JOURNAL OF NATURAL PRODUCTS

ARTICLE

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Dibenzocyclooctadiene Lignans with Antineurodegenerative Potential from *Kadsura ananosma*

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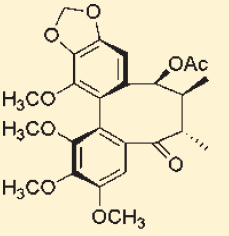
Supporting Information

ABSTRACT: Fourteen new dibenzocyclooctadiene lignans, ananolignans A−N (**1**−**14**), together with five known compounds, were isolated from the seeds of *Kadsura ananosma*. The structures and absolute configurations of **1**−**14** were established using a combination of spectroscopic methods including 1D- and 2D-NMR and CD techniques. The biological activity of the isolated lignans was evaluated, and ananolignan F (**6**) and ananolignan L (**12**) showed significant neuroprotective effects in an in vitro assay.



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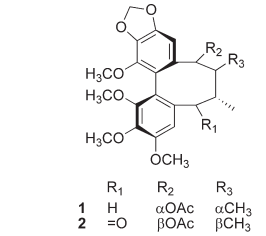
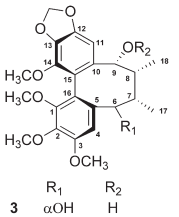
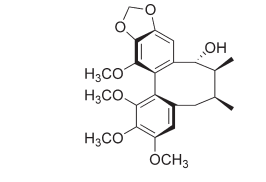
The economically and medicinally important family Schisandraceae contains two genera, *Schisandra* and *Kadsura*. Phytochemical and biological studies have shown that plants in this family are sources of dibenzocyclooctadiene lignans,[1−4] which possess various effects such as antitumor,[5] anti-HIV,[6,7] and cytotoxic[8] bioactivities. *Kadsura ananosma* Kerr is a liana indigenous to Yunnan Province, People's Republic of China.[9] Previous work has led to the isolation of triterpenoids, sesquiterpenoids, and lignans from the stems of this plant.[10−16] In the present study, the seeds of *K. ananosma* were studied for the first time. As a result, 19 dibenzocyclooctadiene lignans were isolated including 14 new compounds, ananolignans A−N (**1**−**14**), along with five known analogues. The structures of these new compounds were established by detailed analysis of their spectroscopic data, especially the 2D-NMR and CD spectra. Our group has initiated a program to discover secondary metabolites with antineurodegenerative activity from plants. In this paper, the isolation and structure elucidation of compounds **1**−**14** and the antineurodegenerative activity in an in vitro assay of 19 dibenzocyclooctadiene lignans are reported.



	R_1	R_2	R_3
1	H	αOAc	αCH_3
2	=O	βOAc	βCH_3



	R_1	R_2
3	αOH	H
4	αOH	Ac
5	βOAc	H
6	βOAc	Ac
7	βOAc	Prop
8	βOAc	Isobut
9	βOAc	But
10	βOAc	Isoval
11	βOAc	Bz
12	βOTig	Ac
13	βOAng	Isobut
14	βOAng	But
15	H	H
16	H	Ac
17	βOTig	H
18	βOAng	Ac



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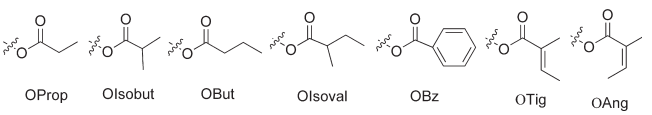

RESULTS AND DISCUSSION

A 70% aqueous acetone extract of the seeds of *K. ananosma* was partitioned between EtOAc and H_2O. The EtOAc layer was subjected repeatedly to column chromatography and HPLC to

Received: December 19, 2010
Published: March 07, 2011

dx.doi.org/10.1021/np1009288 | *J. Nat. Prod.* 2011, 74, 1028–1035

Table 1. ^{1}H NMR Data of 1−7 in $CDCl_3$, δ in ppm (J in Hz)

position	1[a]	2[b]	3[b]	4[a]	5[a]	6[a]	7[b]
4	6.51 (s)	7.70 (s)	7.06 (s)	7.01 (s)	6.74 (s)	6.68 (s)	6.66 (s)
6α	2.04 (m)		4.76 (d, 1.7)	4.75 (s)	5.66 (d, 7.1)	5.70 (d, 8.5)	5.68 (d, 8.5)
6β	2.19 (m)						
7	2.02 (overlap)	3.10 (m)	2.19 (m)	2.15 (overlap)	1.96 (m)	2.01 (m)	2.01 (m)
8	2.01 (overlap)	2.02 (m)	2.08 (m)	2.14 (overlap)	2.07 (m)	2.12 (m)	2.12 (m)
9	5.46 (s)	5.66 (d, 5.0)	4.61 (s)	5.59 (s)	4.65 (d, 4.6)	5.74 (d, 4.6)	5.74 (d, 4.7)
11	6.70 (s)	6.51 (s)	6.33 (s)	6.44 (s)	6.32 (s)	6.44 (s)	6.51 (s)
17	1.02 (d, 6.6)	1.03 (d, 6.7)	0.94 (d, 7.4)	0.89 (d, 6.7)	0.92 (d, 7.9)	0.90 (d, 7.0)	0.94 (d, 7.1)
18	0.96 (d, 6.6)	0.87 (d, 7.2)	1.22 (d, 7.2)	0.98 (d, 6.6)	1.04 (d, 7.9)	0.96 (d, 6.8)	0.98 (d, 7.3)
2′							1.80 (overlap)
3′							0.83 (t, 7.6)
4′							
AcO-6					1.81 (s)	1.78 (s)	1.74 (s)
AcO-9	2.02 (s)	1.40 (s)		1.57 (s)		1.57 (s)	
CH_3O-1	3.61 (s)	3.37 (s)	3.69 (s)	3.64 (s)	3.63 (s)	3.58 (s)	3.54 (s)
CH_3O-2	3.89 (s)	3.96 (s)	3.94 (s)	3.88 (s)	3.89 (s)	3.88 (s)	3.86 (s)
CH_3O-3	3.89 (s)	3.96 (s)	3.94 (s)	3.93 (s)	3.89 (s)	3.88 (s)	3.89 (s)
CH_3O-14	3.85 (s)	3.90 (s)	3.89 (s)	3.84 (s)	3.86 (s)	3.85 (s)	3.84 (s)
OCH_2O	6.00 (d, 0.8)	6.05 (s)	6.00 (s)	5.97 (s)	5.99 (s)	5.99 (s)	5.96 (s)
	5.98 (d, 0.8)	6.04 (s)	5.99 (s)	5.96 (s)		5.97 (s)	5.94 (s)

[a] Recorded at 500 MHz. [b] Recorded at 400 MHz.

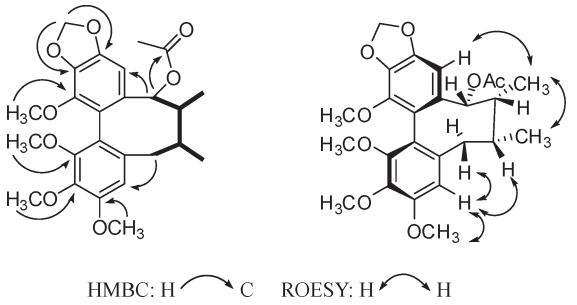

Figure 1. Key HMBC and ROESY correlations of **1**.

afford 14 new dibenzocyclooctadiene lignans, ananolignans A−N (**1**−**14**), together with five known compounds, isogomisin O (**15**),[17] kadsurin (**16**),[18] ananosin A (**17**),[19] interiotherin C (**18**),[5] and yunnankadsurin B (**19**).[20]

Ananolignan A (**1**) was assigned a molecular formula of $C_{25}H_{30}O_8$, according to its HRESIMS (m/z 481.1841 [M + Na]$^+$) and NMR spectroscopic data. The UV data, with absorption maxima at λ_{max} 213 and 241 nm, and its IR spectrum, with absorption bands at 1622 and 1463 cm^{-1} (aromatic moiety), were consistent with **1** being a dibenzocyclooctadiene lignan.[21,22] The ^{1}H NMR spectrum of **1** (Table 1) exhibited two aromatic singlets for a biphenyl moiety at δ_H 6.51 (H-4) and 6.70 (H-11), four singlets for methoxy groups at δ_H 3.89 (6H), 3.85 (3H), and 3.61 (3H), and two singlets characteristic of a methylenedioxy group at δ_H 6.00 (d, J = 0.8 Hz) and 5.98 (d, J = 0.8 Hz). A cyclooctadiene ring was recognized from two secondary methyl doublets at δ_H 1.02 (H_3-17) and 0.96 (H_3-18), two methines at δ_H 2.02 (H-7) and 2.01 (H-8), an oxymethine at δ_H 5.46 (H-9), and a methylene at δ_H 2.19 and 2.04 (H_2-6). This was confirmed by ^{1}H−^{1}H COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17, and H-8/H-18 (Figure 1). A careful analysis of the 2D NMR spectroscopic data of **1** and comparison with kadsurin[23] led to the conclusion that these two compounds possess the same planar structure. HMBC correlations of the methylenedioxy protons with C-12 and C-13 and of the four methoxy group signals with C-1, C-2, C-3, and C-14 showed that the methylenedioxy group is connected to C-12 and C-13, and the four methoxy groups are located at C-1, C-2, C-3, and C-14, respectively. The presence of an acetyl group at C-9 was deduced from the HMBC correlation of H-9 (δ_H 5.46) with the acetate carbonyl (δ_C 170.0) (Figure 1).

The CD spectrum of **1** exhibited a positive Cotton effect at λ_{max} 250 nm and a negative value at λ_{max} 210 nm, indicating an *R*-biphenyl configuration rather than an *S*-biphenyl configuration, as in kadsurin.[23] With the axial chirality defined, a ROESY experiment was used to establish the absolute configuration of the remaining stereocenters in **1**. The observed ROESY correlations of H-11 with H_3-18, H-4 with H-7, and H_3-17 with H_3-18 indicated that CH_3-17 and CH_3-18 are both α-oriented.[24] A characteristic singlet suggested that H-9 is β-oriented, the same as H-8. These conclusions were consistent with **1** being a cyclooctadiene lignan with a twisted boat/chair conformation having C-7 (*R*), C-8 (*R*), and C-9 (*R*) (Figure 1) absolute configurations. Thus, the structure of **1** was established as shown, and this new compound has been named ananolignan A.

The molecular formula of ananolignan B (**2**) was assigned as $C_{25}H_{28}O_9$, on the basis of the HRESIMS (m/z 495.1635 [M + Na]$^+$). The ^{1}H NMR spectrum showed evidence of **1** being a dibenzocyclooctadiene derivative. The CD curve of **2** exhibited a positive Cotton effect at λ_{max} 240 nm and a negative value at λ_{max} 210 nm, indicating an *R*-biphenyl configuration. Comparison of the NMR data of **2** with those of schisantherin Q[25] disclosed that the only structural differences refer to the conformation of the biphenyl ring system and the substituent at C-9. The HMBC correlations from H-9 (δ_H 5.66) to C-7 (δ_C 42.7, d),

C-8 (δ_C 46.3, d), C-10 (δ_C 132.2, s), C-11 (δ_C 101.6, d), and acetate carbonyl led to the positioning of an acetyl group at C-9. The configurations of H-8, H-9, and CH_3-17 were deduced to be α-oriented on the basis of the ROESY correlations from H-11 to H-8 and H-9 and from H_3-17 to H-8. Therefore, the structure of ananolignan B (**2**) was determined as shown.

Ananolignan C (**3**) was assigned as $C_{23}H_{28}O_8$, as deduced from the HRESIMS (*m/z* 455.1683 $[M + Na]^+$) and in accordance with its NMR data. The UV, IR, and NMR spectra of **3** suggested the presence of a dibenzocyclooctadiene lignan with almost identical data to **1**, indicating a similar substitution pattern in the biphenyl ring. However, the signals attributable to the substituents in the cyclooctadiene moiety were different. Thus, the signals of two oxymethines were assigned to C-6 and C-9, which was deduced from the HMBC correlations of H-9 (δ_H 4.61) with C-11 (δ_C 102.2, d) and C-18 (δ_C 20.3, q) and of H-6 (δ_H 4.76) with C-4 (δ_C 106.4, d) and C-17 (δ_C 9.8, q)

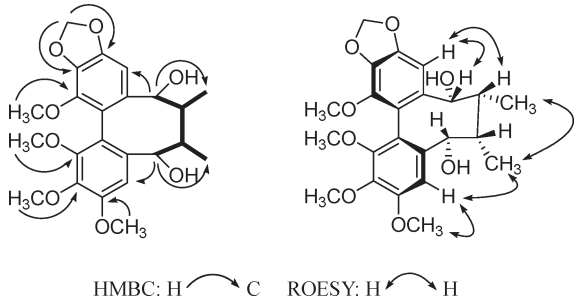

Figure 2. Key HMBC and ROESY correlations of **3**.

(Figure 2). According to the molecular formula, the oxymethines at C-6 and C-9 should both be substituted by hydroxy groups. The CD curve of **3** showed a negative Cotton effect around 254 nm and a positive value around 220 nm, suggesting that **3** possesses an *S*-biphenyl configuration. ROESY correlations of H-11 with H-8 and H-9, H-4 with H_3-17, H_3-18 with H_3-17, and H-8 with H-6 (Figure 2) suggested that CH_3-17 and CH_3-18 are α-oriented and that H-6 and H-9 are both β-oriented. From all of these data, compound **3** (ananolignan C) was assigned as shown.

Comparison of the NMR data of **3** with those of compounds **4**−**6** disclosed that the main structural differences between these compounds concerned the substituents at C-6 and C-9. Ananolignan D gave a molecular formula of $C_{25}H_{30}O_9$ by HRESIMS at *m/z* 497.1772 $[M + Na]^+$ (calcd 497.1787). Detailed comparison of the NMR data of **4** with those of **3** revealed that the only significant difference between them was the type of substituent at C-9. The presence of an acetyl group at C-9 in **4** was established by a HMBC correlation of H-9 (δ_H 5.59) with the acetate carbonyl resonance (δ_C 169.9). The molecular formula of **5** was determined as $C_{25}H_{30}O_9$ by HRESIMS at *m/z* 513.1520 $[M + K]^+$ (calcd 513.1526), and the NMR data of **5** also showed similarities with the analogous values for **3**. However, a signal for an acetyl group was evident at C-6 in **5**, which was confirmed by the HMBC correlations from H-6 (δ_H 5.66) to the acetate carbonyl (δ_C 170.2). The difference between **6** and **5** was evident only in the presence of a further acetyl group substituted at C-9 in **6**, instead of a hydroxy group in **5**. A HMBC correlation was observed from H-9 (δ_H 5.74) to the acetate carbonyl (δ_C 170.0). The CD, UV, and IR data suggested that **4**−**6** are *S*-biphenyl-configured dibenzocyclooctadiene lignans. ROESY correlations

Table 2. ^{1}H NMR Data of 8−14 in $CDCl_3$, δ in ppm (*J* in Hz)

position	8[b]	9[b]	10[b]	11[a]	12[a]	13[b]	14[b]
4	6.69 (s)	6.68 (s)	6.69 (s)	6.82 (s)	6.64 (s)	6.72 (s)	6.71 (s)
6	5.70 (d, 8.8)	5.68 (d, 8.6)	5.71 (d, 8.9)	5.86 (d, 8.6)	5.84 (d, 6.9)	5.83 (d, 8.0)	5.84 (d, 7.8)
7	2.02 (m)	2.02 (m)	2.00 (m)	2.15 (m)	2.18 (m)	2.12 (m)	2.11 (m)
8	2.15 (m)	2.14 (m)	2.18 (m)	2.29 (m)	2.22 (m)	2.22 (m)	2.21 (m)
9	5.78 (d, 5.1)	5.76 (d, 4.8)	5.78 (d, 4.7)	6.05 (d, 4.7)	5.70 (d, 1.7)	5.76 (br s)	5.76 (br s)
11	6.45 (s)	6.44 (s)	6.45 (s)	6.57 (s)	6.47 (s)	6.45 (s)	6.45 (s)
17	0.95 (d, 7.1)	0.90 (d, 8.8)	0.90 (d, 7.0)	1.01 (d, 7.0)	0.92 (d, 7.1)	0.95 (d, 7.1)	0.93 (d, 7.2)
18	1.00 (d, 7.1)	0.95 (d, 8.4)	0.95 (d, 6.9)	1.09 (d, 7.3)	1.05 (d, 6.8)	1.01 (d, br s)	1.02 (d, br s)
2′	1.93 (m)	1.76 (m)	1.73 (m)				
3′	0.85 (d, 7.1)	1.35 (m)	1.38, 1.23 (m)	7.34 (d, 7.3)	6.11 (br s)	5.97 (overlap)	5.97 (overlap)
4′	0.88 (d, 7.1)	0.77 (t, 7.4)	0.73 (t, 7.4)	7.30 (t, 6.6)	1.66 (d, 7.1)	1.86 (d, 7.2)	1.85 (d, 5.9)
5′			0.86 (d, 7.0)	7.44 (t, 7.3)	1.59 (s)	1.52 (s)	1.49 (s)
6′				7.30 (t, 6.6)			
7′				7.34 (d, 7.3)			
2″						1.95 (m)	1.79 (m)
3″						0.88 (d, 6.5)	1.37 (m)
4″						0.87 (d, 6.5)	0.79 (t, 7.4)
AcO-6	1.80 (s)	1.57 (s)	1.80 (s)	1.60 (s)			
AcO-9					1.58 (s)		
CH_3O-1	3.59 (s)	3.56 (s)	3.61 (s)	3.11 (s)	3.58 (s)	3.59 (s)	3.56 (s)
CH_3O-2	3.87 (s)	3.88 (s)	3.88 (s)	3.83 (s)	3.85 (s)	3.86 (s)	3.88 (s)
CH_3O-3	3.88 (s)	3.88 (s)	3.88 (s)	3.97 (s)	3.88 (s)	3.90 (s)	3.90 (s)
CH_3O-14	3.84 (s)	3.84 (s)	3.84 (s)	3.50 (s)	3.72 (s)	3.77 (s)	3.77 (s)
OCH_2O	6.00 (s)	5.99 (s)	5.99 (s)	6.01 (s)	5.97 (s)	5.95 (s)	5.94 (s)
	5.99 (s)	5.96 (s)	5.97 (s)	5.98 (s)	5.91 (s)	5.93 (s)	

[a] Recorded at 400 MHz. [b] Recorded at 500 MHz.

Table 3. ^{13}C NMR Data of 1−7 in $CDCl_3$, δ in ppm

position	1[a]	2[b]	3[a]	4[a]	5[a]	6[b]	7[a]
1	152.0 (s)	152.2 (s)	151.1 (s)	150.3 (s)	152.8 (s)	151.9 (s)	151.8 (s)
2	140.5 (s)	145.7 (s)	140.8 (s)	140.1 (s)	142.0 (s)	141.4 (s)	141.3 (s)
3	153.8 (s)	152.2 (s)	153.0 (s)	152.1 (s)	152.2 (s)	151.5 (s)	151.5 (s)
4	107.8 (d)	107.8 (d)	106.4 (d)	105.8 (d)	111.2 (d)	110.6 (d)	110.5 (d)
5	139.3 (s)	131.9 (s)	135.4 (s)	135.5 (s)	131.0 (s)	131.2 (s)	131.1 (s)
6	35.2 (t)	200.4 (s)	72.6 (d)	72.8 (d)	81.0 (d)	80.9 (d)	80.9 (d)
7	39.2 (d)	42.7 (d)	43.6 (d)	43.4 (d)	38.3 (d)	38.0 (d)	37.9 (d)
8	41.0 (d)	46.3 (d)	41.6 (d)	40.6 (d)	41.5 (d)	39.8 (d)	39.1 (d)
9	76.4 (d)	79.3 (d)	83.8 (d)	82.1 (d)	80.4 (d)	79.6 (d)	79.8 (d)
10	132.0 (s)	132.2 (s)	138.6 (s)	135.2 (s)	133.5 (s)	132.9 (s)	133.0 (s)
11	102.1 (d)	101.6 (d)	102.2 (d)	102.2 (d)	102.0 (d)	102.3 (d)	102.3 (d)
12	148.7 (s)	149.5 (s)	149.0 (s)	149.0 (s)	148.8 (s)	148.6 (s)	148.5 (s)
13	136.0 (s)	136.5 (s)	135.3 (s)	135.8 (s)	136.9 (s)	136.2 (s)	136.1 (s)
14	141.3 (s)	142.2 (s)	141.0 (s)	141.0 (s)	142.5 (s)	141.8 (s)	141.7 (s)
15	121.4 (s)	120.0 (s)	117.5 (s)	118.6 (s)	119.7 (s)	121.4 (s)	121.3 (s)
16	120.9 (s)	125.7 (s)	119.7 (s)	121.1 (s)	122.5 (s)	123.3 (s)	123.0 (s)
17	21.8 (q)	15.5 (q)	9.8 (q)	9.4 (q)	17.4 (q)	16.7 (q)	17.7 (q)
18	9.0 (q)	10.4 (q)	20.3 (q)	20.0 (q)	17.4 (q)	16.8 (q)	18.1 (q)
1′							173.5 (s)
2′							27.1 (t)
3′							8.6 (q)
4′							
AcO-6					170.2 (s)	170.1 (s)	170.1 (s)
					21.0 (q)	20.9 (q)	20.9 (q)
AcO-9	170.0 (s)	169.8 (s)		169.9 (s)		170.0 (s)	
	22.3 (q)	20.1 (q)		20.6 (q)		20.6 (q)	
CH_3O-1	61.3 (q)	59.9 (q)	60.6 (q)	60.3 (q)	60.4 (q)	60.1 (q)	60.1 (q)
CH_3O-2	61.4 (q)	60.9 (q)	61.0 (q)	60.6 (q)	60.8 (q)	60.6 (q)	60.5 (q)
CH_3O-3	56.3 (q)	55.9 (q)	55.9 (q)	55.9 (q)	55.9 (q)	56.0 (q)	55.9 (q)
CH_3O-14	60.2 (q)	60.2 (q)	59.7 (q)	59.6 (q)	59.5 (q)	59.5 (q)	59.5 (q)
OCH_2O	101.4 (t)	101.4 (t)	101.2 (t)	101.2 (t)	101.1 (t)	101.2 (t)	101.1 (t)

[a] Recorded at 100 MHz. [b] Recorded at 125 MHz.

obtained for **4**−**6** were shown from H-11 to H-8 and H-9 and suggested that CH_3-18 has an α-orientation, with H-9 β-oriented. The ROESY correlations in **4** from H-4 to H_3-17, from H-6 to H-8, and from H_3-17 to H_3-18 indicated that HO-6 and CH_3-17 adopt an α-orientation. In compounds **5** and **6**, H-6 and CH_3-17 were assigned as α-oriented, according to the ROESY correlations of H-4 with H-6 and H_3-17. Thus, the structures of ananolignans D (**4**), E (**5**), and F (**6**) were established as shown.

Ananolignans G (**7**) and H (**8**) were determined with the molecular formulas $C_{28}H_{34}O_{10}$ and $C_{29}H_{36}O_{10}$ by HRESIMS (m/z 553.2060 $[M + Na]^+$ and 567.2201 $[M + Na]^+$, respectively). Comparison of the spectroscopic data of **7** with those of **6** revealed these substances to be quite similar structurally, except that the acetyl group at C-9 in **6** was changed to a propionyl group (δ_C 173.5 s, 27.1 t, 8.6 q) in **7**, which was confirmed by HMBC correlations from an oxymethine at δ_H 5.74 (H-9) to δ_C 173.5 (C-1′), 37.9 (C-7), 39.1 (C-8), 133.0 (C-10), and 102.3 (C-11). Compound **8** exhibited an isobutyryl group (δ_C 176.4 s, 33.6 d, 19.3 q, and 17.9 q) at C-9,[26] which was confirmed by the HMBC correlation of H-9 (δ_H 5.78) with the signal at δ_C 176.4. Ananolignans I (**9**), J (**10**), and K (**11**) showed molecular ions at m/z 567.2221, 581.2354, and 601.2046 in their HRESIMS, corresponding to the molecular formulas $C_{29}H_{36}O_{10}$, $C_{30}H_{38}O_{10}$, and $C_{32}H_{34}O_{10}$, respectively. The major differences were in the replacement of an acetyl group at C-9 in **6** by a butyryl group (δ_C 172.7 s, 35.7 t, 18.0 t, and 13.5 q) in **9**, by a isovaleryl group (δ_C 176.0 s, 40.2 d, 26.6 t, 11.1 q, and 15.0 q) in **10**, and by a benzoyloxy group (δ_C 165.7 s, 129.5 s, 129.5 d, 128.1 d, 133.0 d, 128.1 d, and 129.5 d) in **11**.[26,27] The CD, UV, IR, and NMR spectra suggested that **7**−**11** are *S*-biphenyl-configured dibenzocyclooctadiene lignans. ROESY correlations of H-11 with H-8 and H-9, of H-4 with H-6 and H_3-17, and of H_3-18 with H_3-17 in **7**−**11** suggested the absolute configurations as C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*), which were identical with those of **6**. The H-6/H-7 and H-8/H-9 coupling constants for **7**−**11** also confirmed the above deductions.

Ananolignan L (**12**) gave the molecular formula $C_{30}H_{36}O_{10}$ from its HRESIMS data at m/z 579.2221 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra, together with the CD, UV, and IR experiments conducted, suggested that **12** is an *S*-biphenyl-configured dibenzocyclooctadiene lignan. The HMBC correlations of H-9 (δ_H 5.70) with the acetate carbonyl (δ_C 170.0), the methylenedioxy protons with C-12 and C-13, and the four methoxy groups with C-1, C-2, C-3, and C-14, respectively, indicated that the substitution patterns on C-9 and the carbons of the aromatic rings are the same as those of **6**. The ^{13}C NMR

dx.doi.org/10.1021/np1009288 |J. Nat. Prod. 2011, 74, 1028–1035

Table 4. ^{13}C NMR Data of 8−14 in $CDCl_3$, δ in ppm

position	8[a]	9[a]	10[b]	11[a]	12[a]	13[b]	14[a]
1	151.5 (s)	151.4 (s)	151.5 (s)	151.7 (s)	151.6 (s)	151.6 (s)	151.5 (s)
2	141.3 (s)	141.3 (s)	141.4 (s)	141.8 (s)	141.0 (s)	140.4 (s)	140.5(s)
3	151.9 (s)	151.8 (s)	152.0 (s)	152.1 (s)	151.6 (s)	151.9 (s)	151.7 (s)
4	110.5 (d)	110.5 (d)	110.6 (d)	110.5 (d)	110.0 (d)	110.5 (d)	110.3 (d)
5	131.1 (s)	131.1 (s)	131.1 (s)	131.0 (s)	131.2 (s)	131.2 (s)	131.2 (s)
6	81.0 (d)	80.9 (d)	81.0 (d)	80.9 (d)	80.7 (d)	80.7 (d)	80.6 (d)
7	37.8 (d)	37.8 (d)	37.8 (d)	39.1 (d)	38.9 (d)	38.6 (d)	38.5 (d)
8	37.8 (d)	37.8 (d)	37.8 (d)	39.9 (d)	38.3 (d)	39.7 (d)	38.5 (d)
9	79.5 (d)	79.8 (d)	79.4 (d)	80.4 (d)	80.9 (d)	80.3 (d)	80.6 (d)
10	132.9 (s)	132.9 (s)	133.1 (s)	132.7 (s)	133.4 (s)	133.2 (s)	133.1 (s)
11	102.5 (d)	102.3 (d)	102.5 (d)	102.6 (d)	102.3 (d)	102.5 (d)	102.3 (d)
12	148.5 (s)	148.5 (s)	148.6 (s)	148.6 (s)	148.4 (s)	148.6 (s)	148.5 (s)
13	136.1 (s)	136.2 (s)	136.2 (s)	136.4 (s)	135.9 (s)	135.9 (s)	135.9 (s)
14	141.7 (s)	141.7 (s)	141.8 (s)	141.8 (s)	141.3 (s)	143.0 (s)	141.2 (s)
15	121.4 (s)	121.4 (s)	121.6 (s)	121.4 (s)	121.3 (s)	121.3 (s)	121.1 (s)
16	123.0 (s)	123.2 (s)	122.4 (s)	123.5 (s)	122.5 (s)	123.4 (s)	124.3 (s)
17	16.5 (q)	15.6 (q)	17.6 (q)	16.7 (q)	15.8 (q)	19.9 (q)	19.9 (q)
18	16.7 (q)	18.8 (q)	17.6 (q)	16.7 (q)	15.8 (q)	19.3 (q)	19.9 (q)
1′	176.4 (s)	172.7 (s)	176.0 (s)	165.7 (s)	166.8 (s)	166.8 (s)	166.7 (s)
2′	33.6 (d)	35.7 (t)	40.2 (d)	129.5 (s)	128.2 (s)	127.8 (s)	127.7 (s)
3′	17.9 (q)	18.0 (t)	26.6 (t)	129.5 (d)	137.2 (d)	138.3 (d)	138.6 (d)
4′	19.3 (q)	13.5 (q)	11.1 (q)	128.1 (d)	14.2 (q)	15.5 (q)	15.6 (q)
5′			15.0 (q)	133.0 (d)	11.6 (q)	20.4 (q)	19.9 (q)
6′				128.1 (d)			
7′				129.5 (d)			
1″						176.4 (s)	172.8 (s)
2″						33.6 (d)	35.8 (t)
3″						19.3 (q)	17.9 (t)
4″						18.0 (q)	13.6 (q)
AcO-6	170.1 (s)	170.1 (s)	170.1 (s)	170.2 (s)			
	21.0 (q)	20.9 (q)	21.0 (q)	21.0 (q)			
AcO-9					170.0 (s)		
					20.7 (q)		
CH_3O-1	60.2 (q)	60.1 (q)	59.7 (q)	59.6 (q)	60.3 (q)	60.3 (q)	60.2 (q)
CH_3O-2	60.4 (q)	60.5 (q)	60.5 (q)	59.7 (q)	60.5 (q)	60.4 (q)	60.5 (q)
CH_3O-3	55.9 (q)	55.9 (q)	55.9 (q)	56.0 (q)	55.9 (q)	56.0 (q)	55.9 (q)
CH_3O-14	59.5 (q)	59.4 (q)	59.4 (q)	60.1 (q)	59.2 (q)	59.2 (q)	59.3 (q)
OCH_2O	101.1 (t)	101.1 (t)	101.2 (t)	101.2 (t)	101.0 (t)	101.0 (t)	101.0 (t)

[a] Recorded at 100 MHz. [b] Recorded at 125 MHz.

signals at δ_C 166.8 s, 128.2 s, 137.2 d, 14.2 q, and 11.6 q suggested the presence of a tigloyloxy moiety substituted at C-6, which was confirmed by analysis of the HSQC, HMBC, and $^1H-^1H$ COSY spectra. The configuration of **12** was determined through ROESY correlations of H-11/H-8, H-9; H-4/H-6, H_3-17; and H_3-18/H_3-17, as well as the proton coupling constants of H-6 (d, J = 6.9 Hz) and H-9 (d, J = 1.7 Hz), which were in agreement with a cyclooctadiene lignan with a twisted boat/chair conformation having C-6 (*R*), C-7 (*S*), C-8 (*R*), and C-9 (*R*) absolute configurations. Therefore, the structure of ananolignan L (**12**) was determined as shown.

Ananolignans M (**13**) and N (**14**) were assigned with the same molecular formula, $C_{32}H_{40}O_{10}$, as determined by HRESIMS (m/z 607.2526 $[M + Na]^+$ and 607.2522 $[M + Na]^+$, respectively). Both compounds were assigned as *S*-biphenyl-configured dibenzocyclooctadiene lignans by comparison of their CD, UV, and ROESY spectra with those of **8**. The main difference found between **8** and **13** concerned the substituent group located at C-6. Analysis of the 1D NMR data showed an angeloyloxy group (δ_C 166.8 s, 127.8 s, 138.3 d, 15.5 q, and 20.4 q) in **13** instead of an acetyl group in **8**, which was deduced from a HMBC correlation of H-6 (δ_H 5.83) with C-1′ (δ_C 166.8). Comparison of the NMR data of **14** with those of **13** disclosed that the only structural difference was the isobutyryl group located at C-9 in **13** being changed into a butyryl moiety (δ_C 172.8 s, 35.8 t, 17.9 t, and 13.6 q) in **14**. This was confirmed by the HMBC correlations from δ_H 5.76 (H-9) to δ_C 172.8 (C-1″). The configurations of H-6, CH_3-17, and CH_3-18 were assigned as α-oriented, with H-9 β-oriented, on the basis of the ROESY correlations from H-11 to H-8 and H-9, from H-4 to H-6 and H_3-17, and from H_3-17 to

dx.doi.org/10.1021/np1009288 |J. Nat. Prod. 2011, 74, 1028–1035

Table 5. Neuroprotective Effects of Compounds 1−19 on SH-SY5Y Cells

	H_2O_2 (100 μM)				H_2O_2 (100 μM)		
	test concentration (μM)				test concentration (μM)		
compound	1	10	vehicle	compound	1	10	vehicle
1	58.9 ± 2.0[a]	51.7 ± 2.5	50.9 ± 2.1	**11**	68.9 ± 1.1	71.4 ± 1.3	73.0 ± 1.6
2	59.7 ± 0.6	69.6 ± 1.4[a]	59.4 ± 0.7	**12**	79.2 ± 3.7[a]	66.9 ± 0.9	63.1 ± 1.9
3	57.7 ± 1.3[b]	59.6 ± 0.7	64.8 ± 1.9	**13**	74.0 ± 1.7[b]	64.5 ± 2.4	68.7 ± 1.4
4	64.3 ± 3.0	63.0 ± 2.6	63.9 ± 2.0	**14**	75.0 ± 2.1[b]	66.8 ± 1.9	68.7 ± 1.4
5	62.0 ± 1.2[a]	56.3 ± 1.4	52.9 ± 3.8	**15**	56.9 ± 1.5[b]	56.7 ± 1.1[b]	50.9 ± 2.1
6	90.0 ± 2.1[a]	88.6 ± 2.6[a]	71.0 ± 3.4	**16**	56.3 ± 1.4	49.7 ± 3.4	53.7 ± 1.6
7	57.7 ± 1.2	56.7 ± 1.9	53.7 ± 1.6	**17**	66.4 ± 1.4	68.2 ± 1.1	65.4 ± 3.0
8	64.1 ± 1.1	63.9 ± 2.3	63.9 ± 2.0	**18**	45.7 ± 1.1[a]	53.6 ± 1.0	50.9 ± 2.1
9	61.1 ± 1.8	60.1 ± 1.7	63.9 ± 2.0	**19**	41.7 ± 1.0[a]	60.1 ± 1.5[a]	51.3 ± 1.2
10	52.2 ± 1.7[a]	55.1 ± 0.6	58.6 ± 1.9				

[a] $p < 0.01$ vs H_2O_2 group. [b] $p < 0.05$ vs H_2O_2 group.

H_3-18. Accordingly, the structures of **13** and **14** were determined as shown.

The neuroprotective effects of all dibenzocyclooctadiene lignans were evaluated according to a reported in vitro protocol[28] using SH-SY5Y neuroblastoma cells, a neuroblastoma cell line used for the study of neurodegenerative disease.[29,30] As may be seen from Table 5, ananolignan F (**6**) and ananolignan L (**12**) showed the most promising cell survival data against oxidative stress-induced neurotoxicity, of all the compounds tested.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm ×25 cm) column. Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China) and MCI gel (75−150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The seeds of *K. ananosma* were collected in Simao Country of Yunnan Province, People's Republic of China, in October 2008, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 08102010) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered seeds of *K. ananosma* (250 g) were extracted with 70% aqueous Me_2CO (500 mL × 3) at room temperature and concentrated in vacuo to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (6.5 g) was chromatographed on MCI CHP 20P gel (90% CH_3OH−H_2O). The 90% CH_3OH fraction (5.0 g) was subjected to silica gel (200−300 mesh) column chromatography, eluting with a $CHCl_3$−Me_2CO gradient system (9:1, 8:2, 2:1, 1:1, 0:1), to afford fractions 1−5. Fraction 2 (3.5 g) was chromatographed on a silica gel column ($CHCl_3$−Me_2CO, 50:1−25:1) to give three subfractions (2.1−2.3). Fraction 2.1 (1.5 g) was purified by semipreparative HPLC (82% CH_3OH−H_2O) to get three fractions (2.1.1−2.1.3). Fraction 2.1.1 (35 mg) was separated further by semipreparative HPLC (63% CH_3CN−H_2O) to give **2** (2 mg). Fraction 2.1.2 (350 mg) was purified by semipreparative HPLC (65% CH_3CN−H_2O) to give **1** (14 mg), **6** (39 mg), and **16** (31 mg). Fraction 2.1.3 (650 mg) was purified repeatedly by semipreparative HPLC (65% CH_3CN−H_2O) to give **7** (42 mg), **8** (27 mg), **9** (43 mg), **10** (9 mg), **11** (8 mg), **12** (27 mg), **13** (42 mg), **14** (21 mg), and **18** (71 mg). Fraction 2.2 (0.5 g) was subjected to semipreparative HPLC (62% CH_3CN−H_2O) to produce **4** (31 mg), **5** (4 mg), **15** (28 mg), **17** (3 mg), and **19** (10 mg). Finally, fraction 2.3 (0.1 g) was separated by semipreparative HPLC (60% CH_3CN−H_2O) to yield **3** (9 mg).

Ananolignan A (***1***): white solid; $[\alpha]_D^{26}$ +68.1 (*c* 0.17, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 210 (−25), 250 (+30); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.99), 230 (3.67), 226 (3.68), 219 (3.66), 213 (3.64), 208 (3.64), 199 (3.64) nm; IR (KBr) ν_{max} 2929, 1738, 1622, 1463, 1243 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m*/*z* 481 (100) $[M + Na]^+$; positive HRESIMS *m*/*z* 481.1841 $[M + Na]^+$ (calcd for $C_{25}H_{30}O_8Na$, 481.1838).

Ananolignan B (***2***): white solid; $[\alpha]_D^{27}$ +47.8 (*c* 0.19, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 210 (−7), 240 (+7); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.06), 227 (3.83), 220 (3.82), 205 (3.80) nm; IR (KBr) ν_{max} 2937, 1740, 1664, 1235 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m*/*z* 495 (100) $[M + Na]^+$; positive HRESIMS *m*/*z* 495.1635 $[M + Na]^+$ (calcd for $C_{25}H_{28}O_9Na$, 495.1631).

Ananolignan C (***3***): white solid; $[\alpha]_D^{27}$ −35.5 (*c* 0.17, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 220 (+22), 254 (−18); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.99), 222 (3.68), 210 (3.67), 205 (3.66), 198 (3.66) nm; IR (KBr) ν_{max} 3442, 2932, 1621, 1462 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m*/*z* 455 (40) $[M + Na]^+$; positive HRESIMS *m*/*z* 455.1683 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_8Na$, 455.1681).

Ananolignan D (***4***): white solid; $[\alpha]_D^{27}$ −26.6 (*c* 0.20, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 220 (+16), 254 (−15); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.08), 224 (3.73), 214 (3.71) nm; IR (KBr) ν_{max} 3442, 2940, 1741, 1622, 1464, 1236 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m*/*z* 497 (65) $[M + Na]^+$; positive HRESIMS *m*/*z* 497.1772 $[M + Na]^+$ (calcd for $C_{25}H_{30}O_9Na$, 497.1787).

Ananolignan E (***5***): white solid; $[\alpha]_D^{26}$ +58.5 (*c* 0.22, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+40), 254 (−25); UV ($CHCl_3$) λ_{max} (log ε) 242 (3.95), 226 (3.51), 204 (3.58), 192 (3.58) nm; IR (KBr) ν_{max} 3448, 2925, 1732, 1463 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m*/*z* 513 $[M + K]^+$; positive HRESIMS *m*/*z* 513.1520 $[M + K]^+$ (calcd for $C_{25}H_{30}O_9K$, 513.1526).

*Ananolignan F (**6**):* white solid; $[\alpha]_D^{29}$ +74.3 (*c* 0.21, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 237 (+7), 254 (−15); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.03), 226 (3.55), 199 (3.66) nm; IR (KBr) ν_{max} 2935, 1741, 1622, 1232 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 539 (100) $[M + Na]^+$; positive HRESIMS *m/z* 539.1887 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_{10}Na$, 539.1893).

*Ananolignan G (**7**):* white solid; $[\alpha]_D^{27}$ +76.1 (*c* 0.17, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+30), 254 (−12); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.00), 231 (3.68), 204 (3.65), 199 (3.65) nm; IR (KBr) ν_{max} 2940, 1732, 1735, 1237 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 553 (100) $[M + Na]^+$; positive HRESIMS *m/z* 553.2060 $[M + Na]^+$ (calcd for $C_{28}H_{34}O_{10}Na$, 553.2049).

*Ananolignan H (**8**):* white solid; $[\alpha]_D^{27}$ +90.5 (*c* 0.18, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+31), 254 (−12); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.05), 199 (3.67), 193 (3.68) nm; IR (KBr) ν_{max} 2971, 2939, 1731, 1238 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 567 (50) $[M + Na]^+$; positive HRESIMS *m/z* 567.2201 $[M + Na]^+$ (calcd for $C_{29}H_{36}O_{10}Na$, 567.2206).

*Ananolignan I (**9**):* white solid; $[\alpha]_D^{28}$ +57.3 (*c* 0.16, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+17), 254 (−8); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.01), 194 (3.67), 192 (3.68) nm; IR (KBr) ν_{max} 2967, 2939, 1733, 1237 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 567 (80) $[M + Na]^+$; positive HRESIMS *m/z* 567.2221 $[M + Na]^+$ (calcd for $C_{29}H_{36}O_{10}Na$, 567.2206).

*Ananolignan J (**10**):* white solid; $[\alpha]_D^{27}$ +103.3 (*c* 0.19, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+8), 254 (−3); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.97), 232 (3.64), 209 (3.60), 196 (3.61) nm; IR (KBr) ν_{max} 2969, 2938, 1731, 1237 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 581 (100) $[M + Na]^+$; positive HRESIMS *m/z* 581.2354 $[M + Na]^+$ (calcd for $C_{30}H_{38}O_{10}Na$, 581.2362).

*Ananolignan K (**11**):* white solid; $[\alpha]_D^{27}$ +1.6 (*c* 0.40, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+21), 248 (−20); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.95), 224 (3.59), 218 (3.58), 196 (3.59) nm; IR (KBr) ν_{max} 2926, 1719, 1249 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 601 (100) $[M + Na]^+$; positive HRESIMS *m/z* 601.2046 $[M + Na]^+$ (calcd for $C_{32}H_{34}O_{10}Na$, 601.2049).

*Ananolignan L (**12**):* white solid; $[\alpha]_D^{29}$ −22.6 (*c* 0.19, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 195 (+80), 248 (−38); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.01), 202 (3.61), 195 (3.63) nm; IR (KBr) ν_{max} 2935, 1738, 1704, 1251 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 579 (100) $[M + Na]^+$; positive HRESIMS *m/z* 579.2221 $[M + Na]^+$ (calcd for $C_{30}H_{36}O_{10}Na$, 579.2206).

*Ananolignan M (**13**):* white solid; $[\alpha]_D^{27}$ +77.8 (*c* 0.16, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 230 (+11), 250 (−5); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.10), 222 (3.59), 202 (3.68), 194 (3.70) nm; IR (KBr) ν_{max} 2970, 2943, 1733, 1710, 1103 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 607 (100) $[M + Na]^+$; positive HRESIMS *m/z* 607.2526 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_{10}Na$, 607.2519).

*Ananolignan N (**14**):* white solid; $[\alpha]_D^{27}$ +64.8 (*c* 0.21, $CHCl_3$); CD (CH_3OH) λ_{max} nm ($\Delta\varepsilon$) 225 (+35), 250 (−17); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.09), 210 (3.71), 198 (3.71) nm; IR (KBr) ν_{max} 2965, 2938, 1735, 1712, 1463 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; positive ESIMS *m/z* 607 (100) $[M + Na]^+$; positive HRESIMS *m/z* 607.2522 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_{10}Na$, 607.2519).

Neurodegenerative Activity Assay. SH-SY5Y neuroblastoma cells were obtained from ATCC (American Type Culture Collection) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded into 96-well plates (Greiner) at a density of 5×10^4 cells per mL in DMEM/F12 (Gibco), supplemented with 10% heat-inactivated bovine calf serum, 100 units/mL penicillin, and 100 mg/mL streptomycin. All experiments were carried out 24 h after cells were seeded. Appropriate concentrations of hydrogen peroxide (H_2O_2) were prepared in deionized water on the day of application to cultures. The SH-SY5Y cells were preincubated with different compounds 2 h before H_2O_2 (1 mM) was added, and the assay for cell viability was performed 24 h after H_2O_2 was added. Cell survival was evaluated by reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma).[31] The values of cell survival were normalized against the value for the control group, which was set to 100%. Data were evaluated for statistical significance with one-way ANOVA followed by the LSD test by using a computerized statistical package. Differences were considered significant at $p < 0.05$.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of new compounds **1**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was supported financially by the NSFC (No. 30830115 to H.-D.S. and 20902093 to J.-X.P.), the Science and Technology Program of Yunan Province (2008GA031), the Major State Basic Research Development Program of China (Nos. 2009CB522300 and 200940900), the Western Doctoral Foundation of Chinese Academy of Sciences (J.-X.P.), the CAS action-plan for West Development (KZCX2-XB2-15), and the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program of China" (Nos. 2009ZX09301-001 and -063).

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