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Diarylheptanoids from the Seeds of *Alpinia katsumadai* as Heat Shock Factor 1 Inducers

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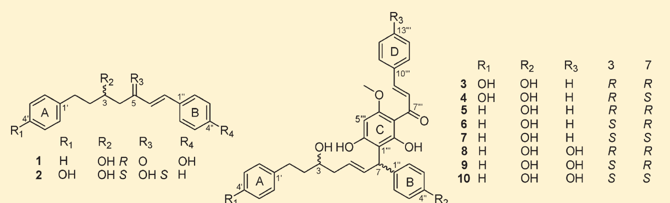
Supporting Information

ABSTRACT: Seven new diarylheptanoids, (−)-(*R*)-4″-hydroxyyashabushiketol (**1**), (3*S*,5*S*)-alpinikatin (**2**), katsumain C (**3**), 7-*epi*-katsumain C (**4**), *ent*-alpinnanin B (**5**), *ent*-alpinnanin A (**6**), and *ent*-calyxin H (**8**), were isolated from the EtOAc extract of the seeds of *Alpinia katsumadai* together with three known compounds, alpinnanin B (**7**), epicalyxin H (**9**), and calyxin H (**10**). Each isomer mixture of **3** and **4**, **5**−**7**, and **8**−**10** was separated successfully by preparative HPLC using a chiral column. The three isomer mixtures (**3** and **4**, **5**−**7**, **8**−**10**) at 1 μM increased expression of heat shock factor 1 (HSF1) with fold increases of 1.438, 1.190, and 1.316, respectively, which was accompanied with increased expression of heat shock protein (HSP) 27 (1.403-, 1.250-, and 1.270-fold, respectively) and HSP70 (1.373-, 1.313-, and 1.229-fold, respectively) without cellular cytotoxicity, suggesting a possible application of these compounds as HSP inducers. Celastrol was used as a positive control of HSP induction, producing fold increases of 1.066 (HSF1), 1.216 (HSP27), and 1.371 (HSP70) at 1 μM. Compounds **1** and **2** did not affect the induction of HSF1 protein.

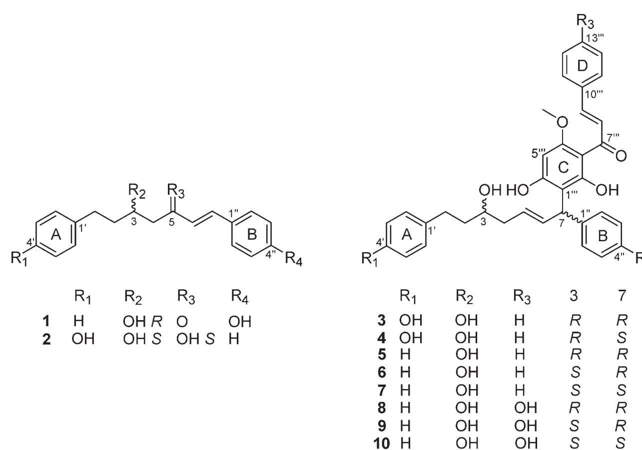


The seeds of *Alpinia katsumadai* Hayata (Zingiberaceae) have been used as an antiemetic and for treatment of gastric disorders in Oriental Medicine.[1] Previous phytochemical investigations of this plant have resulted in the isolation of various types of diarylheptanoids,[2−5] kavalactones,[3] flavonoids,[2,4,6,7] stilbenes,[6] monoterpenes,[6,7] and sesquiterpenes.[8] Some of these compounds have antioxidant,[9] antiemetic,[10,11] antiviral,[5] cytoprotective,[12] or other biological effects.

Heat shock transcription factor 1 (HSF1) plays a key role in the cellular response that leads to the expression of heat shock protein (HSP) genes under stress conditions.[13] HSPs have cytoprotective effects in neurodegenerative diseases and in other types of cellular damage.[14,15] As a part of a collaborative project directed toward the discovery of HSP-modulating agents from natural products, isolates from the seeds of *A. katsumadai* were evaluated for their effects on HSF1 protein expression and on its transcriptional targets, HSP27 and HSP70. Seven new compounds, **1**−**6** and **8**, were isolated from the EtOAc extract of the seeds of this plant together with three known compounds **7**, **9**, and **10**. The three known compounds have not been isolated previously from this plant. We report herein the isolation and structural elucidation of **1**−**6** and **8**. Compounds **1** and **2** and three isomer mixtures of **3** and **4**, **5**−**7**, and **8**−**10** were also evaluated for their HSF1-inducing activities in an H460 system.

■ RESULTS AND DISCUSSION

Compound **1** showed a molecular ion peak at m/z 319.1310 $[M + Na]^+$ in the HR-ESIMS, corresponding to the sodiated



elemental formula $C_{19}H_{20}O_3Na$. The IR spectrum showed absorption bands at 3312 cm^{-1} for one or more hydroxy groups and at 1717 cm^{-1} for conjugated carbonyl groups.[16] The ^1H and ^{13}C NMR spectra exhibited resonances at δ_H 7.24/δ_C 129.3, 7.25/129.2, 7.15/126.5, 7.56/131.3, and 6.90/116.9 and δ_C 143.5, 127.3, and 160.8 for two substituted benzene rings. In the ^1H NMR spectrum of **1**, the protons of an olefinic functionality appeared at δ_H 6.70 and 7.58 with a 16.4 Hz coupling constant, indicating their *trans*-configuration.[16] A ^{13}C NMR

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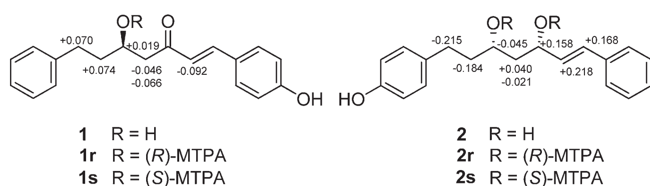

Figure 1. $\Delta\delta$ ($\delta_S - \delta_R$) values of MTPA esters of **1** and **2**.

resonance at δ_C 200.1 indicated the presence of a carbonyl group. The DEPT spectrum demonstrated three methylene groups at δ_C 32.6, 40.0, and 48.5, along with one methine at δ_C 68.1. These NMR data were similar to those of the known compound (−)-(*R*)-yashabushiketol,[17,18] except for the resonances of the aromatic systems. The ^{1}H and ^{13}C NMR resonances at δ_H 7.56 (d, *J* = 8.6 Hz)/δ_C 131.3 (C-2″ and C-6″), 6.90 (d, *J* = 8.6 Hz)/116.9 (C-3″ and C-5″), and δ_C 127.3 (C-1″) and 160.8 (C-4″) indicated the presence of a *p*-hydroxyphenyl ring in the structure of **1** instead of the monosubstituted benzene ring in (−)-(*R*)-yashabushiketol.[17,18] The HMBC correlations of **1** showed three-bond correlations of H-2′ and H-6′/C-1, H-2/C-1′, H-7/C-2″ and C-6″, and H-6/C-1″, so that rings A and B could be assigned at C-1 and C-7, respectively. Further analysis of the DEPT, COSY, NOESY, HSQC, and HMBC data allowed unambiguous assignments for the ^{1}H and ^{13}C NMR resonances. The configuration of **1** was determined by the Mosher ester procedure.[19] Compound **1** was treated with (*S*)- and (*R*)-MTPA-Cl, affording (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**), respectively. The absolute configuration at C-3 was *R* on the basis of the $\Delta\delta$ ($\delta_S - \delta_R$) values presented in Figure 1. Thus, compound **1** was elucidated as a new diarylheptanoid, (3*R*)-3-hydroxy-1-phenyl-7-(4-hydroxyphenyl)-6*E*-hepten-5-one, namely, (−)-(*R*)-4″-hydroxyyashabushiketol.

Compound **2** showed a molecular ion peak at *m*/*z* 321.1463 $[M + Na]^+$ in the HR-ESIMS, corresponding to the sodiated elemental formula $C_{19}H_{22}O_3Na$. The IR spectrum showed an absorption band at 3302 cm^{-1}. The ^{1}H and ^{13}C NMR spectra exhibited resonances at δ_H 7.00/δ_C 130.4, 6.67/116.1, 7.37/127.5, 7.29/129.6, and 7.20/128.6 and δ_C 134.4, 156.3, and 138.4 for two substituted benzene rings. In the ^{1}H NMR spectrum, a *trans*-olefinic group appeared at δ_H 6.20 (dd, *J* = 15.8, 6.8 Hz, H-6) and 6.58 (d, *J* = 15.8 Hz, H-7). The DEPT data revealed the presence of two methines at δ_C 70.1 and 72.3 and three methylenes at δ_C 32.0, 41.1, and 45.3. These NMR data were similar to those of a known compound, (3*S*,5*S*)-3,5-dihydroxy-1,7-diphenyl-6*E*-heptene,[2] except for the presence of a hydroxy group at C-4′ of ring A {δ_H 7.00 (d, *J* = 8.6 Hz), 6.67 (d, *J* = 8.6 Hz)} in **2**. Rings A and B of **2** were assigned to C-1 and C-7, respectively, on the basis of the HMBC correlations of H-2′ and H-6′/C-1, H-2/C-1′, H-7/C-2″ and C-6″, and H-6/C-1″. Further analysis of the DEPT, COSY, NOESY, HSQC, and HMBC data allowed unambiguous assignments for the ^{1}H and ^{13}C NMR resonances. The Mosher ester procedure[19] was performed to determine the absolute configurations at C-3 and C-5, which contain secondary hydroxyls, i.e., a diol. The configurations at both C-3 and C-5 in **2** were "*S*" according to the $\Delta\delta$ values (Figure 1), which revealed the typical pattern of *syn*-acyclic 1,3-diols.[20] Thus, the structure of **2** was assigned to a new compound, (3*S*,5*S*)-3,5-dihydroxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene, namely, (3*S*,5*S*)-alpinikatin.

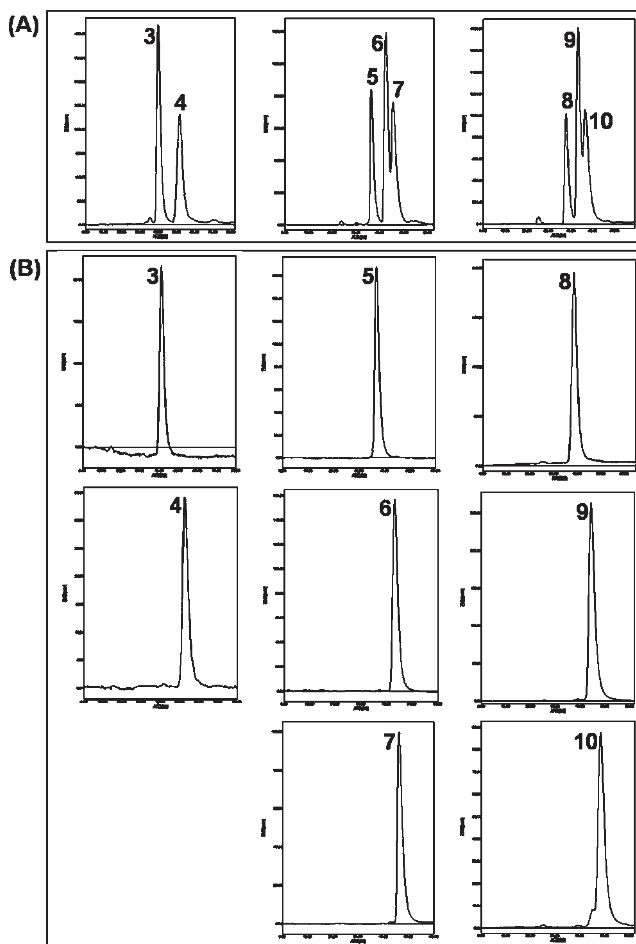

Figure 2. (A) HPLC chromatograms of mixtures of **3** and **4**, **5**−**7**, and **8**−**10**. (B) HPLC chromatograms of separated compounds **3**−**10** [column: ChiralPak IB (10 × 250 mm); mobile phase: *n*-hexane−IPA, 7:3; detection: UV 365 nm].

A stereoisomeric mixture of **3** and **4** was obtained using various column chromatography procedures as described in the Experimental Section. The stereoisomers **3** and **4** were effectively separated by preparative HPLC using a chiral column (ChiralPak IB; 5 μm, 250 mm × 10 mm i.d.) as shown in Figure 2.

Compound **3** showed a molecular ion peak at *m*/*z* 567.2389 $[M + H]^+$ in the HR-ESIMS, corresponding to an elemental formula of $C_{35}H_{35}O_7$. The IR spectrum indicated the presence of one or more hydroxy groups at 3223 cm^{-1} and conjugated carbonyl groups at 1616 cm^{-1}.[16] The ^{1}H and ^{13}C NMR spectra revealed the presence of two sets of *p*-substituted benzene rings at δ_H 6.99/δ_C 130.1, 6.72/115.8, 7.12/129.4, and 6.69/115.3 and δ_C 156.1, 134.2, and 135.7 and a monosubstituted benzene ring at δ_H 7.73/δ_C 129.2, 7.45/129.9, and 7.44/130.9 and δ_C 136.6. Resonances for two sets of *trans*-olefinic functionalities appeared at δ_H 5.64 (dt, *J* = 15.4, 6.7 Hz, H-5)/δ_C 128.5 (C-5), 6.39 (dd, *J* = 15.4, 8.6 Hz, H-6)/134.7 (C-6), 8.03 (d, *J* = 16.0 Hz, H-8‴)/128.9 (C-8‴), and 7.76 (d, *J* = 16.0 Hz, H-9‴)/142.4 (C-9‴). The ^{1}H NMR resonance at δ_H 3.97 (3H, s), which was correlated to the ^{13}C NMR resonance at δ_C 56.3 in the HSQC spectrum, indicated the presence of an aromatic methoxy group. The methoxy group was assigned at C-4‴ due to the three-bond connectivity between the methoxy protons at δ_H 3.97 and C-4‴ in the HMBC spectrum. The NOESY correlation between the

methoxy protons and H-5‴ provided further evidence for the position of the methoxy group in **3**. In the ^{13}C NMR spectrum, a carbonyl carbon appeared at δ_C 193.4, hydrogen-bonded to the hydroxy proton resonating at δ_H 14.74 in the ^1H NMR spectrum in acetone-d_6. The placement of substituents of ring C was deduced by the HMBC correlations of H-5‴/C-1‴, C-3‴, C-4‴, C-6‴; O*H*-2‴/C-1‴, C-2‴, C-3‴; and H-7/C-1‴, C-2‴, C-6‴. These NMR data were similar to those of the known compounds katsumains A and B,[3] except for the presence of a *p*-hydroxy group in rings A and B in **3**. The HMBC data of **3** showed three-bond correlations of H-2′ and H-6′/C-1 and H-2″ and H-6″/C-7 so that rings A and B could be assigned at C-1 and C-7, respectively. The three-bond connectivities of H-11‴ and 15‴/C-9‴ and H-8‴/C-10‴ provided evidence for the position of ring D at C-9‴. The linkage between C-7 of the diarylhepanoid and C-1‴ of the chalcone moiety was deduced by the HMBC cross-peaks of H-7/C-2‴, C-6‴ and H-6/C-1‴. Further analysis of the COSY, NOESY, HSQC, and HMBC data allowed unambiguous assignments for the ^1H and ^{13}C NMR resonances. The established method was applied to determine the absolute configuration at C-3 and C-7 by comparison of the proton splitting patterns of H_2-4 and the optical activity to those of epicalyxin H (**9**) and calyxin H (**10**) as previously described.[4,21] Compound **3** exhibited a specific rotation of $[\alpha]^{25}_D$ +9.4 (*c* 0.2, MeOH) and showed a *triplet*-like splitting pattern for H_2-4 in the ^1H NMR spectrum. Thus, the configuration at C-3 and C-7 was *R* and the structure of **3** was elucidated as a new compound, (2*E*)-1-{2,4-dihydroxy-3-[(1*R*,2*E*,5*R*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)-2-hepten-1-yl]-6-methoxyphenyl}-3-phenyl-2-propen-1-one, namely, katsumain C.

Compound **4** showed a molecular ion peak at *m*/*z* 567.2380 $[M + H]^+$ in the HR-ESIMS, corresponding to an elemental formula of $C_{35}H_{35}O_7$. The ^1H and ^{13}C NMR spectra of **4** were similar to those of **3**, except for the splitting pattern of H_2-4 in the ^1H NMR spectrum, suggesting that compound **4** was a stereoisomer of **3**. Further analysis of the COSY, NOESY, HSQC, and HMBC data confirmed that the gross structure of **4** was the same as **3**. Compound **4** was levorotatory, $[\alpha]^{25}_D$ −10.1 (*c* 0.1, MeOH), and showed a *quartet*-like splitting pattern for H_2-4 in the ^1H NMR spectrum. Thus, the configurations at C-3 and C-7 in **4** were "*R*" and "*S*", respectively. As a result, the structure of **4** was elucidated as a new compound, (2*E*)-1-{2,4-dihydroxy-3-[(1*S*,2*E*,5*R*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)-2-hepten-1-yl]-6-methoxyphenyl}-3-phenyl-2-propen-1-one, namely, 7-*epi*-katsumain C.

A stereoisomeric mixture of **5**−**7** was separated by various column chromatography methods as described in the Experimental Section. Compounds **5**−**7** were each purified by preparative HPLC using a chiral column (ChiralPak IB; 5 μm, 250 mm × 10 mm i.d.) as shown in Figure 2.

Compound **5** showed a molecular ion peak at *m*/*z* 551.2440 $[M + H]^+$ in the HR-ESIMS, corresponding to an elemental formula of $C_{35}H_{35}O_6$. The ^1H and ^{13}C NMR spectra were similar to those of compounds **3** and **4**, except for the absence of a *p*-hydroxy group in ring A in **5**. The ^1H and ^{13}C NMR data exhibited two monosubstituted benzene rings at δ_H 7.17/δ_C 129.3, 7.24/129.3, 7.18/126.4, 7.74/129.2, 7.45/129.9, and 7.44/131.0 and δ_C 143.8 and 136.7, together with a *p*-substituted benzene ring at δ_H 7.12/δ_C 129.5 and 6.69/115.4 and δ_C 135.8 and 156.1. The *p*-substituted benzene ring was assigned as ring B due to the HMBC correlations of H-2″ and H-6″/C-7 and H-3″ and H-5″/C-1″. Further analysis of the COSY, NOESY, HSQC, and HMBC data allowed unambiguous assignments for the ^1H and ^{13}C NMR resonances. The gross structure of **5** was identical to that of the known compound alpinnanin B (**7**),[22] which was also isolated from *A. katsumadai* for the first time in the present study. The spectroscopic data of **5** were similar to those of compound **7**, suggesting that these two compounds are stereoisomers. Compound **5** was dextrorotatory, $[\alpha]^{25}_D$ +7.0 (*c* 0.05, MeOH), and showed a *triplet*-like splitting pattern for H_2-4 in the ^1H NMR spectrum. The configuration at both C-3 and C-7 was *R*.[4,21] Therefore, the structure of **5** was elucidated as the new stereoisomer (2*E*)-1-{2,4-dihydroxy-3-[(1*R*,2*E*,5*R*)-5-hydroxy-1-(4-hydroxyphenyl)-7-phenyl-2-hepten-1-yl]-6-methoxyphenyl}-3-phenyl-2-propen-1-one, namely, *ent*-alpinnanin B.

Compound **6** showed a molecular ion peak at *m*/*z* 551.2427 $[M + H]^+$ in the HR-ESIMS, corresponding to an elemental formula of $C_{35}H_{35}O_6$. The ^1H and ^{13}C NMR spectra were similar to those of **5**, except for the splitting pattern of H_2-4 in the ^1H NMR spectrum, suggesting that these two compounds are stereoisomers. Further analysis of the COSY, NOESY, HSQC, and HMBC data confirmed that the gross structure of **6** was the same as **5**. Compound **6** was dextrorotatory, $[\alpha]^{25}_D$ +5.8 (*c* 0.07, MeOH), and showed a *quartet*-like splitting pattern for H_2-4 in the ^1H NMR spectrum. The configurations at C-3 and C-7 were "*S*" and "*R*",[4,21] respectively, and the structure of **6** was elucidated as the new stereoisomer (2*E*)-1-{2,4-dihydroxy-3-[(1*R*,2*E*,5*S*)-5-hydroxy-1-(4-hydroxyphenyl)-7-phenyl-2-hepten-1-yl]-6-methoxyphenyl}-3-phenyl-2-propen-1-one, namely, *ent*-alpinnanin A.

A stereoisomeric mixture of **8**−**10** was obtained by various column chromatography procedures as described in the Experimental Section. Compounds **8**−**10** were separated by preparative HPLC using a chiral column (ChiralPak IB; 5 μm, 250 mm × 10 mm i.d.) as shown in Figure 2.

Compound **8** showed a molecular ion peak at *m*/*z* 567.2387 $[M + H]^+$ in the HR-ESIMS, corresponding to an elemental formula of $C_{35}H_{35}O_7$. The ^1H and ^{13}C NMR data exhibited two *p*-substituted benzene rings at δ_H 7.12/δ_C 129.4, 6.68/115.3, 7.62/131.3, and 6.92/116.8 and δ_C 135.8, 155.9, 128.1, and 160.7 together with a monosubstituted benzene ring at δ_H 7.17/δ_C 129.3, 7.24/129.1, and 7.18/126.3 and δ_C 143.7. These data were similar to those of **5**, except for the presence of a *p*-hydroxy group in ring D in **8**. The *p*-substituted benzene ring was assigned as ring D due to the HMBC correlations of H-11‴ and H-15‴/C-9‴ and H-12‴ and H-14‴/C-10‴. Further analysis of the COSY, NOESY, HSQC, and HMBC data allowed unambiguous assignments for the ^1H and ^{13}C NMR resonances. The spectroscopic data of **8** were similar to those of compounds **9** and **10**, except for the optical activities or the splitting patterns of H_2-4 in the ^1H NMR spectrum, suggesting that these compounds are stereoisomers. Compound **8** was dextrorotatory, $[\alpha]^{25}_D$ +22.5 (*c* 0.1, MeOH) and had a *triplet*-like splitting pattern for H_2-4 in the ^1H NMR spectrum. Thus, the configuration at C-3 and C-7 was *R*,[4,21] and the structure of **8** was elucidated as a new stereoisomer, (2*E*)-1-{2,4-dihydroxy-3-[(1*R*,2*E*,5*R*)-5-hydroxy-1-(4-hydroxyphenyl)-7-phenyl-2-hepten-1-yl]-6-methoxyphenyl}-3-(4-hydroxyphenyl)-2-propen-1-one, namely, *ent*-calyxin H.

In order to evaluate whether the isolates had inductive effects on HSF1 and its transcriptional targets, HSP27 and HSP70, Western blotting was performed at 12 and 24 h after treatment of NCI-H460 cells with the compounds (12 h data not shown). The results showed that both HSP27 and HSP70 protein expression, as well as HSF1 protein expression, was increased significantly by the isomeric mixtures of compounds **3** and **4**, **5**−**7**, and **8**−**10**,

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Table 1. ^{1}H NMR Data of Compounds **1**−**6** and **8**[a]

position	**1**[b]	**2**[c]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**8**[b]
1	2.71 m	2.55 m	2.57 m	2.57 m	2.66 m	2.66 m	2.65 m
	2.84 m	2.66 m	2.69 m	2.70 m	2.79 m	2.79 m	2.79 m
2	1.80 m	1.70 m	1.65 m	1.64 m	1.70 m	1.68 m	1.70 m
			1.76 m	1.79 m	1.80 m	1.83 m	1.80 m
3	4.13 p (6.0)	3.73 m	3.61 m	3.64 m	3.63 m	3.65 m	3.62 m
4	2.84 d (6.0)	1.80 m	2.27 t-like (6.7)	2.27 q-like (6.8)	2.28 t-like (6.8)	2.29 q-like (6.8)	2.28 t-like (6.6)
5		4.43 q (6.8)	5.64 dt (15.4, 6.7)	5.65 dt (15.4, 6.8)	5.64 dt (15.2, 6.8)	5.66 dt (15.2, 6.8)	5.63 dt (15.2, 6.6)
6	6.70 d (16.4)	6.20 dd (15.8, 6.8)	6.39 dd (15.4, 8.6)	6.39 dd (15.4, 8.5)	6.38 dd (15.2, 8.4)	6.40 dd (15.2, 8.4)	6.38 dd (15.2, 8.4)
7	7.58 d (16.4)	6.58 d (15.8)	5.21 d (8.6)	5.21 d (8.5)	5.22 d (8.4)	5.22 d (8.4)	5.21 d (8.4)
2′,6′	7.24 m	7.00 d (8.6)	6.99 d (8.6)	7.00 d (8.8)	7.17 d (7.2)	7.18 d (7.2)	7.17 d (7.0)
3′,5′	7.25 m	6.67 d (8.6)	6.72 d (8.6)	6.74 d (8.8)	7.24 t (7.2)	7.25 t (7.2)	7.24 t (7.0)
4′	7.15 m				7.18 m	7.19 m	7.18 m
2′′,6”	7.56 d (8.6)	7.37 d (7.4)	7.12 d (8.8)	7.12 d (8.6)	7.12 d (8.4)	7.12 d (8.4)	7.12 d (8.8)
3′′,5′′	6.90 d (8.6)	7.29 t (7.4)	6.69 d (8.8)	6.69 d (8.6)	6.69 d (8.4)	6.69 d (8.4)	6.68 d (8.8)
4′′		7.20 m					
5′′′			6.21 s	6.24 s	6.19 s	6.18 s	6.21 s
8′′′			8.03 d (16.0)	8.03 d (15.8)	8.03 d (15.6)	8.03 d (15.6)	7.90 d (15.4)
9′′′			7.76 d (16.0)	7.75 d (15.8)	7.76 d (15.6)	7.76 d (15.6)	7.75 d (15.4)
11′′′,15′′′			7.73 d (7.4)	7.73 d (7.6)	7.74 d (7.0)	7.74 d (7.2)	7.62 d (8.8)
12′′′,14′′′			7.45 t (7.4)	7.45 t (7.6)	7.45 t (7.0)	7.45 t (7.2)	6.92 d (8.8)
13′′′			7.44 m	7.44 m	7.44 m	7.44 m	
OCH_3-4′′′			3.97 s	3.96 s	3.94 s	3.94 s	3.94 s
OH-2′′′			14.74 s	14.74 s	14.75 s	14.75 s	14.94 s

[a] TMS was used as an internal standard; chemical shifts (δ) are expressed in ppm; *J* values are given in parentheses. [b] Data were measured in acetone-d_6 at 400 MHz. [c] Data were measured in methanol-d_4 at 400 MHz.

compared to those in untreated control cells. Celastrol, an HSP inducer and positive control,[23] also increased the expressions of HSP27 and HSP70; however, celastrol did not affect the expression of HSF1, which indicates that celastrol and our isolates act through different mechanisms. The most effective sample was the mixture of compounds **3** and **4** as shown in Table 3; compounds **1** and **2** did not modulate the expression of HSP27, HSP70, and HSF1. The active compound mixtures of **3** and **4**, **5**−**7**, and **8**−**10** did not exhibit any cytotoxicity to NCI-H460 cells (IC_{50} values >30 μM), while the anticancer drug Taxol and celastrol showed cytotoxicity with IC_{50} values of 8 and 12.3 μM, respectively (Table 3).

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a P-1010 polarimeter (JASCO, Japan) at 25 °C. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA) with tetramethylsilane (TMS) as an internal standard. Mass spectrometry was carried out with 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-A, 12 nm, S-150 μm), and Sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. TLC 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) H_2SO_4 spray followed by heating (120 °C, 5 min). Preparative HPLC was carried out on an Acme 9000 system (Young Lin, South Korea) using YMC J'sphere ODS-H80 (4 μm, 250 mm × 20 mm i.d.) and ChiralPak IB (5 μm, 250 mm ×10 mm i.d.) columns.

Plant Material. The seeds of *A. katsumadai* were purchased from Kyungdong Oriental Herbal market in Seoul, South Korea, in May 2010 and identified by Professor Je-hyun Lee (College of Oriental Medicine, Dongguk University). A voucher specimen (no. EA299) was deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation. The seeds of *A. katsumadai* (5.4 kg) were extracted with MeOH (3 × 9 L) overnight at room temperature. The solvent was evaporated *in vacuo* to afford a concentrated MeOH extract (788 g). This extract was suspended in distilled H_2O and successively fractionated with *n*-hexane, EtOAc, and *n*-BuOH. A portion of the EtOAc extract (150 g) was separated by silica gel flash column chromatography (CC; 230−400 mesh, 2 kg) using CH_2Cl_2−MeOH (95.5:0.5 to 9:1) as a gradient solvent system to afford 16 fractions (F01−F16). Fraction F11 (6.0 g), eluted with CH_2Cl_2−MeOH (85:15), was subjected to reversed-phase CC (100 g) with MeCN−H_2O (5:5) as a solvent system to yield 17 subfractions (F11.01−F11.17). Subfraction F11.03 (130 mg) was chromatographed using Sephadex LH-20 with 100% MeOH to give compound **2** (56.0 mg, 0.00104% w/w). Subfraction F11.06 (80 mg) was subjected to silica gel CC (10 g) using gradient mixtures of *n*-hexane−EtOAc (9:1 → 7:3) to afford compound **1** (43.0 mg, 0.000796% w/w). Fractions F11.10 (200 mg) and F11.13 (110 mg) were chromatographed on preparative HPLC using an isocratic mixture of MeOH−0.1% formic acid in water (82:18, 2 mL/min) as a solvent system to afford isomer mixtures of compounds **8**−**10** (t_R 50.3 min, 32.0 mg, 0.000593% w/w) and compounds **3** and **4** (t_R 43.2 min, 7.8 mg, 0.000144% w/w), respectively. Fraction F11.15 (520 mg) was subjected to silica gel CC (50 g) with a gradient of *n*-hexane−EtOAc (9:1 → 5:5) as a solvent system to afford 15 subfractions (F11.15.01−F11.15.15). Fraction F11.15.10 (75 mg) was

Table 2. ^{13}C NMR Data of Compounds 1−6 and 8[a]

position	1[b]	2[c]	3[b]	4[b]	5[b]	6[b]	8[b]
1	32.6, CH_2	32.0, CH_2	31.7, CH_2	31.8, CH_2	32.7, CH_2	32.7, CH_2	32.6, CH_2
2	40.0, CH_2	41.1, CH_2	39.9, CH_2	39.9, CH_2	39.7, CH_2	39.7, CH_2	39.6, CH_2
3	68.1, CH	70.1, CH	70.7, CH	70.8, CH	70.8, CH	70.9, CH	70.7, CH
4	48.5, CH_2	45.3, CH_2	41.7, CH_2	41.7, CH_2	41.8, CH_2	41.8, CH_2	41.6, CH_2
5	200.1, C	72.3, CH	128.5, CH	128.4, CH	128.5, CH	128.4, CH	128.3, CH
6	124.9, CH	133.3, CH	134.7, CH	134.7, CH	134.9, CH	134.8, CH	134.9, CH
7	143.6, CH	131.5, CH	43.0, CH	42.9, CH	43.1, CH	43.0, CH	43.0, CH
1′	143.5, C	134.4, C	134.2, C	134.3, C	143.8, C	143.8, C	143.7, C
2′,6′	129.3, CH	130.4, CH	130.1, CH	130.1, CH	129.3, CH	129.3, CH	129.3, CH
3′,5′	129.2, CH	116.1, CH	115.8, CH	115.8, CH	129.3, CH	129.3, CH	129.1, CH
4′	126.5, CH	156.3, C	156.1, C	156.1, C	126.4, CH	126.4, CH	126.3, CH
1″	127.3, C	138.4, C	135.7, C	135.9, C	135.8, C	135.9, C	135.8, C
2″,6″	131.3, CH	127.5, CH	129.4, CH	129.3, CH	129.5, CH	129.4, CH	129.4, CH
3″,5″	116.9, CH	129.6, CH	115.3, CH	115.3, CH	115.4, CH	115.4, CH	115.3, CH
4″	160.8, C	128.6, CH	156.1, C	156.1, C	156.1, C	156.1, C	155.9, C
1‴			111.8, C	111.8, C	111.9, C	112.0, C	112.0, C
2‴			166.5, C	166.5, C	165.9, C	166.5, C	166.5, C
3‴			106.2, C	106.2, C	106.1, C	106.3, C	106.3, C
4‴			162.3, C	162.3, C	162.4, C	162.4, C	162.2, C
5‴			92.3, CH	92.3, CH	92.3, CH	92.4, CH	92.1, CH
6‴			164.2, C	164.0, C	164.2, C	163.9, C	163.1, C
7‴			193.4, C	193.4, C	193.5, C	193.5, C	193.4, C
8‴			128.9, CH	128.9, CH	129.0, CH	128.9, CH	125.5, CH
9‴			142.4, CH	142.3, CH	142.5, CH	142.4, CH	143.1, CH
10‴			136.6, C	136.6, C	136.7, C	136.6, C	128.1, C
11‴,15‴			129.2, CH	129.2, CH	129.2, CH	129.2, CH	131.3, CH
12‴,14‴			129.9, CH	129.9, CH	129.9, CH	129.9, CH	116.8, CH
13‴			130.9, CH	130.9, CH	131.0, CH	131.0, CH	160.7, C
OCH_3-4‴′			56.3, CH_3	56.3, CH_3	56.3, CH_3	56.3, CH_3	56.2, CH_3

[a] TMS was used as an internal standard; Chemical shifts (δ) are expressed in ppm. [b] Data were measured in acetone-d_6 at 100 MHz. [c] Data were measured in methanol-d_4 at 100 MHz.

chromatographed over reversed-phase CC (8 g) using an isocratic solvent system of MeOH−H_2O (85:15) to yield an isomer mixture of compounds **5**−**7** (31.0 mg, 0.000574% w/w). The mixtures of compounds **3** and **4**, **5**−**7**, and **8**−**10** were subjected to preparative HPLC using a chiral selective column with *n*-hexane−IPA (7:3, 1 mL/min) as the solvent system to provide **3** (0.90 mg, t_R 41.1 min, 0.000017% w/w), **4** (2.00 mg, t_R 52.4 min, 0.000037% w/w), **5** (2.10 mg, t_R 36.7 min, 0.000039% w/w), **6** (2.20 mg, t_R 43.1 min, 0.000041% w/w), **7** (1.30 mg, t_R 46.9 min, 0.000024% w/w), **8** (1.10 mg, t_R 38.5 min, 0.000020% w/w), **9** (2.00 mg, t_R 44.3 min, 0.000037% w/w), and **10** (2.30 mg, t_R 48.3 min, 0.000043% w/w).

Western Blot Analysis. The ability of compounds isolated from *A. katsumadai* to modulate HSF1 and HSPs expression was evaluated by established protocol.[24] Proteins in lysates were separated by SDS-PAGE, electrotransferred to nitrocellulose membranes (GE Healthcare, UK), subsequently blotted with the specified antibodies, and visualized with an ECL detection system (Thermo Scientific, USA). Anti-HSF1, -Hsp27, and -Hsp70 and β-actin antibodies were purchased from Santa Cruz Biotechnology (USA).

MTT Assay. The cells were assayed for their cytotoxicity in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT; Sigma) test according to established protocol.[25]

*(−)-(R)-4″-Hydroxyyashabushiketol (**1**):* yellow powder; $[\alpha]^{25}_D$ −18.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 325 (4.7), 233 (4.6) nm; IR (KBr) ν_{max} 3312, 2929, 1717, 1541 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-7/H-2″ and H-6″; HMBC correlations H-4′/C-3′; H-3′ and H-5′/C-1′; H-2′ and H-6′/C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1′, C-1, C-3, C-4; H-3/C-1, C-2, C-4, C-5; H-4/C-2, C-3, C-5; H-6/C-4, C-5, C-7, C-1″, C-2″, C-6″; H-7/C-5, C-6, C-1″, C-2″, C-6″; H-2″ and H-6″/C-7, C-3″, C-4″, C-5″; H-3″ and H-5″/C-1″, C-4″; HR-ESIMS *m/z* 319.1310 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_3Na$, 319.1305).

*(3S,5S)-Alpinikatin (**2**):* yellow powder; $[\alpha]^{25}_D$ +19.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 252 (4.4), 227 (4.2) nm; IR (KBr) ν_{max} 3302, 2926, 1554, 1455 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-3/H-5, H-7/H-2″ and H-6″; HMBC correlations H-3′ and H-5′/C-1′, C-4′; H-2′ and H-6′/C-3′, C-4′, C-5′, C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1′, C-1, C-4; H-3/C-1, C-2, C-4, C-5; H-4/C-2, C-3, C-5, C-6; H-5/C-3, C-4, C-6, C-7; H-6/C-4, C-5, C-1″, C-2″, C-6″; H-7/C-5, C-6, C-1″, C-2″, C-6″; H-2″ and H-6″/C-7, C-1″, C-4″; H-3″ and H-5″/C-1″, C-2″, C-6″; H-4″/C-2″, C-3″, C-5″, C-6″; HR-ESIMS *m/z* 321.1463 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_3Na$, 321.1461).

*Katsumain C (**3**):* yellow, amorphous solid; $[\alpha]^{25}_D$ +9.4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 348 (4.7), 288 (4.5), 217 (4.9) nm; IR (KBr) ν_{max} 3223, 1616, 1559, 1509, 1454 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-5/H-7, H-7/H-2″ and H-6″, OCH_3−4‴/H-5‴, H-9‴/H-11‴ and H-15‴; HMBC correlations H-3′ and H-5′/ C-1′, C-4′; H-2′ and H-6′/C-3′, C-4′, C-5′, C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1′,

Table 3. Induction of HSF1 and HSPs by Isolates from *A. katsumadai*

compound(s)	fold increase[b] HSF1	HSP27	HSP70	IC_{50} (μM)[c]
3 and **4**[a]	1.438 ± 0.032	1.403 ± 0.023	1.373 ± 0.012	47.4
5−**7**[a]	1.190 ± 0.005	1.250 ± 0.011	1.313 ± 0.047	39.8
8−**10**[a]	1.316 ± 0.053	1.270 ± 0.031	1.229 ± 0.009	45.7
celastrol[d]	1.066 ± 0.009	1.216 ± 0.022	1.371 ± 0.037	12.3
Taxol	ND[e]	ND[e]	ND[e]	8.0

[a] Protein expressions were assayed after treatment with isomeric mixtures. [b] Quantitative immunoblotting results for HSF1, HSP27, and HSP70 in NCI-H460 (human non-small-cell lung cancer cells) after normalization to the β-actin signal are summarized. Data shown represent the mean ± SD of three independent experiments performed in triplicate at 24 h of treatment. Statistically significant difference ($p < 0.05$) in comparison with the quantitative value of HSP70, HSP27, or HSF1 level between treated and untreated control cells. [c] IC_{50} values were the concentrations (μM) necessary for 50% inhibition of cell growth in NCI-H460 cells. [d] Celastrol was used as a positive control. [e] ND; not detected

C-2, C-3; H-3/C-1, C-5; H-4/C-2, C-3, C-5, C-6; H-5/C-4, C-7; H-6/C-4, C-7, C-1″, C-1‴; H-7/C-5, C-2″, C-6″, C-1‴, C-2‴, C-6‴; H-2″ and H-6″/C-7, C-4″; H-3″ and H-5″/C-1″, C-4″; O*H*-2‴/C-1‴, C-2‴, C-3‴; OC*H*₃-4‴/C-4‴; H-5‴/C-1‴, C-3‴, C-4‴, C-6‴; H-8‴/C-7‴, C-10‴; H-9‴/C-7‴, C-8‴, C-10‴, C-11‴, C-15‴; H-11‴ and H-15‴/C-9‴, C-13‴; H-12‴ and H-14‴/C-10‴; H-13‴/C-12‴; HR-ESIMS m/z 567.2389 $[M + H]^+$ (calcd for $C_{35}H_{35}O_7$, 567.2377).

7-epi-Katsumain C (**4**): yellow, amorphous solid; $[\alpha]^{25}_D$ −10.1 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 347 (4.7), 288 (4.5), 216 (4.8) nm; IR (KBr) ν_{max} 3225, 1616, 1560, 1511, 1458 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-5/H-7, H-7/H-2″ and H-6″, OC*H*₃−4‴/H-5‴, H-9‴/H-11‴ and H-15‴; HMBC correlations H-3′ and H-5′/C-1′, C-4′; H-2′ and H-6′/ C-3′, C-4′, C-5′, C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1, C-3, C-4; H-3/C-1; H-4/C-2, C-3, C-5; H-5/C-3, C-4, C-7; H-6/C-4, C-7, C-1‴; H-7/C-5, C-6, C-1″, C-2″, C-6″, C-1‴, C-2‴, C-6‴; H-2″ and H-6″/C-7, C-4″; H-3″ and H-5″/C-1″, C-4″; O*H*-2‴/C-1‴, C-2‴, C-3‴; OC*H*₃-4‴/C-4‴; H-5‴/C-1‴, C-3‴, C-4‴, C-6‴, C-7‴; H-8‴/C-7‴, C-10‴; H-9‴/C-7‴, C-10‴, C-11‴, C-15‴; H-11‴ and H-15‴/C-9‴, C-13‴; H-12‴ and H-14‴/C-10‴; H-13‴/C-12‴; HR-ESIMS m/z 567.2380 $[M + H]^+$ (calcd for $C_{35}H_{35}O_7$, 567.2377).

Ent-alpinnanin B (**5**): yellow, amorphous solid; $[\alpha]^{25}_D$ +7.0 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 350 (4.8), 289 (4.5), 217 (4.9) nm; IR (KBr) ν_{max} 3255, 1616, 1559, 1509, 1427 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-5/H-7, H-7/H-2″ and H-6″, OC*H*₃−4‴/H-5‴, H-9‴/H-11‴ and H-15‴; HMBC correlations H-4′/C-3′, C-5′; H-3′ and H-5′/C-1′; H-2′ and H-6′/C-4′, C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1′, C-1, C-3, C-4; H-3/C-1, C-5; H-4/C-2, C-3, C-6; H-5/C-3, C-4, C-7; H-6/C-4, C-7, C-1″; H-7/C-6, C-2″, C-6″, C-1‴, C-2‴, C-6‴; O*H*-2‴/C-1‴, C-2‴, C-3‴; OC*H*₃-4‴/C-4‴, C-5‴; H-5‴/C-1‴, C-3‴, C-4‴, C-6‴, C-7‴; H-8‴/C-7‴, C-9‴, C-10‴; H-9‴/C-7‴, C-10‴, C-11‴, C-15‴; H-11‴ and H-15‴/C-13‴; H-12‴ and H-14‴/C-10‴; H-13‴/C-12‴, C-14‴; HR-ESIMS m/z 551.2440 $[M + H]^+$ (calcd for $C_{35}H_{35}O_6$, 551.2428).

Ent-alpinnanin A (**6**): yellow, amorphous solid; $[\alpha]^{25}_D$ +5.8 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 351 (4.8), 289 (4.5), 217 (4.9) nm; IR (KBr) ν_{max} 3255, 1616, 1561, 1507, 1427 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-5/H-7, H-7/H-2″ and H-6″, OC*H*₃-4‴/H-5‴, H-9‴/H-11‴ and H-15‴; HMBC correlations H-4′/C-3′, C-5′; H-3′ and H-5′/C-1′; H-2′ and H-6′/C-4′, C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1′, C-1, C-3, C-4; H-3/C-1, C-5; H-4/C-2, C-3, C-6; H-5/C-3, C-4, C-7; H-6/C-4, C-7, C-1″; H-7/C-6, C-2″, C-6″, C-1‴, C-2‴, C-6‴; O*H*-2‴/C-1‴, C-2‴, C-3‴; OC*H*₃-4‴/C-4‴, C-5‴; H-5‴/C-1‴, C-3‴, C-4‴, C-6‴, C-7‴; H-8‴/C-7‴, C-9‴, C-10‴; H-9‴/C-7‴, C-10‴, C-11‴, C-15‴; H-11‴ and H-15‴/C-13‴; H-12‴ and H-14‴/C-10‴; H-13‴/C-12‴, C-14‴; HR-ESIMS m/z 551.2427 $[M + H]^+$ (calcd for $C_{35}H_{35}O_6$, 551.2428).

Alpinnanin B (**7**): $[\alpha]^{25}_D$ −8.0 (c 0.1, MeOH); HR-ESIMS m/z 551.2434 $[M + H]^+$ (calcd for $C_{35}H_{35}O_6$, 551.2428); physical and spectroscopic data were comparable to literature values.[22]

Ent-calyxin H (**8**): yellow, amorphous solid; $[\alpha]^{25}_D$ +22.5 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 371 (4.8), 228 (4.7) nm; IR (KBr) ν_{max} 3260, 1615, 1558, 1510, 1437 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; NOESY correlations H-1/H-2′ and H-6′, H-7/H-2″ and H-6″, OC*H*₃-4‴/H-5‴, H-9‴/H-11‴ and H-15‴; HMBC correlations H-4′/C-3′, C-5′; H-3′ and H-5′/C-1′; H-2′ and H-6′/C-4′, C-1; H-1/C-1′, C-2′, C-6′, C-2, C-3; H-2/C-1, C-3; H-4/C-2, C-3, C-5, C-6; H-5/C-4, C-7; H-6/C-4, C-7, C-1‴; H-7/C-5, C-6, C-1″, C-2″, C-6″, C-1‴, C-2‴, C-6‴; H-2″ and H-6″/C-7, C-4″; H-3″ and H-5″/C-1″, C-4″; OC*H*₃-4‴/C-4‴; H-5‴/C-1‴, C-3‴, C-4‴, C-6‴; H-8‴/C-7‴, C-9‴, C-10‴; H-9‴/C-7‴, C-8‴, C-10‴, C-11‴, C-15‴; H-11‴ and H-15‴/C-9‴, C-13‴; H-12‴ and H-14‴/C-10‴, C-13‴; HR-ESIMS m/z 567.2387 $[M + H]^+$ (calcd for $C_{35}H_{35}O_7$, 567.2377).

Epicalyxin H (**9**): $[\alpha]^{25}_D$ +9.3 (c 0.09, MeOH); HR-ESIMS m/z 567.2382 $[M + H]^+$ (calcd for $C_{35}H_{35}O_7$, 567.2377); physical and spectroscopic data were comparable to literature values.[21]

Calyxin H (**10**): $[\alpha]^{25}_D$ −14.9 (c 0.07, MeOH); HR-ESIMS m/z 567.2370 $[M + H]^+$ (calcd for $C_{35}H_{35}O_7$, 567.2377); physical and spectroscopic data were comparable to literature values.[21]

Preparation of the (*S*)- and (*R*)-MTPA Ester Derivatives of 1 and 2 by the Mosher Ester Procedure. (*S*)- and (*R*)-MTPA esters of compounds **1** and **2** were prepared using the Mosher ester procedure.[19] Compounds **1** and **2** (1 mg each) were dried under vacuum, resuspended in pyridine-d_5 (1 mL each), and transferred into clean NMR tubes, respectively. (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl] (10 μL) and DMAP were immediately added into each NMR tube under an N_2 gas stream, and then the NMR tubes were shaken to ensure even mixing. The NMR tubes were incubated in a water bath for 4 h (40 °C). The reactions afforded (*R*)-MTPA ester derivatives **1r** and **2r**, respectively. In the same manner except for the treatment with (*R*)-MTPA-Cl (10 μL), (*S*)-MTPA ester derivatives (**1s** and **2s**) were prepared. The spectra of **1r**, **1s**, **2r**, and **2s** were obtained directly from the reaction NMR tubes. ^{1}H NMR data (pyridine-d_5, 400 MHz) for **1s**: δ 6.954 (1H, d, J = 16.4 Hz, H-6), 6.109 (1H, m, H-3), 3.404 (1H, dd, J = 17.2, 8.0 Hz, H-4a), 3.156 (1H, dd, J = 17.2, 4.0 Hz, H-4b), 2.766 (2H, m, H-1), 2.200 (2H, m, H-2). **1r**: δ 7.046 (1H, d, J = 16.4 Hz, H-6), 6.090 (1H, m, H-3), 3.450 (1H, dd, J = 17.2, 8.0 Hz, H-4a), 3.222 (1H, dd, J = 17.2, 4.0 Hz, H-4b), 2.696 (2H, m, H-1), 2.126 (2H, m, H-2). **2s**: δ 7.044 (1H, d, J = 16.0 Hz, H-7), 6.549 (1H, dd, J = 16.0, 8.0 Hz, H-6), 6.121 (1H, q, J = 8.0 Hz, H-5), 5.445 (1H, m, H-3), 2.558 (1H, m, H-4a), 2.549 (2H, m, H-1), 2.276 (1H, m, H-4b), 2.008 (2H, m, H-2). **2r**: δ 6.876 (1H, d, J = 15.8 Hz, H-7), 6.331 (1H, dd, J = 15.8, 7.4 Hz, H-6), 5.963 (1H, q, J = 7.4 Hz, H-5), 5.490 (1H, m, H-3), 2.764 (2H, m, H-1), 2.518 (1H, m, H-4a), 2.297 (1H, m, H-4b), 2.192 (2H, m, H-2).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Spectroscopic data including ^{1}H, ^{13}C, DEPT, and 2D NMR of compounds **1** and **2**; ^{1}H, ^{13}C, and 2D NMR of compounds **3**−**6** and **8**; and ^{1}H NMR of the

(*R*)- and (*S*)-MTPA esters of compounds **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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