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Chalcones from *Angelica keiskei*: Evaluation of Their Heat Shock Protein Inducing Activities

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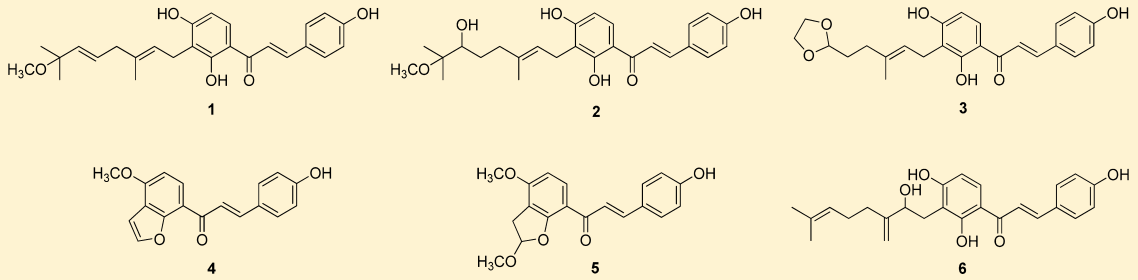
Supporting Information



ABSTRACT: Five new chalcones, 4,2′,4′-trihydroxy-3′-[(2*E*,5*E*)-7-methoxy-3,7-dimethyl-2,5-octadienyl]chalcone (**1**), (±)-4,2′,4′-trihydroxy-3′-[(2*E*)-6-hydroxy-7-methoxy-3,7-dimethyl-2-octenyl]chalcone (**2**), 4,2′,4′-trihydroxy-3′-[(2*E*)-3-methyl-5-(1,3-dioxolan-2-yl)-2-pentenyl]chalcone (**3**), 2′,3′-furano-4-hydroxy-4′-methoxychalcone (**4**), and (±)-4-hydroxy-2′,3′-(2,3-dihydro-2-methoxyfurano)-4′-methoxychalcone (**5**), were isolated from the aerial parts of *Angelica keiskei* Koidzumi together with eight known chalcones, **6**−**13**, which were identified as (±)-4,2′,4′-trihydroxy-3′-[(6*E*)-2-hydroxy-7-methyl-3-methylene-6-octenyl]chalcone (**6**), xanthoangelol (**7**), xanthoangelol F (**8**), xanthoangelol G (**9**), 4-hydroxyderricin (**10**), xanthoangelol D (**11**), xanthoangelol E (**12**), and xanthoangelol H (**13**), respectively. Chalcones **1**−**13** were evaluated for their promoter activity on heat shock protein 25 (*hsp25*, murine form of human *hsp27*). Compounds **1** and **6** activated the *hsp25* promoter by 21.9- and 29.2-fold of untreated control at 10 μM, respectively. Further protein expression patterns of heat shock factor 1 (HSF1), HSP70, and HSP27 by **1** and **6** were examined. Compound **6** increased the expression of HSF1, HSP70, and HSP27 by 4.3-, 1.5-, and 4.6-fold of untreated control, respectively, without any significant cellular cytotoxicities, whereas **1** did not induce any expression of these proteins. As a result, **6** seems to be a prospective HSP inducer.

Angelica keiskei Koidzumi (Umbelliferae), an edible plant, is well known as a health food in Korea. Its Korean name is "Shin-Sun Cho" or "Myeong-Il Yeob", and the Japanese refer to it as "Ashitaba". Previous phytochemical studies on *A. keiskei* reported the presence of chalcones,[1−9] flavanones,[2,4] coumarins,[2,4,9] acetylenes,[2,4] and a sesquiterpenoid[10] with biological effects such as antioxidant,[1,4] antitumor-preventive,[2,9] antiobesity,[3] antiviral,[5] anti-inflammatory,[6] and antihypertensive activities.[7] In particular, two major chalcones, xanthoangelol and 4-hydroxyderricin, have been proposed as effective antitumor agents by inducing apoptosis in cancer cells[11−13] and inhibiting tumor-induced angiogenesis.[14,15] The administration of these two chalcones suppressed metastasis and prolonged the survival time after surgically removing implanted tumors in mice.[14,15] The green juice of *A. keiskei* protects against lymphocytic DNA damage caused by smoking, which is relevant to reducing the risk of cancer,[16] and is, therefore, recommended as a source of green juice for the chemoprevention and convalescence of cancer diseases.

Heat shock proteins (HSPs) are a group of ubiquitous proteins, induced by various stress conditions such as temperature, hypoxia, heat, ischemia, heavy metals, and ionizing radiation.[17−19] Previous biological studies on HSPs have suggested their protective effects on damaged cells.[20−24] Chemotherapy and irradiation used as cancer treatments cause severe cytotoxic side effects on normal cells, which have led to many efforts to reduce these toxicities.[25−27] Our ongoing project on HSPs is aimed at discovering HSP-inducible agents from natural products, suggesting them as prospective agents to protect normal cells from damage caused by cancer treatment.[28,29] The chalcones isolated from *A. keiskei* were assessed for their effects on HSP expression. In a recent study, the known 3-methyl-4′-hydroxychalcone induced expression of HSP70 and showed protective effects on gastric ulcer disease caused by indomethacin, a nonsteroidal anti-inflammatory drug,[30] which motivated a study involving the HSP modulating activity of chalcones. Five new compounds (**1**−**5**) were isolated from the EtOAc extract of the aerial parts of *A. keiskei*, together with eight known compounds (**6**−**13**). The known compounds were identified as (±)-4,2′,4′-trihydroxy-3′-[(6*E*)-2-hydroxy-7-methyl-3-methylene-6-octenyl]chalcone (**6**),[31] xanthoangelol (**7**),[9] xanthoangelol F (**8**),[8] xanthoangelol G (**9**),[8] 4-

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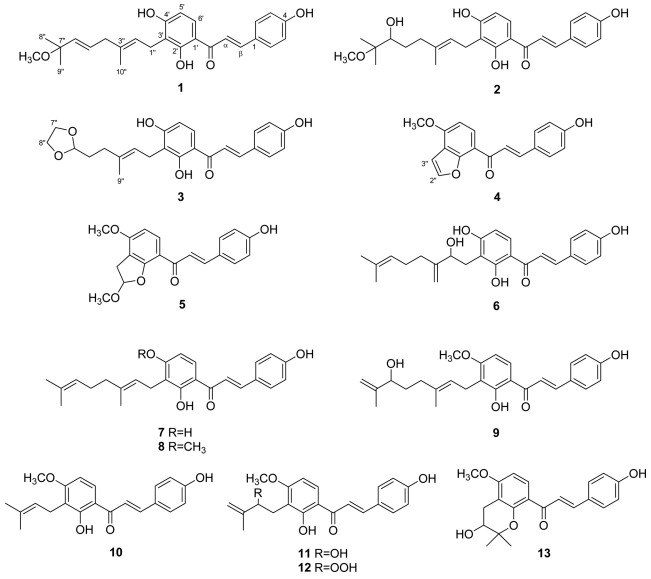
hydroxyderricin (**10**),[32] xanthoangelol D (**11**),[33] xanthoangelol E (**12**),[33] and xanthoangelol H (**13**).[8] Herein, we report the isolation and structure elucidation of **1**−**5** along with the evaluation of the effects of chalcones **1**−**13** on HSP expression.



RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow, amorphous powder with a molecular formula of $C_{26}H_{30}O_5$, based on the ^{13}C NMR data and an $[M + Na]^+$ ion at m/z 445.1986 (calcd 445.1985) in the HRESIMS. The ^1H and ^{13}C NMR spectra of **1** showed resonances at δ_H 6.42 (1H, d, J = 8.8 Hz)/δ_C 107.7 (C-5′), 7.73 (1H, d, J = 8.8 Hz)/129.2 (C-6′), 6.88 (2H, d, J = 8.4 Hz)/116.0 (C-3 and C-5), 7.57 (2H, d, J = 8.4 Hz)/130.6 (C-2 and C-6), 7.46 (1H, d, J = 15.6 Hz)/118.1 (C-α), 7.84 (1H, d, J = 15.6 Hz)/144.0 (C-β), and δ_C 192.2 (C═O), indicating the presence of a chalcone skeleton.[1,2,31,34] One hydrogen-bonded hydroxy group resonated at δ_H 13.86 (1H, s), and a methoxy functionality was observed at δ_H 3.14 (3H, s)/δ_C 74.9 (OCH_3-7″). The ^1H NMR resonances at δ_H 1.26 (6H, s, H_3-8″ and H_3-9″), 1.82 (3H, d, J = 1.2 Hz, H_3-10″), 2.77 (2H, d, J = 6.1 Hz, H-4″), 3.50 (2H, d, J = 7.0 Hz, H_2-1″), 5.35 (1H, tq, J = 7.0, 1.2 Hz, H-2″), 5.54 (1H, dt, J = 15.7, 6.1 Hz, H-5″), and 5.64 (1H, d, J = 15.7 Hz, H-6″) (Table 1) indicated a modified geranyl group in **1**.[34] The three-bond HMBC correlation of OCH_3-7″/C-7″ confirmed the C-7″ location of the methoxy group on the modified geranyl group. Thus, the side chain of **1** was defined as a 7″-methoxy-3″,7″-dimethyl-2″,5″-octadienyl moiety. The HMBC connectivities of H-1″/C-2′, C-3′, C-4′ showed that C-1″ of the 7″-methoxy-3″,7″-dimethyl-2″,5″-octadienyl moiety is attached to C-3′.[34] The hydrogen-bonded hydroxy group at δ_H 13.86 was assigned to C-2′ according to the HMBC correlations of O*H*-2′/C-1′, C-2′, C-3′ (Figure 1). The data of **1** are similar to those of xanthokeismin A,[1,34] except for the presence of the methoxy group at C-7″. Thus, the structure of **1** was defined as the new 4,2′,4′-trihydroxy-3′-[(2*E*,5*E*)-7-methoxy-3,7-dimethyl-2,5-octadienyl]chalcone.

Compound **2** was isolated as a yellow, amorphous powder with a molecular formula of $C_{26}H_{32}O_6$, based on the ^{13}C NMR

Table 1. ^{13}C (100 MHz) and ^1H (400 MHz) NMR Spectroscopic Data for Compounds 1−3[a]

	1[b]		2[b]		3[c]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
α	118.1, CH	7.46, d (15.6)	117.9, CH	7.43, d (15.4)	118.5, CH	7.76, d (15.6)
β	144.0, CH	7.84, d (15.6)	144.1, CH	7.81, d (15.4)	145.0, CH	7.84, br d (15.6)
C═O	192.2, C		192.2, C		193.1, C	
1	127.8, C		127.6, C		127.7, C	
2, 6	130.6, CH	7.57, d (8.4)	130.6, CH	7.52, d (8.4)	131.8, CH	7.74, d (8.6)
3, 5	116.0, CH	6.88, d (8.4)	116.0, CH	6.86, d (8.4)	116.8, CH	6.93, d (8.6)
4	157.9, C		158.2, C		161.0, C	
1′	114.07,[d] C		114.0, C		114.5, C	
2′	163.9, C		163.9, C		165.2, C	
3′	114.12,[d] C		114.3, C		116.1, C	
4′	161.4, C		161.6, C		162.7, C	
5′	107.7, CH	6.42, d (8.8)	107.8, CH	6.39, d (8.8)	108.0, CH	6.53, br d (8.8)
6′	129.2, CH	7.73, d (8.8)	129.2, CH	7.69, d (8.8)	130.3, CH	7.98, d (8.8)
1″	21.8, CH_2	3.50, d (7.0)	21.7, CH_2	3.47[e]	22.2, CH_2	3.39, d (7.4)
2″	122.1, CH	5.35, tq (7.0, 1.2)	121.6, CH	5.32, br t (6.6)	123.2, CH	5.33, tq (7.4, 1.3)
3″	137.7, C		138.7, C		135.0, C	
4″	42.7, CH_2	2.77, d (6.1)	37.0, CH_2	2.13, m, 2.34, ddd (13.9, 8.7, 5.5)	34.8, CH_2	2.06[e]
5″	127.7, CH	5.54, dt (15.7, 6.1)	29.3, CH_2	1.48, 1.56, m	33.4, CH_2	1.66, ddd (9.6, 6.4, 4.9)
6″	137.3, CH	5.64, d (15.7)	76.5, CH	3.44, br d (10.2)	104.7, CH	4.76, t (4.9)
7″	74.9, C		77.5, C		65.4, CH_2	3.75, 3.87, m
8″	25.8, CH_3	1.26, s	20.8, CH_3	1.12, s	65.4, CH_2	3.75, 3.87, m
9″	25.8, CH_3	1.26, s	18.9, CH_3	1.10, s	16.3, CH_3	1.81, d (1.3)
10″	16.3, CH_3	1.82, d (1.2)	16.3, CH_3	1.83, br s		
OH-2′		13.86, s		13.85, s		14.00, s
OCH_3-7″	50.3, CH_3	3.14, s	49.1, CH_3	3.21, s		

[a]TMS was used as internal standard. [b]Data were measured in $CDCl_3$. [c]Data were measured in acetone-d_6. [d]Data are exchangeable in the same column. [e]Multiplicity patterns were unclear due to signal overlapping; chemical shifts were estimated by HSQC NMR spectra.

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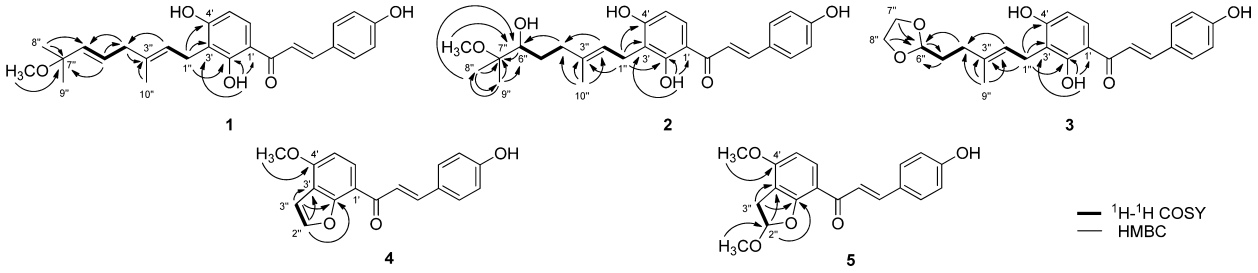

Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of **1**−**5**.

data and an $[M + H]^+$ ion at m/z 441.2278 (calcd 441.2272) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the resonances of the geranyl functionality. A methylene group was observed at δ_H 1.48 (1H, m) and 1.56 (1H, m)/δ_C 29.3 (C-5″), and an oxygenated methine group was at δ_H 3.44 (1H, br d, J = 10.2 Hz)/δ_C 76.5 (C-6″) instead of the $\Delta^{5'',6''}$ olefinic group in **1**. The ^{1}H NMR resonances of the C-4″ methylene protons in **2** showed its diastereotopic characteristics at δ_H 2.13 (1H, m, H-4″a) and 2.34 (1H, ddd, J = 13.9, 8.7, 5.5 Hz, H-4″b), compared to its magnetic equivalence at δ_H 2.77 (2H, d, J = 6.1 Hz) in **1** (Table 1). The diastereotopicity was also observed for the methylene protons at C-5″ (δ_H 1.48 and 1.56). The data were similar to those of 4,2′,4′-trihydroxy-3′-[(2*E*)-3,7-dimethyl-6,7-dihydroxy-2-octenyl]chalcone[3] except for the presence of a methoxy functionality at C-7″ in **2**. Compound **2** had a specific rotation of $[\alpha]^{25}_D$ ±0 (*c* 0.1, MeOH), indicative of its racemic nature. Therefore, the structure of **2** was defined as the new (±)-4,2′4′-trihydroxy-3′-[(2*E*)-6-hydroxy-7-methoxy-3,7-dimethyl-2-octenyl]chalcone.

Compound **3** was obtained as a yellow, amorphous powder with a molecular formula of $C_{24}H_{26}O_6$, based on the ^{13}C NMR data and an $[M + H]^+$ ion at m/z 411.1805 (calcd 411.1802) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra exhibited similar chemical shifts to those of **1** and **2** except for the modified geranyl functionality. Two sets of methylene protons resonated at δ_H 3.75 (2H, m) and 3.87 (2H, m)/δ_C 65.4 (C-7″ and C-8″) together with a methine proton at δ_H 4.76 (1H, t, J = 4.9 Hz)/δ_C 104.7 (C-6″), indicating the presence of a 2-substituted 1,3-dioxolane group (Table 1). The NMR data of **3** were also compared with those of xanthokeistal A, which had two methoxy groups at C-6″[5] instead of the two oxygenated methylenes in **3**. Thus, the structure of **3** was defined as the new 4,2′,4′-trihydroxy-3′-[(2*E*)-3-methyl-5-(1,3-dioxolan-2-yl)-2-pentenyl]chalcone.

Compound **4** was obtained as a yellow, amorphous powder with a molecular formula of $C_{18}H_{14}O_4$, based on the ^{13}C NMR data and an $[M + H]^+$ ion at m/z 295.0965 (calcd 295.0965) in the HRESIMS. The ^{1}H and ^{13}C NMR data of **4** are reminiscent of the presence of a chalcone skeleton at δ_H 6.93 (1H, d, J = 8.8 Hz)/δ_C 105.1 (C-5′), 8.02 (1H, d, J = 8.8 Hz)/129.8 (C-6′), 6.86 (2H, d, J = 8.4 Hz)/117.0 (C-3 and C-5), 7.62 (2H, d, J = 8.4 Hz)/131.8 (C-2 and C-6), 7.80 (1H, d, J = 15.4 Hz)/145.7 (C-β), 7.90 (1H, d, J = 15.4 Hz)/122.7 (C-α), and δ_C 188.9 (C═O). In addition, two olefinic methine groups resonated at δ_H 7.86 (1H, d, J = 2.4 Hz)/δ_C 145.8 (C-2″) and 7.00 (1H, d, J = 2.4 Hz)/δ_C 105.0 (C-3″), suggesting the presence of a furan ring.[35] An aromatic methoxy group resonated at δ_H 4.04 (3H, s)/δ_C 56.6 (Table 2).[28] The HMBC correlations of H-2″/C-2′, C-3′ and H-3″/C-2′, C-3′ confirmed the presence of a furan ring, which was fused to the chalcone skeleton at C-2′ and C-3′.

Table 2. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 4 and 5[a]

	4[b]		5[c]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
α	122.7, CH	7.90, d (15.4)	123.9, CH	7.85, d (15.4)
β	145.7, CH	7.80, d (15.4)	143.0, CH	7.69, d (15.4)
C═O	188.9, C		186.5, C	
1	128.0, C		128.1, C	
2, 6	131.8, CH	7.62, d (8.4)	131.0, CH	7.61, d (8.4)
3, 5	117.0, CH	6.86, d (8.4)	116.8, CH	6.92, d (8.4)
4	161.7, C		160.5, C	
1′	118.7, C		117.2, C	
2′	156.0,[d] C		159.9, C	
3′	119.8, C		113.1,[d] C	
4′	159.5, C		161.0, C	
5′	105.1,[e] CH	6.93, d (8.8)	105.6, CH	6.72, d (9.2)
6′	129.8, CH	8.02, d (8.8)	132.0, CH	7.84, d (9.2)
2″	145.8, CH	7.86, d (2.4)	109.6, CH	5.95, dd (6.7, 2.0)
3″	105.0,[e] CH	7.00, d (2.4)	34.0, CH_2	2.93, br dd (16.5, 2.0), 3.31, br dd (16.5, 6.7)
OCH_3-4′	56.6, CH_3	4.04, s	56.2, CH_3	3.94, s
OCH_3-2″			56.3, CH_3	3.61, s

[a]TMS was used as internal standard. [b]Data were measured in methanol-d_4. [c]Data were measured in acetone-d_6. [d]Chemical shifts were estimated by HMBC NMR spectra. [e]Data are exchangeable in the same column.

The methoxy group was assigned at C-4′ on the basis of the HMBC connectivity between the methoxy protons and C-4′ (Figure 1). The NOE correlation of the methoxy protons with H-5′ provided further evidence for the positions of both the furan ring (C-2′ and C-3′) and the methoxy group (C-4′) in **4** (Figure 2). Thus, the structure of **4** was defined as the new 2′,3′-furano-4-hydroxy-4′-methoxychalcone.

Compound **5** was isolated as a yellow, amorphous powder with a molecular formula of $C_{19}H_{18}O_5$, based on the ^{13}C NMR data and an $[M + H]^+$ ion at m/z 327.1227 (calcd 327.1227) in the HRESIMS. Compound **5** displayed similar ^{1}H and ^{13}C NMR data to **4** except for a methylene and a methoxymethine group replacing the $\Delta^{2'',3''}$ olefinic moiety of **4**. The methylene group resonated at δ_H 2.93 (1H, br dd, J = 16.5, 2.0 Hz) and 3.31 (1H, br dd, J = 16.5, 6.7 Hz)/δ_C 34.0 (C-3″), and the methoxymethine group was observed at δ_H 5.95 (dd, J = 6.7, 2.0 Hz)/δ_C 109.6 (C-2″) (Table 2), indicating the presence of a

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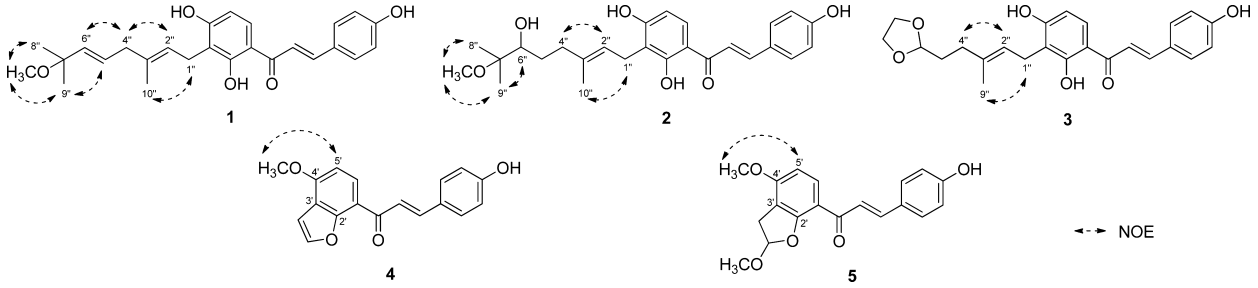

Figure 2. Key NOE correlations of **1**−**5**.

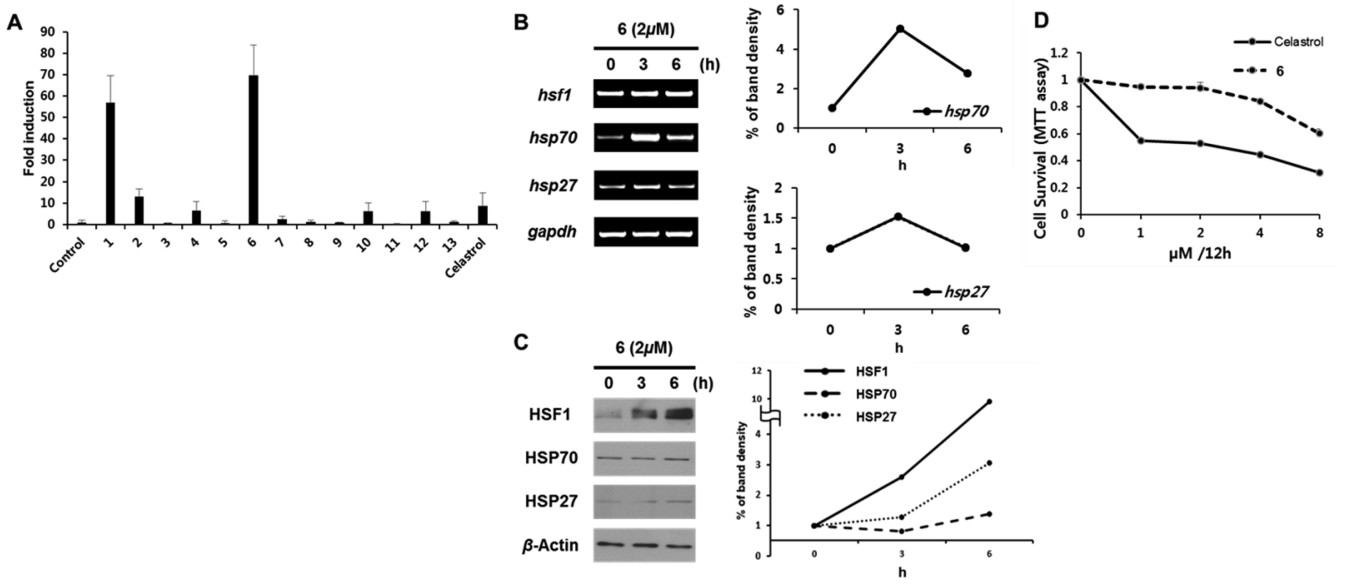

Figure 3. (A) Effects of **1**−**13** on *hsp25* promoter activity. NCI-H460 cells were treated with 10 μM of **1**−**13** for 5 h prior to a luciferase assay. Luciferase activity was expressed as the fold induction, normalized to the β-galactosidase expression. (B and C) Effects of **6** on mRNA and protein expression levels of HSF1, HSP70, and HSP27. L132 cells were treated with 2 μM **6** for the indicated periods (0, 3, 6 h) prior to mRNA or protein extraction. (B) RT-PCR was performed with HSF1, HSP70, and HSP27 primers, and mRNA band intensity was normalized to the loading control band (*gapdh*). (C) Western blot analysis was performed with anti-HSF1, anti-HSP27, anti-HSP70, and β-actin antibodies, and protein band intensity was normalized to the loading control band (β-actin). (D) Cytotoxic effect of **6** in L132 cells. Cell viability was measured using the MTT assay after treatment with the indicated concentrations (1, 2, 4, or 8 μM) for 12 h. Values were expressed as cell survival relative to no treatment control (DMSO).

substituted 2,3-dihydrofuran ring in **5**. The methoxy group resonated at δ_H 3.61 (3H, s)/δ_C 56.3. The 2,3-dihydrofuran ring was assigned to C-2′ and C-3′ according to the HMBC correlations of H-2″/C-2′, C-3′, H-3″a/C-2′, C-3′, and H-3″b/C-2′, C-3′. The methoxy group was located at C-2″ via the HMBC correlation between the methoxy protons and C-2″ (Figure 1). Compound **5** was isolated as a racemate, evidenced by its specific rotation value, $[\alpha]^{25}_D$ ±0 (*c* 0.1, MeOH). Therefore, the structure of **5** was defined as the new (±)-4-hydroxy-2′,3′-(2,3-dihydro-2-methoxyfurano)-4′-methoxychalcone.

To identify HSP-modulating activities, celastrol was used as an HSP inducer (positive control),[36] and a luciferase assay was performed. The luciferase assay was conducted at 5 h after treatment with 10 μM of **1**−**13** using human lung cancer NCI-H460 cells transfected with luciferase reporter construct for *hsp25* (murine form of human *hsp27*). Among the 13 compounds, **1** and **6** increased luciferase activity for the *hsp25* promoter by 21.9- and 29.2-fold of untreated control (Figure 3A). The protein expression patterns on HSF1, HSP70, and HSP27 were examined for **1** and **6**. Compound **6** (2 μM) increased protein expressions of HSF1 (4.3-fold), HSP70 (1.5-fold), and HSP27 (4.6-fold) at 6 h of treatment (Figure 3C). Increased expression of mRNA for HSP70 (5.0-fold) and HSP27 (1.5-fold) at 3 h was also observed (Figure 3B). Moreover, **6** exhibited lower cytotoxicity than celastrol (Figure 3D). Compound **1** did not elicit increased expression of HSF1, HSP70, and HSP27. The structural difference of **6** compared to the other compounds is the presence of a hydroxy group at C-2″ and an exomethylene functionality at C-3″ on the geranyl side chain. No significant structure activity relationship (SAR) can be established between these chalcones and their HSP-inducing activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a P-1010 polarimeter. UV spectra were recorded on a U-3000 spectrophotometer. 1D and 2D NMR spectra were recorded on a UNITY INOVA 400 MHz FT-NMR instrument with TMS as internal standard. Mass spectrometry was performed on a Waters ACQUITY UPLC system coupled to a Micromass Q-Tof Micro mass spectrometer and Agilent 6220 Accurate-Mass TOF LC/MS system. Silica gel (230−400 mesh, Merck, Germany), RP-18 (YMC gel ODS-

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A, 12 nm, S-150 μm, YMC Co., Japan), and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden) were used for column chromatography (CC). Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F_{254} (silica gel, 0.25 mm layer thickness, Merck, Germany) and RP-18 F_{254s} (Merck, Germany) plates, with visualization under UV light (254 and 365 nm) and 10% (v/v) sulfuric acid spray followed by heating (120 °C, 5 min). Preparative HPLC was conducted using an Acme 9000 system (Young Lin, Korea), equipped with a YMC-Pack Pro C_{18} column (5 μm, 250 mm × 20 mm i.d.).

Plant Material. The aerial parts of *A. keiskei* were purchased from the Chodamchae in Seoul, Korea, and identified by Professor Je-hyun Lee (College of Oriental Medicine, Dongguk University). A voucher specimen (no. EA327) has been deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation. The aerial parts of *A. keiskei* (8 kg) were extracted with MeOH (2 × 12 L) at room temperature overnight. The solvent was evaporated under reduced pressure to afford 2.5 kg of a concentrated MeOH extract. The crude extract was suspended in MeOH−H_2O (19:1, 4 L) and fractionated with hexanes (5 × 4 L). The concentrated MeOH−H_2O-soluble extract was suspended in distilled H_2O and partitioned with EtOAc (5 × 4 L). The EtOAc extract (142 g) was chromatographed over silica gel by elution with gradient mixtures of hexanes−EtOAc (99:1 → 1:1) and EtOAc−MeOH (1:0 → 0:1) to afford 16 fractions (F01−F16). Fraction F07 (7.6 g), eluted with hexanes−EtOAc (9:1), was subjected to silica gel CC, using a $CHCl_3$−MeOH gradient solvent system (199:1 → 9:1), to yield 12 subfractions (F07.01−F07.12). Subfraction F07.11 (4.2 g), eluted with $CHCl_3$−MeOH (199:1), was purified by RP-18 CC, using MeOH−H_2O (7:3, 4:1, 9:1) solvent mixtures, to yield **8** (500 mg), **10** (1.8 g), and **12** (3.8 mg). Fraction F11 (13.8 g), eluted with hexanes−EtOAc (22:3 → 4:1) from the first separation, was chromatographed over silica gel using gradient mixtures of $CHCl_3$−MeOH (199:1 → 4:1) to obtain 16 subfractions (F11.01−F11.16). Subfraction F11.12 (9.0 g), eluted with $CHCl_3$−MeOH (199:1), was subjected to RP-18 CC, eluted by a MeOH−H_2O gradient solvent system (2:3 → 9:1), to yield **3** (0.9 mg) and **7** (5.0 g) with 22 subfractions (F11.12.01−F11.12.22). Subfraction F11.12.08 (585 mg), eluted with MeOH−H_2O (1:1), was chromatographed over Sephadex LH-20 using MeOH 100% and then purified by separation over RP-18 CC with MeOH−H_2O (4:1), to yield **5** (1.0 mg). Subfraction F11.12.09 (135 mg), eluted with MeOH−H_2O (1:1), was chromatographed using Sephadex LH-20 with MeOH 100%, to yield **4** (1.0 mg). Subfraction F11.12.18 (1.2 g), eluted with MeOH−H_2O (7:3), was subjected to RP-18 CC (MeOH−H_2O, 4:1), providing five subfractions (F11.12.18.01−11.12.18.05). A part (20 mg) of subfraction F11.12.18.03 (74 mg) was purified by preparative HPLC with MeOH−H_2O (9:1, 2 mL/min) to afford **6** (13.9 mg, t_R 47.8 min). Fraction F13 (3.3 g), eluted with hexanes−EtOAc (1:1) from the first separation, was subjected to silica gel CC using a $CHCl_3$−acetone gradient solvent system (49:1 → 4:1) to yield 21 subfractions (F13.01−F13.21). Subfraction F13.13 (79 mg), eluted with $CHCl_3$−acetone (49:1 → 9:1), was purified by RP-18 CC using MeOH−H_2O (3:2, 7:3, 4:1) solvent mixtures to obtain **9** (6.5 mg) and **11** (17.8 mg). Subfraction F13.16 (169 mg), eluted with $CHCl_3$−acetone (9:1), was subjected to RP-18 CC eluted with gradient mixtures of MeOH−H_2O (1:1 → 4:1) to yield **13** (28.0 mg). Compound **1** (2.0 mg) was separated from subfraction F13.18 (64 mg), eluted with $CHCl_3$−acetone (4:1), by RP-18 CC using mixtures of MeOH−H_2O (3:2, 7:3, 4:1) as eluent. Subfraction F13.20 (528 mg), eluted with $CHCl_3$−acetone (4:1), was also applied on RP-18 CC using gradient mixtures of MeOH−H_2O (9:11 → 4:1) to afford **2** (8.8 mg).

Promoter Activity of *hsp25*. The promoter activities of *hsp25* were evaluated using luciferase reporter constructs, as described previously.[37] The human lung cancer NCI-H460 cells were plated at 1 × 10^5 cells in 12-well plates. After 24 h incubation, cells were treated with chemicals and harvested 5 h later. Luciferase activity was assayed from cell lysates, and the results were normalized to the β-galactosidase expression (Promega).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis. Total RNA was extracted using TRIzol (Invitrogen). cDNA was synthesized using the ReverTra Ace RT-PCR kit (Toyobo, Osaka, Japan). HSF1, HSP70, HSP27, and β-actin transcript levels were measured by RT-PCR (GenDEPOT, Barker, TX, USA). GAPDH was used as an internal control gene. The change in relative gene expression was normalized to GAPDH mRNA levels using the NIH ImageJ program.

Western Blot Analysis. The ability of **6** to modulate HSF1 and HSPs expression was evaluated by an established protocol.[38] L132 human lung fibroblast cells were treated with 2 μM **6** for 0, 3, and 6 h. β-Actin was used as an internal control. Proteins in lysates were separated by SDS-PAGE, electrotransferred to nitrocellulose membranes (GE Healthcare, Little Chalfont, UK), subsequently blotted with the specified antibodies, and visualized with the ECL detection system (Thermo Scientific, Rockford, IL, USA). Anti-HSF1, anti-HSP27, anti-HSP70, and β-actin antibodies were purchased from Santa Cruz Biotechnology (Danvers, MA, USA).

Cell Viability Assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT; Sigma-Aldrich) assay was used as an indirect measure of death, as described in previous literature.[27] Briefly, the medium was removed and replaced with MTT reagent (0.5 mg/mL) in PBS. The plates were incubated for 3 h at 37 °C, and 100 μL of DMSO was added to dissolve the formazan crystals. The absorbance was detected at a wavelength of 540 nm. A calibration curve was used to convert absorbance to number of cells. Cell viability was determined as a ratio relative to no treatment control (DMSO).

Statistical Analysis. All results represent the mean ± SD of three independent experiments. The statistical significance of differences between untreated control and each treated group was compared by analysis of a Student's *t* test.

*4,2′,4′-Trihydroxy-3′-[(2E,5E)-7-methoxy-3,7-dimethyl-2,5-octadienyl]chalcone (**1**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 368 (4.58) nm; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; NOESY correlations H-2 and H-6/H-β, H-α/H-6′, H_2-1″/H_3-10″, H_2-4″/H-2″, H-6″, and H_3-10″, H-5″/H_3-9″, H_3-8″/OCH_3-7″, H_3-9″/OCH_3-7″; HMBC correlations H-2 and H-6/C-4, C-β; H-3 and H-5/C-1, C-4; H-α/*C*═O, C-1; H-β/*C*═O, C-2, C-6; H-5′/C-1′, C-3′; H-6′/*C*═O, C-2′, C-4′; H_2-1″/C-2′, C-3′, C-4′, C-2″, C-3″; H-2″/C-1″, C-4″, C-10″; H_2-4″/C-2″, C-3″, C-5″, C-6″, C-10″; H-5″/C-4″, C-7″; H-6″/C-4″, C-8″, C-9″; H_3-8″/C-6″, C-7″; H_3-9″/C-6″, C-7″; H_3-10″/C-2″, C-3″, C-4″; O*H*-2′/C-1′, C-2′, C-3′; OCH_3-7″/C-7″; HRESIMS *m*/*z* 445.1986 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_5Na$, 445.1985).

*(±)-4,2′4′-Trihydroxy-3′-[(2E)-6-hydroxy-7-methoxy-3,7-dimethyl-2-octenyl]chalcone (**2**):* yellow, amorphous powder; $[\alpha]^{25}_D$ ±0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 369 (4.49) nm; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; NOESY correlations H-2 and H-6/H-β, H-α/H-6′, H_2-1″/H_3-10″, H-2″/H-4″a and H-4″b, H_3-8″/H-5″a, H-5″b, H-6″, and OCH_3-7″, H_3-9″/H-5″a, H-5″b, H-6″, and OCH_3-7″; HMBC correlations H-2 and H-6/C-4, C-β; H-3 and H-5/C-1, C-4; H-α/*C*═O, C-β, C-1; H-β/*C*═O, C-α, C-1, C-2, C-6; H-5′/C-1′, C-3′, C-4′; H-6′/*C*═O, C-1′, C-2′, C-4′; H_2-1″/C-2′, C-3′, C-4′, C-2″, C-3″; H-2″/C-1″, C-4″, C-10″; H-4″a/C-2″, C-3″, C-5″, C-6″; H-4″b/C-2″, C-3″, C-5″, C-6″, C-10″; H-5″a/C-3″, C-4″, C-6″; H-5″b/C-3″, C-4″, C-6″; H-6″/C-4″, C-5″, C-7″, C-8″, C-9″; H_3-8″/C-6″, C-7″, C-9″; H_3-9″/C-6″, C-7″, C-9″; H_3-10″/C-2″, C-3″, C-4″; O*H*-2′/C-1′, C-2′, C-3′; OCH_3-7″/C-7″; HRESIMS *m*/*z* 441.2278 $[M + H]^+$ (calcd for $C_{26}H_{33}O_6$, 441.2272).

*4,2′,4′-Trihydroxy-3′-[(2E)-3-methyl-5-(1,3-dioxolan-2-yl)-2-pentenyl]chalcone (**3**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 368 (3.91) nm; ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data, see Table 1; NOESY correlations H-α/H-6′, H_2-1″/H_3-9″, H_2-4″/H-2″; HMBC correlations H-2 and H-6/C-4, C-β; H-3 and H-5/C-1, C-4; H-α/*C*═O, C-β, C-1; H-β/*C*═O, C-α, C-2, C-6; H-6′/*C*═O, C-2′, C-4′; H_2-1″/C-2′, C-3′, C-4′, C-2″, C-3″; H-2″/C-1″, C-4″, C-9″; H_2-4″/C-2″, C-3″, C-5″, C-6″, C-9″; H-5″/C-3″, C-4″, C-6″; H-6″/C-5″; H-7″a and H-8″a/C-6″; H-7″b and H-8″b/C-6″; H_3-9″/C-2″, C-3″, C-4″; O*H*-2′/C-1′, C-2′, C-

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3′; HRESIMS m/z 411.1805 $[M + H]^+$ (calcd for $C_{24}H_{27}O_6$, 411.1802).

2′,3′-Furano-4-hydroxy-4′-methoxychalcone (4): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 359 (4.11), 243 (3.90) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 2; NOESY correlations H-2 and H-6/H-α and H-β, H-5′/OCH_3-4′; HMBC correlations H-2 and H-6/C-4, C-β; H-3 and H-5/C-1, C-4; H-α/C═O, C-β, C-1; H-β/ C═O, C-α, C-1, C-2, C-6; H-5′/C-1′, C-3′; H-6′/C═O, C-2′, C-4′; H-2″/C-2′, C-3′; H-3″/C-2′, C-3′, C-2″; OCH_3-4′/C-4′; HRESIMS m/z 295.0965 $[M + H]^+$ (calcd for $C_{18}H_{15}O_4$, 295.0965).

(±)-4-Hydroxy-2′,3′-(2,3-dihydro-2-methoxyfurano)-4′-methoxychalcone (5): yellow, amorphous powder; $[\alpha]^{25}_D$ ±0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 352 (4.05) nm; ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data, see Table 2; NOESY correlations H-2 and H-6/H-α, H-5′/OCH_3-4′; HMBC correlations H-2 and H-6/C-4, C-β; H-3 and H-5/C-1, C-4; H-α/C═O, C-β, C-1; H-β/C═O, C-α, C-1; H-5′/C-1′, C-3′; H-6′/C-2′, C-4′; H-2″/C-2′, C-3′; H-3″a/C-2′, C-3′, C-2″; H-3″b/C-2′, C-3′, C-2″; OCH_3-4′/C-4′; OCH_3-2″/C-2″; HRESIMS m/z 327.1227 $[M + H]^+$ (calcd for $C_{19}H_{19}O_5$, 327.1227).

(±)-4,2′,4′-Trihydroxy-3′-[(6E)-2-hydroxy-7-methyl-3-methylene-6-octenyl]chalcone (6): yellow, amorphous powder; $[\alpha]^{22}_D$ ±0 (c 0.1, MeOH); HRESIMS m/z 409.2012 $[M + H]^+$ (calcd for $C_{25}H_{29}O_5$, 409.2010); physical and spectroscopic data were comparable with literature values.[31]

Xanthoangelol (7): yellow, amorphous powder; HRESIMS m/z 393.2067 $[M + H]^+$ (calcd for $C_{25}H_{29}O_4$, 393.2060); physical and spectroscopic data were comparable with literature values.[9]

Xanthoangelol F (8): yellow, amorphous powder; HRESIMS m/z 407.2224 $[M + H]^+$ (calcd for $C_{26}H_{31}O_4$, 407.2217); physical and spectroscopic data were comparable with literature values.[8]

Xanthoangelol G (9): yellow, amorphous powder; $[\alpha]^{22}_D$ ±0 (c 0.1, MeOH); HRESIMS m/z 423.2169 $[M + H]^+$ (calcd for $C_{26}H_{31}O_5$, 423.2166); physical and spectroscopic data were comparable with literature values.[8]

4-Hydroxyderricin (10): yellow, amorphous powder; HRESIMS m/z 339.1593 $[M + H]^+$ (calcd for $C_{21}H_{23}O_4$, 339.1591); physical and spectroscopic data were comparable with literature values.[32]

Xanthoangelol D (11): yellow, amorphous powder; $[\alpha]^{23}_D$ ±0 (c 0.1, MeOH); HRESIMS m/z 355.1540 $[M + H]^+$ (calcd for $C_{21}H_{23}O_5$, 355.1540); physical and spectroscopic data were comparable with literature values.[33]

Xanthoangelol E (12): yellow, amorphous powder; $[\alpha]^{23}_D$ ±0 (c 0.1, MeOH); HRESIMS m/z 371.1487 $[M + H]^+$ (calcd for $C_{21}H_{23}O_6$, 371.1489); physical and spectroscopic data were comparable with literature values.[33]

Xanthoangelol H (13): yellow, amorphous powder; $[\alpha]^{23}_D$ ±0 (c 0.1, MeOH); HRESIMS m/z 355.1543 $[M + H]^+$ (calcd for $C_{21}H_{23}O_5$, 355.1540); physical and spectroscopic data were comparable with literature values.[8]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00633.

Spectroscopic data for compounds **1**−**5** (PDF)

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Notes

The authors declare no competing financial interest.

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