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Diterpenoids from the Twigs and Leaves of *Fokienia hodginsii*

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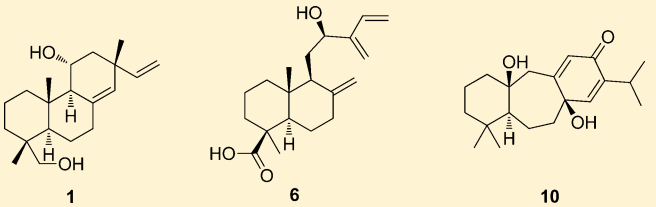
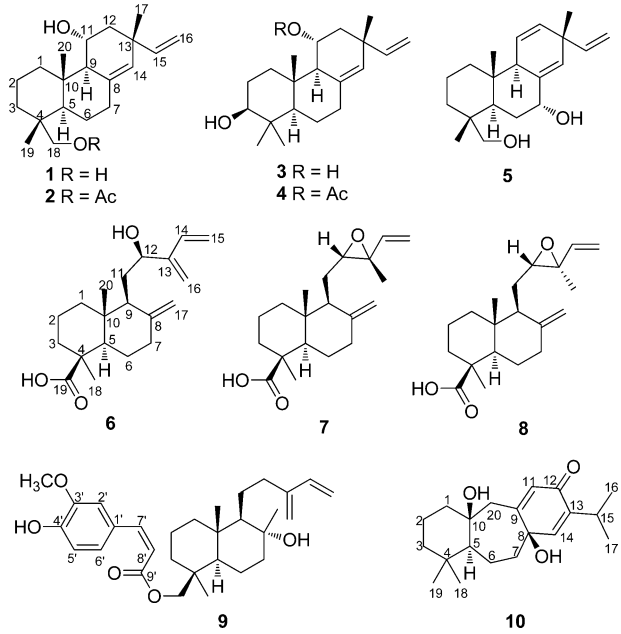
Supporting Information

ABSTRACT: Five new isopimarane diterpenoids, fokihodgins A–E (**1**–**5**), four new labdane diterpenoids, fokihodgins F–I (**6**–**9**), and one new icetexane diterpenoid, fokihodgin J (**10**), as well as 18 known diterpenoids were isolated from *Fokienia hodginsii*. The structures of the new compounds were determined on the basis of their spectroscopic analysis, and the absolute configurations of **1** and **6** were established by X-ray crystallographic analysis. Compound **9** showed moderate cytotoxicity against HL-60 and SMMC-7721 cell lines, with IC_{50} values of 9.10 and 7.50 μM, respectively.



The family Cupressaceae contains about 22 genera and 150 species, of which eight genera and 29 species are distributed in China.[1] Many species of this family are economically and medicinally valuable and are used to treat a variety of ailments in traditional Chinese medicine.[2] Phytochemically, plants of the family Cupressaceae are rich sources of structurally diverse diterpenoids, many of which have been reported to possess cytotoxic and antimicrobial activities.[3−7]

Fokienia hodginsii, belonging to the monotypic genus *Fokienia* of the Cupressaceae family, is mainly distributed in southwestern China and northern Vietnam.[8] The heartwood of *F. hodginsii* has been used in Chinese folk medicine for the treatmeat of stomach pain, nausea, and vomiting.[9] Previous chemical investigations of this plant have been mainly focused on the composition of the essential oil and lipids.[10−13] As part of our research on bioactive compounds from monotypic genus species,[14−17] phytochemical investigation of the twigs and leaves of *F. hodginsii* led to the isolation of five new isopimarane diterpenoids, fokihodgins A–E (**1**–**5**), four new labdane diterpenoids, fokihodgins F–I (**6**–**9**), and one new icetexane diterpenoid, fokihodgin J (**10**), as well as 18 known diterpenoids. Herein, we describe the isolation, structural elucidation, and cytotoxicity evaluation of these compounds.



RESULTS AND DISCUSSION

The 95% EtOH extract of the twigs and leaves of *F. hodginsii* was partitioned between H_2O and EtOAc. The EtOAc fraction was subjected repeatedly to column chromatography over silica gel, MCI gel, RP-C_{18} gel, Sephadex LH-20, and semipreparative HPLC to afford 10 new (**1**–**10**) and 18 known (**11**–**28**) diterpenoids. The known compounds (Supporting Information, Figure S1) were identified as sandaracopimaric acid (**11**),[18] isopimara-8(14),15-dien-11α-ol (**12**),[19] isopimara-8(14),15-diene-3β,18-diol (**13**),[20] 12*S*-hydroxylabda-8(17),13(16),14-trien-19-oic acid (**14**),[21] *trans*-communic acid (**15**),[22] *trans*-communal (**16**),[23] isocupressic acid (**17**),[24] acetylisocupressic acid (**18**),[25] 13-*epi*-cupressic acid (**19**),[24] 15-*nor*-labda-8(17),12*E*-diene-14-carboxaldehyde-19-oic acid (**20**),[26] pinuliso-lidic acid (**21**),[22] 8α-hydroxylabda-13(16),14-dien-19-yl (*E*)-coumarate (**22**),[27] 8α-hydroxylabda-13(16),14-dien-19-yl (*E*)-ferulate (**23**),[28] 8α-hydroxylabda-13(16),14-dien-19-yl (*Z*)-

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Table 1. ^{1}H NMR Data of Compounds 1−5 (δ_H, J in Hz)

no.	1[a]	2[a]	3[a]	4[b]	5[b]
1α	1.35, m	1.54, overlap	1.50, m	1.44, m	1.25, m
1β	1.86, m	1.86, m	1.89, m	1.51, m	1.76, overlap
2α	1.58, m	1.55, overlap	1.69, m	1.60, m	1.59, overlap
2β	1.58, m	1.55, overlap	1.58, m	1.60, m	1.59, overlap
3α	1.30, overlap	1.39, overlap	3.29, dd (11.3, 4.2)	3.20, dd (11.3, 4.4)	1.58, overlap
3β	1.46, overlap	1.39, overlap			1.29, m
5α	1.47, overlap	1.39, overlap	1.10, dd (12.5, 2.5)	1.14, dd (12.5, 2.3)	1.96, dd (13.2, 2.7)
6α	1.53, m	1.51, m	1.66, m	1.69, m	1.74, overlap
6β	1.31, overlap	1.32, m	1.36, ddd (17.3, 12.5, 4.4)	1.40, ddd (17.3, 12.5, 4.5)	1.59, overlap
7α	2.08, dt (13.2, 5.4)	2.02, m	2.04, dt (13.4, 5.3)	2.07, m	4.19, t (2.7)
7β	2.30, m	2.30, m	2.34, m	2.37, m	
9α	1.81, d, (4.7)	1.79, d, (4.5)	1.71, d, (4.7)	1.96, d, 6.4	2.75, br s
11	4.03, dt (6.7, 4.7)	4.03, dt (6.5, 4.5)	4.01, dt (6.7, 4.7)	5.21, m	5.69, dd (10.2, 3.4)
12α	1.64, d (6.7)	1.64, d (6.5)	1.64, d (6.7)	1.47, m	5.57, m
12β	1.64, d (6.7)	1.64, d (6.5)	1.64, d (6.7)	1.71, m	
14	5.30, br s	5.31, br s	5.30, br s	5.34, br s	5.46, br s
15	5.87, dd (17.5, 10.5)	5.86, dd (17.5, 10.5)	5.87, dd (17.5, 10.5)	5.80, dd (17.5, 10.6)	5.79, dd (17.5, 10.5)
16a	4.99, dd (17.5, 1.2)	4.99, dd (17.5, 1.1)	5.01, dd (17.5, 1.2)	5.01, dd (17.5, 1.2)	4.95, dd (17.5, 1.2)
16b	4.91, dd (10.5, 1.2)	4.91, dd (10.5, 1.1)	4.91, dd (10.5, 1.2)	4.91, dd (10.6, 1.2)	4.92, dd (10.5, 1.2)
17	1.05, s	1.04, s	1.05, s	1.10, s	1.11, s
18a	3.40, d (10.9)	3.87, d (10.9)	1.01, s	1.00, s	3.38, d (11.4)
18b	3.10, d (10.9)	3.62, d (10.9)			2.99, d (11.4)
19	0.79, s	0.85, s	0.82, s	0.81, s	0.76, s
20	0.86, s	0.85, s	0.82, s	0.88, s	0.79, s
OAc		2.06, s		1.99, s	

[a]Recorded in $CDCl_3$ at 400 MHz. [b]Recorded in methanol-d_4 at 400 MHz.

coumarate (**24**),[28] ferruginol (**25**),[29] pisiferol (**26**),[30] pisiferal (**27**),[30] and 5-*epi*-pisiferol (**28**),[7] respectively, by comparison of observed and reported spectroscopic data.

Fokihodgin A (**1**) was obtained as colorless crystals. Its molecular formula was established as $C_{20}H_{32}O_2$ by HREIMS at m/z 304.2397 $[M]^+$ (calcd 304.2402), requiring five indices of hydrogen deficiency. The IR absorption bands of **1** revealed the presence of hydroxy (3419 cm^{-1}) and olefinic (1635 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of three tertiary methyls at δ_H 1.05 (s, H_3-17), 0.79 (s, H_3-19), and 0.86 (s, H_3-20), a characteristic isolated AB methylene group at δ_H 3.10 and 3.40 (both d, both J = 10.9 Hz), an oxygenated methine at δ_H 4.03 (dt, J = 6.7, 4.7 Hz), an olefinic methine at δ_H 5.30 (br s), and a monosubstituted olefinic moiety at δ_H 5.87 (dd, J = 17.5, 10.5 Hz), 4.99 (dd, J = 17.5, 1.2 Hz), and 4.91 (d, J = 10.5, 1.2 Hz). The ^{13}C NMR and DEPT spectra of **1** (Table 3) displayed 20 carbon signals, consisting of three methyl, eight methylene (one oxygenated and one sp^2), five methine (one oxygenated and two sp^2), and four quaternary (one sp^2) carbons. The aforementioned spectroscopic analysis suggested **1** was an isopimarane diterpenoid with one secondary hydroxy and one hydroxymethyl group. Detailed analysis of the NMR spectroscopic data of **1** with those of isopimara-8(14),15-dien-11α-ol (**12**) indicated their structural similarity, except for the hydroxymethyl group (δ_C 72.0, C-18) in **1** instead of a C-18 methyl group in **12**. This deduction was supported by HMBC correlations (Figure 1) from H-18a (δ_H 3.40, d, J = 10.9 Hz) and H-18b (δ_H 3.10, d, J = 10.9 Hz) to C-4 (δ_C 37.8), C-5 (δ_C 47.8), and C-19 (δ_C 17.9).

The relative configuration of **1** was established by a ROESY experiment (Figure 1). The ROESY correlations of H-5/H-9 and H-5/H_2-18 indicated the α-orientation of H-5, H-9, and the hydroxymethyl group, while the correlations of H-11/H_3-17, H-11/H_3-20, and H_3-19/H_3-20 suggested the β-orientation of H-11, H_3-17, H_3-19, and H_3-20. Finally, the absolute configuration of **1** was determined by X-ray crystallography based on an anomalous dispersion of Cu Kα radiation (Figure 2). The C-4, C-5, C-9, C-10, C-11, and C-13 absolute configurations were thus assigned as *R*, *R*, *S*, *S*, *R*, and *R*, respectively. Therefore, the structure of **1** was assigned as 11*R*-isopimara-8(14),15-diene-11,18-diol.

The molecular formula of fokihodgin B (**2**) was assigned as $C_{22}H_{34}O_3$, according to its HREIMS ($[M]^+$ m/z 346.2513, calcd 346.2508). The ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 3) were similar to those of **1**. The only difference was the hydroxy group at C-18 in **1** was replaced by an acetoxy group in **2**, as deduced from the correlations of H-18a (δ_H 3.87, J = 10.9 Hz) and H-18b (δ_H 3.62, J = 10.9 Hz) with an ester carbonyl carbon (δ_C 171.3) in the HMBC experiment. The relative configurations of both **2** and **1** were identical according to the observed ROESY correlations. Consequently, **2** was determined as 18-*O*-acetylisopimara-8(14),15-dien-11α-ol.

Fokihodgin C (**3**) exhibited the molecular formula $C_{20}H_{32}O_2$, as determined on the basis of its HREIMS ($[M]^+$ m/z 304.2397, calcd 304.2402). The ^{1}H and ^{13}C NMR data of **3** (Tables 1 and 3) were similar to those of **12**, suggesting that they were structural analogues. The main difference was the presence of an additional hydroxy group in **3** as compared with **12**. The HMBC correlations from H-3 (δ_H 3.29, dd, J = 11.3, 4.2 Hz) to C-1 (δ_C 37.9), C-2 (δ_C 27.6), C-4 (δ_C 39.0), C-5 (δ_C 54.2), and C-19 (δ_C 15.8) indicated the location of the hydroxy group at C-3. The ROESY correlations of H-3/H-5 and H-3/H_3-18 indicated the α-orientation of H-3. In addition, H-11 was determined as β-oriented in view of the ROESY correlations of

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Table 2. ^{1}H NMR Data of Compounds 6−8 and 10 (δ_H, J in Hz)

no.	6[a]	7[b]	8[b]	10[c]
1α	1.16, dt (12.0, 3.2)	1.08, overlap	1.16, m	1.43, dt (13.0, 3.7)
1β	1.80, m	1.70, m	1.81, overlap	1.57, br d (12.7)
2α	1.51, m	1.49, m	1.49, m	1.32, m
2β	1.84, m	1.89, overlap	1.89, overlap	1.77, m
3α	1.06, dt (13.3, 3.6)	1.07, overlap	1.07, m	1.02, m
3β	2.14, br d (13.3)	2.14, m	2.14, m	1.27, m
5α	1.41, br d (11.2)	1.41, m	1.36, m	0.60, d (7.0)
6α	2.01, m	2.01, m	1.89, overlap	1.81, m
6β	1.89, m	1.89, overlap	2.01, m	1.28, m
7α	2.42, m	2.43, m	2.43, m	0.95, m
7β	1.99, m	1.98, m	1.98, m	2.07, m
9α	2.09, m	1.74, m	1.81, overlap	
11a	1.67, m	1.72, m	1.76, m	5.84, br s
11b	1.67, m	1.65, m	1.76, m	
12	4.41, m	2.95, dd, (7.2, 3.1)	2.80, t like	
14	6.33, dd (17.8, 11.1)	5.86, dd (17.2, 11.3)	5.63, dd (17.4,10.8)	6.61, br s
15a	5.41, d (17.8)	5.35, dd (17.2, 1.4)	5.30, dd (10.8, 1.1)	2.78, hep (7.0)
15b	5.12, d (11.1)	5.30, dd (11.3, 1.4)	5.28, dd (17.4, 1.1)	
16a	5.21, br s	1.35, s	1.39, s	0.98, d (6.8)
16b	5.14, br s			
17a	4.52, br s	4.92, br s	4.92, br s	1.01, d (6.8)
17b	4.88, br s	4.74, br s	4.74, br s	
18	1.24, s	1.20, s	1.21, s	0.74, s
19				0.90, s
20α	0.57, s	0.62, s	0.65, s	3.11, d (12.7)
20β				2.14, d (12.7)
8-OH				5.53, br s
10-OH				4.33, br s

[a]Recorded in $CDCl_3$ at 400 MHz. [b]Recorded in methanol-d_4 at 400 MHz. [c]Recorded in DMSO-d_6 at 600 MHz.

H-11/H_3-17 and H-11/H_3-20. Thus, compound **3** was elucidated as isopimara-8(14),15-diene-3β,11α-diol.

Fokihodgin D (**4**) had the molecular formula $C_{22}H_{34}O_3$, based on the HRESIMS ([M + Na]$^+$ m/z 369.2395, calcd 369.2405), which was 42 mass units greater than **3**. Comparing the NMR data of **4** (Tables 1 and 3) with those of **3** indicated that the two compounds were related, except for the existence of an acetoxy group in **4**. The acetoxy group was located at C-11 based on the HMBC correlations from H-11 (δ_H 5.21, m) to C-9 (δ_C 56.6), C-12 (δ_C 43.4), and an acetoxy carbonyl carbon (δ_C 172.4), which was further confirmed by the downfield shift of C-11 from δ_C 66.2 in **3** to δ_C 70.4 in **4**. The relative configuration of **4** was determined to be the same as **3** based on the detailed analysis of the ROESY spectrum. Accordingly, the structure of compound **4** was elucidated as 11α-*O*-acetylisopimara-8(14),15-dien-3β-ol.

Fokihodgin E (**5**) gave a molecular formula of $C_{20}H_{30}O_2$ by HREIMS ([M]$^+$ m/z 302.2249, calcd 302.2246). The ^{1}H and ^{13}C NMR data of **5** (Tables 1 and 3) were similar to those of **1**, with the main differences being that an oxygenated methine at C-11 and a methylene at C-12 in **1** were changed into a double bond in **5**, as inferred from the HMBC correlations from H-11 (δ_H 5.69, dd, J = 10.2, 3.4 Hz) to C-9 (δ_C 48.3), C-10 (δ_C 40.6), and C-12 (δ_C 134.8) and from H-12 (δ_H 5.57, m) to C-11 (δ_C 124.1), C-13 (δ_C 40.5), and C-14 (δ_C 130.5). The other difference between these two compounds was that an oxymethine at C-7 (δ_C 73.9) in **5** replaced a methylene in **1**, as deduced from the HMBC correlations of H-7 (δ_H 4.19, t, J = 2.7 Hz) with C-5 (δ_C 41.5), C-6 (δ_C 30.9), C-8 (δ_C 136.8), and C-14. The ROESY correlations of H-5/H_2-18 and H-5/H-9 indicated the α-orientation of H_2-18. Additionally, the hydroxy group at C-7 was α-oriented because of the small coupling constant (2.7 Hz) between H-7 and H_2-6, which was further confirmed by the cross-peak of H-7 with H-14 in the ROESY spectrum. Hence, the structure of **5** was assigned as isopimara-8(14),11,15-triene-7α,18-diol.

Fokihodgin F (**6**) was obtained as colorless crystals and had a molecular formula of $C_{20}H_{30}O_3$ as deduced from the HREIMS at m/z 318.2200 [M]$^+$ (calcd 318.2195), indicating six indices of hydrogen deficiency. The IR spectrum showed the presence of a hydroxy (3440 cm^{-1}) and double-bond (1641 and 1650 cm^{-1}) groups. Analysis of the ^{1}H NMR, ^{13}C NMR, and DEPT data (Tables 2 and 3) revealed the existence of two tertiary methyl groups, six methylene carbons, two methine carbons, two sp^3 quaternary carbons, one oxymethine carbon [δ_C 69.9 (d, C-12)], three terminal double bonds [δ_C 148.9 (s, C-8), 150.4 (s, C-13), 136.0 (d, C-14), 114.7 (t, C-15), 113.3 (t, C-16), 106.5 (t, C-17)], and one carboxy group [δ_C 183.9 (s, C-18)] in **6**. The above spectroscopic data showed that **6** had a structure similar to 12*S*-hydroxylabda-8(17),13(16),14-trien-19-oic acid (**13**). Their 1D NMR data showed many similarities except for obvious differences of the chemical shifts of the protons and carbons around C-12 in the side chain, suggesting that **6** was likely the stereoisomer of **13** at C-12. The proposed structure was rigorously determined by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Figure 3), and its absolute configuration was established as 4*S*, 5*R*, 9*S*, 10*R*, and 12*R*. Accordingly, compound **6** was elucidated as 12*R*-hydroxylabda-8(17),13(16),14-trien-19-oic acid.

Fokihodgin G (**7**) and fokihodgin H (**8**) were isolated as a pair of epimers both having the molecular formula $C_{20}H_{30}O_3$ from their HREIMS ([M]$^+$ m/z 318.2177, calcd 318.2195), corresponding to six indices of hydrogen deficiency. Their NMR data (Tables 2 and 3) were similar to those of *trans*-communic acid (**14**), except that a double bond in **14** was replaced by a trisubstituted epoxide moiety in **7** [δ_C 65.4 (d, C-12), 60.6 (s, C-13)] and **8** [δ_C 64.8 (d, C-12), 59.5 (s, C-13)]. The HMBC correlations from H-14 and H_3-16 to C-12 and C-13 and from H-12 to C-9, C-11, and C-13 confirmed the location of the epoxide group between C-12 and C-13. The absolute configuration of **7** and **8** at C-12 could be elucidated from the chemical shifts of the vinyl protons at C-17. Owing to the deshielding effects of the epoxide group at C-12 and C-13, H_2-17 in a 12*S*-isomer appeared at lower field (near δ_H 4.75 and 4.90) than those (near δ_H 4.48 and 4.86) in a 12*R*-isomer.[31] Therefore, the H_2-17 signals at δ_H 4.74 and 4.92 of **7** and **8** indicated that both compounds had a 12*S* configuration. In the ROESY spectra of **7** and **8**, H-12 correlated to H-14 and H_3-16, respectively, suggesting the respective 13*R* and 13*S* configuration in **7** and **8**. Accordingly, the structure of compound **7** was elucidated as (12*S*,13*R*)-12,13-epoxylabda-8(17),14-dien-19-oic acid, and that of compound **8** as (12*S*,13*S*)-12,13-epoxylabda-8(17),14-dien-19-oic acid.

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Table 3. ^{13}C NMR Data of Compounds 1−8 and 10 (δ_C)

no.	1[a]	2[a]	3[a]	4[b]	5[b]	6[a]	7[b]	8[b]	10[c]
1	39.7, CH_2	39.5, CH_2	37.9, CH_2	38.4, CH_2	39.1, CH_2	38.7, CH_2	39.0, CH_2	39.1, CH_2	39.2, CH_2
2	18.3, CH_2	18.2, CH_2	27.6, CH_2	28.2, CH_2	19.4, CH_2	19.8, CH_2	19.5, CH_2	19.6, CH_2	18.6, CH_2
3	35.3, CH_2	35.8, CH_2	78.8, CH	79.3, CH	36.5, CH	37.8, CH_2	37.7, CH_2	37.7, CH_2	40.9, CH_2
4	37.8, qC	37.4, qC	39.0, qC	40.0, qC	38.7, qC	44.2, qC	43.6, qC	43.6, qC	34.2, qC
5	47.8, CH	48.5, CH	54.2, CH	55.2, CH	41.5, CH	56.2, CH	55.6, CH	55.6, CH	53.8, CH
6	22.8, CH_2	22.9, CH_2	22.6, CH_2	23.7, CH_2	30.9, CH_2	26.1, CH_2	25.8, CH_2	25.8, CH_2	17.5, CH_2
7	36.0, CH_2	35.9, CH_2	36.0, CH_2	37.0, CH_2	73.9, CH	38.9, CH_2	38.0, CH_2	38.0, CH_2	44.6, CH_2
8	136.1, qC	135.9, qC	135.8, qC	136.6, qC	136.8, qC	148.9, qC	147.9, qC	148.1, qC	69.8, qC
9	59.8, CH	59.7, CH	59.5, CH	56.6, CH	48.3, CH	51.7, CH	53.6, CH	53.9, CH	162.2, qC
10	38.9, qC	38.8, qC	38.8, qC	40.2, qC	40.6, qC	40.1, qC	39.0, qC	39.0, qC	72.3, qC
11	66.1, CH	66.1, CH	66.2, CH	70.4, CH	124.1, CH	31.2, CH_2	22.9, CH_2	23.3, CH_2	125.9, CH
12	43.4, CH_2	43.4, CH_2	43.4, CH_2	43.4, CH_2	134.8, CH	69.9, CH	65.4, CH	64.8, CH	184.9, qC
13	37.5, qC	37.4, qC	37.5, qC	38.8, qC	40.5, qC	150.4, qC	60.6, qC	59.5, qC	139.6, qC
14	127.5, CH	127.7, CH	127.7, CH	129.6, CH	130.5, CH	136.0, CH	136.0, CH	140.6, CH	147.6, CH
15	149.0, CH	148.9, CH	148.9, CH	149.1, CH	147.3, CH	114.7, CH_2	116.7, CH_2	114.6, CH_2	25.2, CH
16	110.6, CH_2	110.7, CH_2	110.7, CH_2	110.9, CH_2	112.1, CH_2	113.3, CH_2	20.2, CH_3	13.8, CH_3	21.5, CH_3
17	26.9, CH_3	27.0, CH_3	27.0, CH_3	26.3, CH_3	28.1, CH_3	106.5, CH_2	106.3, CH_2	106.5, CH_2	21.5, CH_3
18	72.0, CH_2	72.7, CH_2	28.5, CH_2	29.1, CH_3	71.7, CH_2	26.1, CH_3	27.9, CH_3	27.9, CH_3	31.4, CH_3
19	17.9, CH_3	17.8, CH_3	15.8, CH_3	16.3, CH_3	17.8, CH_3	183.9, qC	179.6, qC	179.6, qC	21.5, CH_3
20	16.4, CH_3	16.4, CH_3	15.7, CH_3	16.3, CH_3	15.5, CH_3	12.8, CH_3	11.6, CH_3	11.6, CH_3	46.8, CH_2
−OAc		21.0, CH_3		21.6, CH_3					
		171.3, qC		172.4, qC					

[a]Recorded in $CDCl_3$ at 100 MHz. [b]Recorded in methanol-d_4 at 100 MHz. [c]Recorded in DMSO-d_6 at 150 MHz.

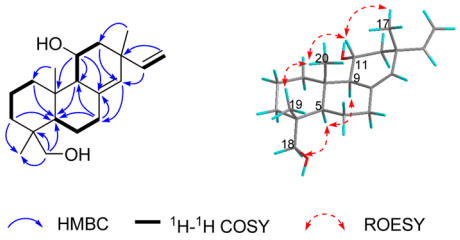

Figure 1. Key 2D NMR correlations of compound 1.

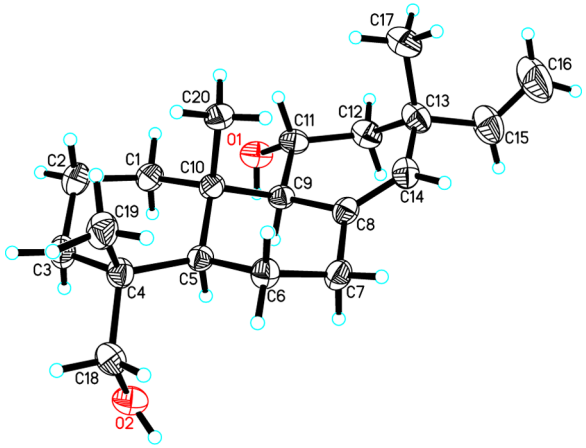

Figure 2. X-ray crystallographic structure of compound 1.

The HREIMS of fokihodgin I (**9**) exhibited a molecular ion peak at m/z 482.3034 $[M]^+$ (calcd 482.3032), suggesting a molecular formula of $C_{30}H_{42}O_5$, with 10 indices of hydrogen deficiency. Detailed comparison of the NMR data of **9** (Table 4) with those of 8α-hydroxylabda-13(16),14-dien-19-yl (*E*)-ferulate (**22**) indicated that they were structural analogues, and the only difference was the presence of a (*Z*)-feruloyl unit [δ_H 7.73 (1H, d, J = 1.6 Hz, H-2′), 6.78 (1H, d, J = 8.2 Hz, H-5′),

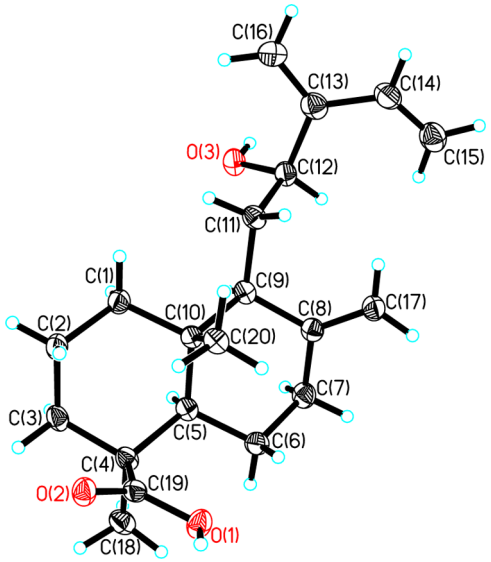

Figure 3. X-ray crystallographic structure of compound 6.

7.10 (1H, dd, J = 8.2, 1.6 Hz, H-6′), 6.88 (1H, d, J = 12.9 Hz, H-7′), 5.80 (1H, d, J = 12.9 Hz, H-8′), and 3.88 (s, OCH_3)] at C-19 in **9** rather than an (*E*)-feruloyl moiety in **22**. This assignment was inferred from the HMBC correlations of H-19a (δ_H 4.28, d, J = 11.1 Hz) and H-19b (δ_H 3.95, d, J = 11.1 Hz) with an ester carbonyl carbon at δ_C 168.6 of the (*Z*)-feruloyl group. Hence, the structure of **9** was assigned as 8α-hydroxylabda-13(16),14-dien-19-yl (*Z*)-ferulate.

The molecular formula of fokihodgin J (**10**) was established as $C_{20}H_{30}O_3$ on the basis of its HREIMS at m/z 318.2198 $[M]^+$ (calcd 318.2195), indicating six indices of hydrogen deficiency. The IR spectrum of **10** showed absorption bands at 3441 cm^{-1} (hydroxy) and 1666 cm^{-1} (conjugated carbonyl). The ^{13}C NMR and DEPT spectrum of **10** (Table 3) showed 20 carbons

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Table 4. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of Compound 9 in Methanol-d_4, δ in ppm, J in Hz

no.	δ_H	δ_C	no.	δ_H	δ_C
1α	1.08, m	41.0, CH_2	14	6.38, dd (17.6, 10.7)	140.2, CH
1β	1.74, m		15a	5.34, d (17.6)	115.9, CH_2
2α	1.45, overlap	19.1, CH_2	15b	5.05, d (10.7)	
2β	1.64, m		16a	5.03, br s	113.8, CH_2
3α	1.03, m	17.5, CH_2	16b	4.99, br s	
3β	1.66, m		17	1.12, s	23.7, CH_3
4		38.2, qC	18	0.96, s	28.0, CH_3
5α	1.14, m	58.0, CH	19a	4.28, d (11.1)	67.9, CH_2
6α	1.76, m	21.8, CH_2	19b	3.95, d (11.1)	
6β	1.40, m		20	0.85, s	16.6, CH_3
7α	1.84, m	45.5, CH_2	1′		128.3, qC
7β	1.44, overlap		2′	7.73, d (1.6)	114.8, CH
8		74.8, qC	3′		149.2, qC
9α	1.18, t (3.5)	62.9, CH	4′		148.4, qC
10		40.1, qC	5′	6.78, d (8.2)	115.7, CH
11a	1.38, m	26.3, CH_2	6′	7.10, dd, (8.2, 1.6)	126.4, CH
11b	1.68, m		7′	6.88, d (12.9)	145.1, CH
12a	2.45, dt (13.4, 4.8)	46.2, CH_2	8′	5.80, d (12.9)	116.9, CH
12b	2.22, dt (13.4, 4.9)		9′		168.6, qC
13		149.4, qC	OCH_3	3.88, s	56.3, CH_3

attributed to four methyl, six methylene, four methine (two sp^2), and six quaternary (one conjugated carbonyl, two oxygenated, and two sp^2) carbons. In the ^{1}H NMR spectrum (Table 1), two methyl signals at δ_H 0.98 and 1.01 (each 3H, d, J = 6.8 Hz, Me-16 and Me-17) and a methine signal at δ_H 2.78 (1H, hep, J = 7.0 Hz, H-11) suggested the presence of an isopropyl group. The above NMR data resembled those of sawaradienone, with the only difference being the presence of a methylene at δ_C 46.8 (C-20) in **10** instead of an oxymethine in sawaradienone,[32] which was confirmed by the HMBC correlations (Figure 4) from H_2-20 (δ_H 2.14, d, J = 12.7 Hz; 3.11, d, J = 12.7 Hz) to C-5 (δ_C 53.8), C-9 (δ_C 162.2), and C-10 (δ_C 72.3). The ROESY correlations (Figure 4) between H-5 and H_3-18 suggested the α-orientation of H_3-18 and H-5. In addition, the 8-OH and 10-OH were assigned as β-oriented based on the cross-peaks of H-6β/8-OH, H-6β/10-OH, 8-OH/H-20β, H-20β/10-OH, and OH-10/H_3-19 in the ROESY experiment. Thus, the structure of **10** was assigned as 8β,10β-dihydroxyicetexa-9(11),13-dien-12-one.

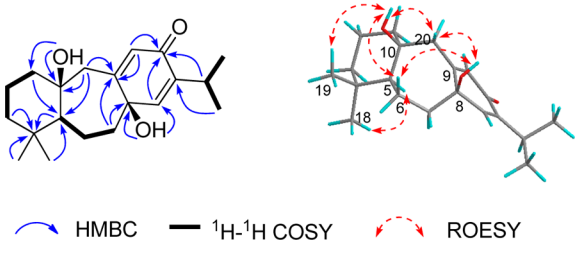

Figure 4. Key 2D NMR correlations of compound **10**.

All compounds were tested for their cytotoxicity against human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW-480) cell lines using the MTT method as previously reported.[33] Cisplatin (Sigma, USA) was used as the positive control. The results showed that compounds **22**–**24**, **27**, and **28** exhibited weak cytotoxicity against the above cancer cell lines. Compound **9** also showed weak cytotoxicity against A-549, MCF-7, and SW-480 cell lines; however, it exhibited moderate cytotoxicity against HL-60 and SMMC-7721 cell lines, with IC_{50} values of 9.10 and 7.50 μM, respectively (Table 5). The other compounds were inactive (IC_{50} > 40 μM).

Table 5. Cytotoxicity of Compounds 9, 22–24, 27, and 28 against Five Tumor Cell Lines (IC_{50} μM)

compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480
9	9.10	7.50	17.32	15.37	17.61
22	11.77	15.48	20.35	16.58	19.30
23	14.28	14.05	16.77	13.45	16.94
24	14.05	16.24	17.05	15.05	13.64
27	14.28	19.70	21.01	15.62	14.36
28	15.48	15.23	20.68	15.02	15.12
cisplatin[a]	1.14	14.51	12.76	19.61	17.54

[a]Positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained by a Tensor 27 spectrophotometer with KBr pellets. 1D and 2D spectra were run on a Bruker AM-400 or an Avance III 600 spectrometer with TMS as the internal standard. X-ray data were collected using a Bruker APEX DUO instrument. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS were performed on an API QSTAR time-of-flight spectrometer. EIMS and HREIMS were recorded on a Waters Autospec Premier P776 spectrometer. Semipreparative HPLC was performed on an Agilent 1200 apparatus equipped with a UV detector and a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. MPLC was performed on a Lisui EZ Purify III System including pump manager P03, detector modules P02, and fraction collector P01 (Shanghai Lisui Chemical Engineering Co., Ltd., Shanghai, China). Column chromatography (CC) was performed using silica gel (200–300 mesh and H, Qingdao Marine Chemical Co. Ltd., Qingdao, China), RP-C_{18} gel (40–63 μm, Merck, Darmstadt, Germany), MCI gel (75–150 μm; Mitsubishi Chemical Corporation, Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Co. Ltd., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents were distilled prior to use.

Plant Material. The twigs and leaves of *F. hodginsii* were collected from the Kunming Botany Garden, Yunnan Province, People's Republic of China, in December 2010, and identified by one of the authors (X. Gong). A voucher specimen (KIB20101215f01) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered twigs and leaves of *F. hodginsii* (15 kg) were extracted three times with 95%

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EtOH (60 L × 3) at room temperature and concentrated in vacuo to yield a residue (1.4 kg), which was partitioned between H_2O and EtOAc. The EtOAc fraction (840 g) was subjected to silica gel CC with a gradient elution of petroleum ether–acetone (1:0 to 0:1) to afford six fractions, A–F. Fraction B (180 g) was crystallized from petroleum ether–acetone (1:1) to yield **15** (11.5 g). The filtrate was condensed to give a yellow residue, which was chromatographed repeatedly over silica gel eluted with petroleum ether–EtOAc (9.5:0.5 to 7:3) to obtain **12** (5.5 mg), **16** (7.0 mg), **18** (11.4 mg), and **25** (4.5 mg). Fraction C (85 g) was fractionated by MPLC (RP-C_{18}), eluting with MeOH–H_2O (60:40 to 100:0), to provide six subfractions, C1–C6. Subfraction C2 was subjected to silica gel CC eluted with petroleum ether–acetone (9:1 to 6:3), then recrystallized from MeOH, and compounds **21** (22.6 mg) and **22** (185.3 mg) were obtained. The mother liquor was further chromatographed over silica gel repeatedly and then purified by semipreparative HPLC (MeOH–H_2O, 64:33) to afford **6** (13.7 mg) and **14** (2.5 mg). Compounds **1** (8.2 mg), **3** (27.5 mg), **5** (14.5 mg), and **13** (18.4 mg) were obtained from subfraction C3 by CC over silica gel using $CHCl_3$–Me_2CO (9.5:0.5 to 7:3). Subfraction C4 was further subjected to CC over silica gel eluted with petroleum ether–acetone (9:1 to 7:3) and purified on Sephadex LH-20 (MeOH) to provide **2** (18.4 mg), **4** (24.3 mg), **17** (154.5 mg), and **23** (8.7 mg). Compound **11** (5.5 g) was obtained by recrystallization in MeOH from subfraction C5 directly. Fraction D (55 g) was chromatographed on MPLC (MCI gel) eluting with MeOH–H_2O (60:40 to 100:0) to yield five subfractions, D1–D5. Subfraction D2 was separated by silica gel CC ($CHCl_3$–Me_2CO, 9:1 to 1:1) and then by Sephadex LH-20 (MeOH) to give **26** (35.3 mg) and **28** (12.5 mg). Compounds **9** (18.2 mg), **19** (22.3 mg), and **24** (15.5 mg) and the pair of epimers **7** and **8** (2.1 mg) were isolated from subfraction D3 by repeated CC over silica gel eluted with petroleum ether–acetone (9:1 to 1:1). Fraction E (25 g) was subjected to repeated column chromatography and purified by Sephadex LH-20 and semipreparative HPLC to afford **10** (22.6 mg), **20** (5.4 mg), and **27** (11.6 mg).

*Fokihodgin A (**1**):* colorless needles; mp 87–89 °C; $[\alpha]_D^{26}$ −30.2 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.89) nm; IR (KBr) ν_{max} 3419, 2932, 2867, 1635, 1458, 1411, 1384, 1134, 1026, 908, 861, 686, 668 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 304 $[M]^+$ (42), 286 (33), 274 (45), 273 (78), 256 (62), 255 (97), 121 (100), 95 (89); HREIMS $[M]^+$ *m*/*z* 304.2397 (calcd for $C_{20}H_{32}O_2$, 304.2402).

*Fokihodgin B (**2**):* colorless oil; $[\alpha]_D^{26}$ −25.6 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.95), 244 (3.0) nm; IR (KBr) ν_{max} 3433, 2928, 2867, 1738, 1634, 1459, 1411, 1380, 1242, 1038, 911, 863, 668 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 346 $[M]^+$ (53), 303 (8), 328 (28), 274 (15), 273 (49), 256 (22), 255 (61), 135 (100), 121 (68); HREIMS $[M]^+$ *m*/*z* 346.2513 (calcd for $C_{22}H_{34}O_3$, 346.2508).

*Fokihodgin C (**3**):* amorphous powder; $[\alpha]_D^{26}$ −63.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.12) nm; IR (KBr) ν_{max} 3396, 2939, 2870, 1635, 1451, 1382, 1365, 1181, 1090, 1035, 995, 909, 618 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 304 $[M]^+$ (18), 286 (25), 268 (15), 153 (20), 152 (56), 135 (100), 105 (37); HREIMS $[M]^+$ *m*/*z* 304.2397 (calcd for $C_{20}H_{32}O_2$, 304.2402).

*Fokihodgin D (**4**):* colorless oil; $[\alpha]_D^{26}$ −65.3 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.91), 263 (2.68) nm; IR (KBr) ν_{max} 3385, 2939, 2872, 1772, 1637, 1459, 1435, 1382, 1239, 1090, 1022, 916, 668 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m*/*z* 369 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m*/*z* 369.2395 (calcd for $C_{22}H_{34}O_3Na$, 369.2405).

*Fokihodgin E (**5**):* colorless oil; $[\alpha]_D^{26}$ −28.1 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.69) nm; IR (KBr) ν_{max} 3423, 2927, 2867, 1726, 1630, 1452, 1384, 1360, 1287, 1041, 912, 871, 731 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m*/*z* 302 $[M]^+$ (8), 284 (9), 254 (10), 253 (32), 157 (37), 145 (40), 144 (100), 131 (58); 123 (81); HREIMS $[M]^+$ *m*/*z* 302.2249 (calcd for $C_{20}H_{30}O_2$, 302.2246).

*Fokihodgin F (**6**):* colorless cubic crystals; mp 81–83 °C; $[\alpha]_D^{26}$ +62.9 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.92), 222 (3.98) nm; IR (KBr) ν_{max} 3440, 2934, 2851, 1693, 1650, 1641, 1467, 1447, 1386, 1269, 1174, 1034, 890, 668 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* 318 $[M]^+$ (22), 300 (29), 285 (25), 222 (45), 221 (67), 133 (82), 121 (100), 81 (89); HREIMS $[M]^+$ *m*/*z* 318.2200 (calcd for $C_{20}H_{30}O_3$, 318.2195).

*Fokihodgins G (**7**) and H (**8**):* colorless oil; $[\alpha]_D^{27}$ +25.6 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.58) nm; IR (KBr) ν_{max} 3449, 2934, 2848, 1692, 1642, 1467, 1450, 1385, 1271, 1176, 1128, 890, 668 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* 318 $[M]^+$ (7), 303 (6), 248 (10), 247 (15), 189 (48), 133 (38), 121 (88), 81 (86), 55 (100); HREIMS $[M]^+$ *m*/*z* 318.2177 (calcd for $C_{20}H_{30}O_3$, 318.2195).

*Fokihodgin I (**9**):* colorless oil; $[\alpha]_D^{27}$ +9.9 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.35), 324 (4.14) nm; IR (KBr) ν_{max} 3441, 2930, 2853, 1709, 1631, 1515, 1452, 1388, 1271, 1161, 1124, 1033 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4; EIMS *m*/*z* 482 $[M]^+$ (5), 444 (4), 270 (5), 194 (31), 177 (100), 145 (23), 121 (100), 81 (27), 55 (28); HREIMS $[M]^+$ *m*/*z* 482.3034 (calcd for $C_{30}H_{42}O_5$, 482.3032).

*Fokihodgin J (**10**):* colorless oil; $[\alpha]_D^{27}$ − 122.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.70), 245 (4.05) nm; IR (KBr) ν_{max} 3441, 2934, 2869, 1666, 1628, 1466, 1387, 1251, 1161, 1110, 1045, 921 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* 318 $[M]^+$ (38), 300 (89), 285 (57), 275 (59), 174 (66), 165 (100), 153 (80), 69 (78); HREIMS $[M]^+$ *m*/*z* 318.2198 (calcd for $C_{20}H_{30}O_3$, 318.2195).

X-ray Crystal Structure Analysis. Colorless crystals of **1** and **6** were obtained from MeOH. The intensity data were collected on a Bruker APEX DUO diffractometer with Cu Kα radiation. The crystal structures of **1** and **6** were solved by the direct method (SHLXS-97), expanded using difference Fourier technique, and refined by the program and the full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions. Crystallographic data for the structures of **1** and **6** have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers: CCDC 914619 and 914620). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).

*Crystal data of fokihodgin A (**1**):* $C_{20}H_{32}O_2$, *M* = 304.5, orthorhombic, space group, $P2_12_12_1$, *a* = 8.3140(2) Å, *b* = 10.5467(3) Å, *c* = 20.9187(6) Å; $\alpha = \beta = \gamma = 90.00°$, *V* = 1834.26(9) $Å^3$, *Z* = 4, μ(Cu Kα) = 0.53 mm^{-1}, ρ_{calc} =1.10 g/cm^3, *F*(000) = 672, 3068 reflections independent and 3068 reflections observed ($w = 1/\sigma|F|^2$). The final R_1 = 0.034, wR_2 = 0.098, Flack parameter = 0.2(2).

*Crystal data of fokihodgin F (**6**):* $C_{20}H_{32}O_2{\cdot}H_2O$, *M* = 336.46, tetragonal, space group, I_4, *a* = 20.0401(8) Å, *b* = 20.0401(8) Å, *c* = 9.4689(4) Å; $\alpha = \beta = \gamma = 90°$, *V* = 3802.8(3) $Å^3$, *Z* = 8, ρ_{calc} =1.175 g/cm^3, μ(Cu Kα) = 0.638 mm^{-1}, *F*(000) = 1472, 3327 reflections independent and 3197 reflections observed ($w = 1/\sigma|F|^2$). The final R_1 = 0.051, wR_2 = 0.135, Flack parameter = 0.0(3).

Cytotoxicity Assay. The cytotoxicity of the compounds against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines was assessed using the MTT method. Cells were plated in 96-well plates 12 h before treatment and continuously exposed to different concentrations of compounds. After 48 h, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well, which were incubated for another 4 h. Then 20% SDS (100 μL) were added to each well. After 12 h at room temperature, the OD value of each well was recorded at 595 nm. The IC_{50} value of each compound was calculated by the Reed and Muench method.[34]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Structures of known compounds, 1D and 2D NMR spectra, and MS spectra of fokihodgins A–E (**1**–**10**). These materials 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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