye, R. A.; Pandita, T. K.; Guarente, L.; Weinberg, R. A. *Cell* **2001**, *107*, 149−159.
(4) Yeung, F.; Hoberg, J. E.; Ramsey, C. S.; Keller, M. D.; Jones, D. R.; Frye, R. A.; Mayo, M. W. *EMBO J.* **2004**, *23*, 2369−2380.
(5) Vu, C. B.; Bernis, J. E.; Disch, J. S.; Ng, P. Y.; Nunes, J. J.; Milne, J. C.; Carney, D. P.; Lynch, A. V.; Smith, J. J.; Lavu, S.; Lambert, P. D.; Gagne, D. J.; Jirousek, M. R.; Schenk, S.; Olefsky, J. M.; Perni, R. B. *J. Med. Chem.* **2009**, *52*, 1275−1283.
(6) Bernis, J. E.; Vu, C. B.; Xie, R.; Nunes, J. J.; Ng, P. Y.; Disch, J. S.; Milne, J. C.; Carney, D. P.; Lynch, A. V.; Jin, L.; Smith, J. J.; Lavu, S.; Iffland, A.; Jirousek, M. R.; Perni, R. B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2350−2353.
(7) Yang, H.; Baur, J. A.; Chen, A.; Miller, C.; Adams, J. K.; Kisielewski, A.; Howitz, K. T.; Zipkin, R. E.; Sinclair, D. A. *Aging Cell* **2007**, *6*, 35−43.
(8) Milne, J. C.; Lambert, P. D.; Schenk, S.; Carney, D. P.; Smith, J. J.; Gagne, D. J.; Jin, L.; Boss, O.; Perni, R. B.; Vu, C. B.; Bernis, J. E.; Xie, R.; Disch, J. S.; Ng, P. Y.; Nunes, J. J.; Lynch, A. V.; Yang, H.; Galonek, H.; Israelian, K.; Choy, W.; Iffland, A.; Lavu, S.; Medvedik, O.; Sinclair, D. A.; Olefsky, J. M.; Jirousek, M. R.; Elliott, P. J.; Westphal, C. H. *Nature* **2007**, *450*, 712−716.
(9) Wood, J. G.; Rogina, B.; Lavu, S.; Howitz, K.; Helfand, S. L.; Tatar, M.; Sinclair, D. *Nature* **2004**, *430*, 686−689.
(10) Bauer, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. *Nature* **2006**, *444*, 337−342.
(11) Guerrero, R. F.; Garcia-Parrilla, M. C.; Puertas, B.; Cantos-Villar, E. *Nat. Prod. Commun.* **2009**, *4*, 635−658.
(12) Borra, M. T.; Smith, B. C.; Denu, J. M. *J. Biol. Chem.* **2005**, *17*, 17187−17195.
(13) Pacholec, M.; Chrunyk, B.; Cunningham, D.; Flynn, D.; Griffith, D.; Griffor, M.; Loulakis, P.; Pabst, B.; Qiu, X.; Stockman, B.; Thanabal, V.; Varghese, A.; Ward, J.; Withka, J.; Ahn, K. *J. Biol. Chem.* **2010**, *285*, 8340−8351.
(14) Kowarik, I. *J. Veg. Sci.* **1995**, *6*, 853−856.
(15) De Feo, V.; De Martino, L.; Quaranta, E.; Pizza, C. *J. Agric. Food Chem.* **2003**, *51*, 1177−1180.
(16) Jin, M. H.; Yook, J.; Lee, E.; Lin, C. X.; Quan, Z.; Son, K. H.; Bae, K. H.; Kim, H. P.; Kang, S. S.; Chang, H. W. *Biol. Pharm. Bull.* **2006**, *29*, 884−888.
(17) Okano, M.; Fukamiya, N.; Lee, K. H. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science Publishers: Amsterdam, 1990; Vol. 7, pp 369−404.
(18) Hwang, S. W.; Lee, J. R.; Lee, J.; Kwon, H. S.; Yang, M. S.; Park, K. H. *Heterocycles* **2005**, *65*, 1963−1966.
(19) Banthorpe, D. V.; Brown, G. D. *Phytochemistry* **1989**, *28*, 3003−3007.
(20) Ito, C.; Itoigawa, M.; Onoda, S.; Hosokawa, A.; Ruangrungsi, N.; Okuda, T.; Tokuda, H.; Nishino, H.; Furukawa, H. *Phytochemistry* **2005**, *66*, 567−572.
(21) Xin, Z. Q.; Lu, J. J.; Ke, C. Q.; Hu, C. X.; Lin, L. P.; Ye, Y. *Chem. Pharm. Bull.* **2008**, *56*, 827−830.
(22) Phoopichayanun, C.; Phuwapraisirisan, P.; Tip-Pyang, S.; Jongaramruong, J. *Nat. Prod. Res.* **2008**, *22*, 1297−1303.
(23) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17−117.
(24) Talapatra, S. K.; Chaudhuri, M. K.; Talapatra, B. *Phytochemistry* **1973**, *12*, 236−237.
(25) Langley, E.; Pearson, M.; Faretta, M.; Bauer, U. M.; Frye, R. A.; Minucci, S.; Pelicci, P. G.; Kouzarides, T. *EMBO J.* **2002**, *21*, 2383−2396.
(26) Kim, E. J.; Kho, J. H.; Kang, M. R.; Um, S. J. *Mol. Cell* **2007**, *28*, 277−290.
(27) Liu, Y.; Gerber, R.; Wu, J.; Tsuruda, T.; McCarter, J. D. *Anal. Biochem.* **2008**, *378*, 53−59.
(28) Um, J. H.; Park, S. J.; Kang, H.; Yang, S.; Foretz, M.; McBurney, M. W.; Kim, M. K.; Viollet, B.; Chung, J. H. *Diabetes* **2010**, *59*, 554−563.
(29) Houtkooper, R. H.; Canto, C.; Wanders, R. J.; Auwerx, J. *Endocr. Rev.* **2009**, *31*, 194−223.
(30) Smith, J. J.; Kenney, R. D.; Gagne, D. J.; Frushour, B. P.; Ladd, W.; Galonek, H. L.; Israelian, K.; Song, J.; Razvadauskaite, G.; Lynch, A. V.; Carney, D. P.; Johnson, R. J.; Lavu, S.; Iffland, A.; Elliott, P. J.;

Lambert, P. D.; Elliston, K. O.; Jirousek, M. R.; Milne, J. C.; Boss, O. *BMC Syst. Biol.* **2009**, *3*, 31−44.

(31) Yamazaki, Y.; Usui, I.; Kanatani, Y.; Matsuya, Y.; Tsuneyama, K.; Fujisaka, S.; Bukhari, A.; Suzuki, H.; Senda, S.; Imanishi, S.; Hirata, K.; Ishiki, M.; Hayashi, R.; Urakaze, M.; Nemoto, H.; Kobayashi, M.; Tobe, K. *Am. J. Physiol. Endocrinol. Metab.* **2009**, *297*, 1179−1186.

(32) Mai, A.; Valente, S.; Meade, S.; Carafa, V.; Tardugno, M.; Nebbioso, A.; Galmozzi, A.; Mitro, N.; De Fabiani, E.; Altucci, L.; Kazantsev, A. *J. Med. Chem.* **2009**, *52*, 5496−5504.

(33) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. *Nature* **2003**, *425*, 191−196.