Oxygenated Lignans from the Fruits of *Schisandra arisanensis*

Yuan-Bin Cheng,[†] Meng-Ting Chang,[†] Yi-Wen Lo,[†] Ching-Jen Ho,[†] Yuh-Chi Kuo,[‡] Ching-Te Chien,[§] Shun-Ying Chen,[§] Shorong-Shii Liou,[⊥] Yao-Haur Kuo,[∥] and Ya-Ching Shen*[,†]

School of Pharmacy, College of Medicine, National Taiwan University, Jen-Ai Rd. Sec. 1, Taipei 100, Taiwan, Republic of China, Department of Life Science, Fu-Jen University, No. 510, Chung-Cheng Road, Hsinchuang, 242, Taipei Hsien, Taiwan, Republic of China, Division of Silviculture, Taiwan Forestry Research Institute, Taipei 100, Taiwan, Republic of China, Department of Pharmacy, Tajen University, Yen-Pou, Ping Tung Shien, Taiwan, Republic of China, and National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China

Received June 18, 2009

An acetone extract of the fruits of the Taiwanese medicinal plant *Schisandra arisanensis* has yielded 11 new oxygenated lignans. Four of these, named arisantetralones A−D (**1**−**4**), possess the aryltetralone skeleton, while the other seven, named arisanschinins F−L (**5**−**11**), are polyoxygenated C_{18}-dibenzocyclooctadiene lignans. Structures were determined on the basis of spectroscopic analyses, especially 2D-NMR techniques. The structure of compound **1** was confirmed by X-ray crystallographic analysis. Immunomodulatory activity of the isolated lignans was tested and evaluated.

The genera *Kadsura* and *Schisandra* (family Schisandraceae) have been commonly used in traditional Chinese medicine for the treatment of hepatitis, diabetes, diarrhea, and cough and used as an astringent remedy.[1] These two genera were reported to contain unique C_{18}-dibenzocyclooctadiene lignans[2,3] and lanostane- and cycloartane-type triterpenoids.[4] Pharmacological studies revealed that certain of these these metabolites possess hepatoprotective,[5] cancer protective,[6] anti-HIV,[7] antioxidative,[8] and antiviral activities.[9] In our research on Taiwanese schisandraceous plants, we previously reported novel lignans and triterpenoids from *Kadsura philippinensis*.[10−12] Herein we report some constituents of *Schisandra arisanensis* Hayata (Schisandraceae), which is an endemic species growing in the mountainous area of central Taiwan. Phytochemical investigation of the fruits of *S. arisanensis* has resulted in the isolation of 11 new oxygenated lignans (**1**−**11**). Six known compounds, (−)-holostyligone (**12**),[13] pregomisin (**13**),[14] gomisin F (**14**),[15] gomisin G (**15**),[15] epigomisin O (**16**),[16] and (+)-gomisin K3 (**17**),[17] were also isolated and identified. The structures of the new compounds were established by detailed analysis of their spectroscopic data, especially 2D-NMR and CD spectra. Structures of the known compounds (**12**−**17**) are given in the Supporting Information. The relative configuration of compound **1** was determined by X-ray crystallographic analysis. Inhibition of proliferation of peripheral blood mononuclear cells (PBMC) induced by phytohemaglutinin (PHA) for these compounds (**1**−**17**), in vitro, was also evaluated.

Results and Discussion

Arisantetralone A (**1**), $[\alpha]^{25}_{D}$ −35 (*c* 0.1, CH_2Cl_2), had the molecular formula $C_{20}H_{22}O_5$ and 10 degrees of unsaturation, as deduced from HRESIMS (*m/z* 365.1368 $[M + Na]^+$) and DEPT spectra. The IR spectrum indicated the presence of OH (3512 cm^{-1}), ketone (1743 cm^{-1}), and phenyl (1666 cm^{-1}) groups. The ^1H NMR data of **1** (Table 1) revealed the presence of two methyl (δ 0.98, d, $J = 6.9$ Hz; δ 1.11, d, $J = 6.9$ Hz), five aromatic methine (δ 6.43 s; δ 6.54 d, $J = 7.8$ Hz; δ 6.55 s; δ 6.84, d, $J = 7.8$ Hz; δ 7.62, s), and two methoxy groups (δ 3.80, s; δ 3.81, s). The ^{13}C NMR (Table 2) and DEPT spectra of **1** showed 20 carbon signals, consisting of two methyl (δ 11.9, 15.9), two methoxy (δ 55.9, 56.0), three aliphatic methine (δ 42.6, 42.6, 50.5), and one carbonyl (δ 200.0) carbon. By considering the biogenic pathway of lignans, which are characterized by coupling two C_6-C_3 moieties, the remaining 12 aromatic carbons suggested the presence of two aryl groups, and the remaining one degree of unsaturation suggested that **1** contained

Table 1. ^1H NMR Data (400 MHz) of Compounds **1**−**4**[a]

position	**1**	**2**	**3**	**4**
3	6.43 s	6.51 s	6.42 s	6.16 s
6	7.62 s	7.57 s	7.62 s	7.58 s
8	2.77 m	2.78 m	2.77 m	2.37 m
9	1.11 d (6.9)	1.13 d (6.4)	1.11 d (6.9)	1.31 d (6.5)
2′	6.55 s	6.55 s	6.62 s	6.58 s
5′	6.84 d (7.8)	6.83 d (7.8)	6.78 d (7.7)	6.86 d (8.0)
6′	6.54 d (7.8)	6.54 d (7.8)	6.54 d (7.7)	6.76 d (8.0)
7′	3.96 d (5.1)	3.90 d (5.5)	3.97 d (5.0)	3.67 d (10.8)
8′	2.40 m	2.42 m	2.40 m	2.07 m
9′	0.98 d (6.9)	0.96 d (6.1)	0.98 d (6.9)	0.93 d (6.3)
4-OMe	3.81 s		3.79 s	3.65 s
5-OMe		3.96 s		
3′-OMe	3.80 s	3.81 s	3.81 s	3.82 s
4′-OMe			3.86 s	3.92 s

[a] Chemical shifts are in ppm (δ); *J* values in Hz are in parentheses.

Table 2. ^{13}C NMR Data (δ) (100 MHz) of Compounds **1**−**4**[a]

position	**1**[b]	**2**	**3**	**4**
1	126.3 s	125.2 s	126.2 s	126.4 s
2	137.9 s	140.0 s	137.8 s	140.7 s
3	111.3 d	115.4 d	111.3 d	110.6 d
4	151.3 s	150.7 s	151.4 s	150.8 s
5	144.7 s	145.8 s	144.7 s	144.4 s
6	111.9 d	108.0 d	111.9 d	111.8 d
7	200.0 s	200.3 s	200.0 s	198.8 s
8	42.6 d	43.2 d	42.5 d	48.6 d
9	11.9 q	11.7 q	11.8 q	12.5 q
1′	135.8 s	135.5 s	136.4 s	136.3 s
2′	111.0 d	111.0 d	111.8 d	111.7 d
3′	146.6 s	146.6 s	149.0 s	149.2 s
4′	144.3 s	144.3 s	147.7 s	147.8 s
5′	114.1 d	114.1 d	110.9 d	110.9 d
6′	121.8 d	121.8 d	121.0 d	122.1 d
7′	50.5 d	49.8 d	50.4 d	53.4 d
8′	42.6 d	42.0 d	42.5 d	43.7 d
9′	15.9 q	16.0 q	15.9 q	18.0 q
4-OMe	56.0 q		56.0 q	55.8 q
5-OMe		56.1 q		
3′-OMe	55.9 q	55.9 q	55.9 q	55.9 q
4′-OMe			55.8 q	55.8 q

[a] Assignments were made using HMQC and HMBC techniques. [b] Multiplicities, s = C, d = CH, q = CH_3.

* To whom correspondence should be addressed. Tel.: 886-2-23123456, ext. 62226. Fax: 886-02-2391-9098. E-mail: ycshen@ntu.edu.tw.
[†] National Taiwan University.
[‡] Fu-Jen University.
[§] Taiwan Forestry Research Institute.
[⊥] Tajen University.
[∥] National Research Institute of Chinese Medicine.

10.1021/np9003678 CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 08/12/2009

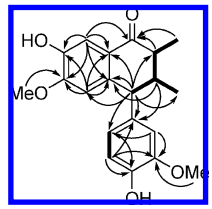


Figure 1. Selected COSY (bold bonds) and HMBC correlations (arrows) of **1**.

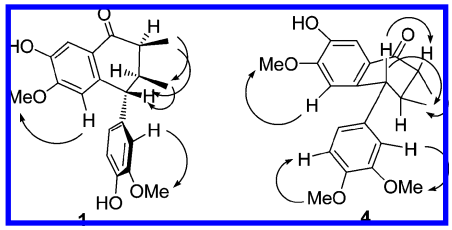


Figure 2. Selected NOESY correlations of **1** and **4**.

an additional ring. Comparing the ^{1}H and ^{13}C NMR data and double-bond equivalence with those of published lignans,[18−20] compound **1** was classified as an aryltetralone-type lignan. Detailed analysis of the COSY spectrum (Figure 1) revealed the proton sequence of H-9 (δ 1.11)/H-8 (δ 2.77)/H-8′ (δ 2.40)/H-7′ (δ 3.96)/H-9′ (δ 0.98). HMBC correlations (Figure 1) of H-7′ (δ 3.96) with C-1 (δ 126.3), C-2 (δ 137.9), and C-3 (δ 111.3) and correlation of H-9 (δ 1.11) with C-7 (δ 200.0) confirmed the presence of an α-tetralone moiety. HMBC correlations of H-7′ with C-1′ (δ 135.8), C-2′ (δ 111.0), and C-6′ (δ 121.8) revealed that an aryl group was attached at C-7′. In one aryl group (C ring), two downfield carbons (δ 144.3, 146.6) and three aromatic protons were observed. This suggested the presence of two oxygenated substituents. COSY correlation between H-5′/H-6′ and the HMBC correlations of H-5′ (δ 6.84) with C-4′ (δ 144.3), and the methoxy (δ 3.80) with C-3′ (δ 146.6), as well as NOE cross-peaks between a methoxy and H-2′ (δ 6.55) placed an OCH_3 group and an OH group at C-3′ and C-4′, respectively. Furthermore, the HMBC spectrum of **1** showed correlations of H-3 (δ 6.43) with C-4 (δ 151.3); H-6 (δ 7.62) with C-5 (δ 144.7); and the OCH_3 (δ 3.81) with C-4, as well as the NOE cross-peaks between an OCH_3 and H-3, indicating an OCH_3 group and an OH group attached at C-4 and C-5, respectively. Thus, the structure of compound **1** was established as indicated.

The relative configuration of **1** at C-7′, C-8′, and C-8 was determined by NOESY experiment (Figure 2) and X-ray crystallographic analysis. NOE correlations of H-7′/H-9 and H-9/H-9′ suggested that H-7′ and two methyl groups were on the same face and were β-oriented. An ORTEP stereodrawing (Figure 3) from the X-ray analysis of compound **1** shows the relative configuration as 7′*R**, 8′*S**, and 8*S**, and the compound was named arisantetralone A.

The HRESIMS of **2** revealed the molecular formula $C_{20}H_{22}O_5$, identical to that of **1**. The similarity of IR, UV data, and optical rotations of **1** and **2** suggested that **2** was an isomer of **1**. ^{1}H and ^{13}C NMR spectra showed that the number of methyl, methylene, methine, and quaternary carbons was the same as those of **1** (Tables 1 and 2). The signal of H-6 was shifted downfield (δ 7.57), the OCH_3 signal at δ 3.96 showed HMBC correlations with C-5 (δ 145.8), and an NOE cross -peak was observed between the OCH_3 and H-6. These findings implied that the difference between **1** and **2** was an interchange of the OCH_3 and OH groups at C-4 and C-5. NOESY correlations of H-7′ (δ 3.90)/H-9 (δ 1.13)/H-9′ (δ 0.96) indicated the same relative configuration as **1**. Compound **2** was named arisantetralone B.

Compound **3** was assigned the molecular formula $C_{21}H_{24}O_5$, with 10 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **3**

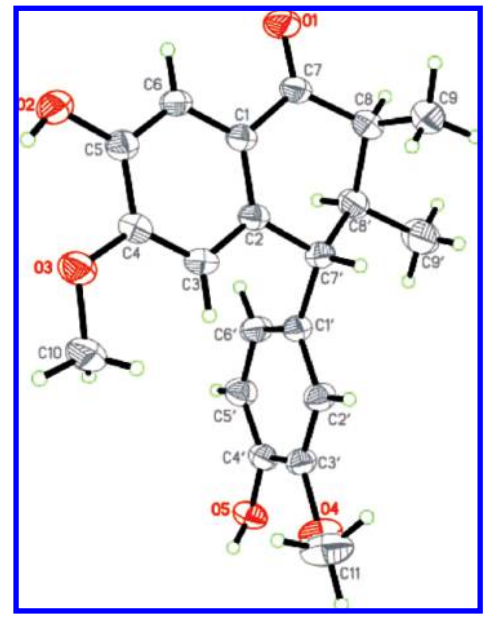


Figure 3. Perspective drawing of the X-ray structure of **1**.

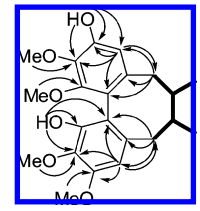


Figure 4. Selected COSY (bold bonds) and HMBC correlations (arrows) of **5**.

suggested that it was also an analogue of **1** and that the OH group of **1** had been replaced with an OCH_3 group in **3**. The OCH_3 group (δ_H 3.86, δ_C 55.8) attached at C-4′ (δ 147.7) was determined by virtue of its HMBC correlation with C-4′ and NOE correlation with H-5′ (δ 6.78). The NOESY spectrum of **3** also exhibited correlations of H-7′ (δ 3.97)/H-9 (δ 1.11)/H-9′ (δ 0.98), indicating the same configuration as **1**. Compound **3** was named arisantetralone C.

The molecular formula of **4** ($C_{21}H_{24}O_5$) was identical to that of **3**. The NMR, COSY, and HMBC data were quite similar to those of **3** and indicated that they were closely related isomers (Tables 1 and 2). Comparison of the ^{13}C NMR data of **4** with those of **3** revealed a difference at C-8 (δ 48.6 in **4**; δ 42.5 in **3**), suggesting that the configuration at C-8 was different. The presence of cross-peaks between H-8, H-9′ and H-7′ and the absence of correlation between H-9 and H7′ confirmed the *R** relative configuration at C-8. Compound **4** was given the name arisantetralone D.

Arisanschinin F (**5**) was obtained as a white, amorphous powder and had the molecular formula $C_{22}H_{28}O_6$, as inferred from the HRESIMS (*m*/*z* 411.1785 $[M + Na]^+$). The IR spectrum revealed OH (3427 cm^{-1}) and aromatic (1583 cm^{-1}) functionalities. The UV spectrum showed bands at 278, 242, and 218 nm, while its CD spectrum exhibited negative Cotton effects at λ_{max} 250 (+7.5) and 231 (−6.3) nm, suggesting that **5** was a dibenzocyclooctadiene lignan with an *R*-biphenyl configuration.[20] The ^{1}H NMR spectrum of **5** (Table 3) exhibited two aromatic singlets (δ 6.38, 6.66), two OH singlets (δ 5.71, 5.73), and four OCH_3 singlets (δ 3.60, 3.89, 3.93, 3.93) on the biphenyl moiety. The oxygenated substituents on the biphenyl rings were assigned by NMR interpretations, including the HMBC correlations of δ_H 3.60/C-14, δ_H 3.89/C-3, δ_H 3.93/C-13, δ_H 3.93/C-2, δ_H 5.73/C-11/C-12/C-13, and δ_H 5.71/C-1/C-2/C-16. Moreover, the COSY correlations of H-9 (δ 2.04, δ 2.24)/H-8 (δ 1.80)/H-18 (δ 0.98)/H-7 (δ 1.90)/H-17 (δ 0.75)/

Table 3. ^{1}H NMR Data (400 MHz) of Compounds **5**−**11**[a]

position	5	6	7	8	9	10	11
4	6.38 s	6.38 s	6.37 s	6.55 s	6.74 s	6.77 s	6.71 s
6	2.50 d (13.4)	2.05 d (13.1)	2.18 d (13.8)	5.64 s	5.72 s	5.84 s	5.68 s
	2.57 dd (7.4, 13.4)	2.28 dd (9.8, 13.1)	2.39 dd (10.1, 13.8)				
7	1.90 m	1.83 m	1.96 m				
8	1.80 m	1.90 m		1.99 m	2.04 m	2.17 m	2.02 m
9	2.04 d (13.1)	2.45 d (13.5)	5.49 s	2.21 d (13.9)	2.24 d (3.7)	2.31 d (7.3)	2.25 m
	2.24 dd (9.6, 13.1)	2.54 dd (7.5, 13.5)		2.30 dd (9.0, 13.9)			
11	6.66 s	6.67 s	6.74 s	6.50 s	6.62 s	6.57 s	6.51 s
17	0.75 d (7.0)	1.00 d (7.0)	1.16 d (7.1)	1.11 s	1.08 s	1.14 s	1.09 s
18	0.98 d (7.1)	0.74 d (7.0)	1.35 s	1.13 d (7.2)	1.13 d (7.2)	1.19 d (7.0)	1.14 d (7.1)
19			5.98 s	5.90 s		5.72 s	5.88 s
			6.01 s	5.90 s		5.81 s	5.94 s
3′			5.90 q (7.0)	6.00 q (7.3)	5.97 q (6.6)	7.51 t (7.7)	6.00 q (6.9)
4′			1.83 d (7.0)	1.86 d (7.3)	1.83 d (6.6)	7.32 t (7.7)	1.68 d (6.9)
5′			1.34 s	1.40 s	1.33 s	7.48 d (7.9)	1.58 s
1-OMe					3.51 s	3.55 s	3.54 s
2-OMe	3.93 s	3.91 s	3.84 s	3.93 s	3.89 s	3.88 s	3.88 s
3-OMe	3.89 s	3.91 s	3.88 s	3.92 s	3.92 s	3.94 s	3.91 s
13-OMe	3.93 s	3.94 s			3.85 s		
14-OMe	3.60 s	3.60 s	3.86 s	3.75 s	3.42 s	3.27 s	3.66 s
1-OH	5.71 s	5.75 s	5.49 s	5.75 s			
12-OH	5.73 s	5.70 s					

[a] Chemical shifts are in ppm (δ); J values in Hz are in parentheses.

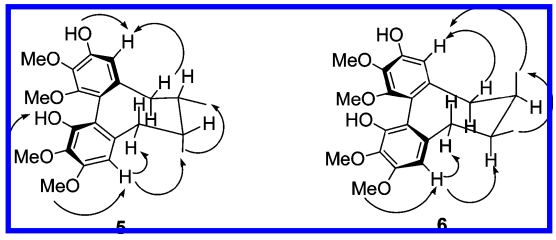


Figure 5. Selected NOESY correlations of **5** and **6**.

H-6 (δ 2.50, δ 2.57) completed the proton sequence of the cyclooctadiene ring. The HMBC spectrum of **5** revealed the correlations of H-6/C-4 (δ 107.3)/C-5 (δ 134.4)/C-16 (δ 116.7) and H-9/C-10 (δ 140.6)/C-11 (δ 110.6)/C-15 (δ 120.4), assembling the biphenyl and cyclooctadiene moieties to the C_{18}-dibenzocyclooctadiene structure [the relative configuration of **5** was determined by the NOESY experiment (Figure 5)]. On the basis of the above discussion, the chiral centers of **5** were identified as 7*R** and 8*S**. Thus the structure **5** was established for arisanschinin F.

Arisanschinin G (**6**) also had a molecular formula of $C_{22}H_{28}O_6$. The UV, IR, and NMR spectroscopic data were similar to those of **5**. The difference between **6** and **5** was the CD spectrum, which showed positive Cotton effects at 240.8 (−28.4) and 235.2 (+15.5). This suggested that compound **6** was a C_{18}-dibenzocyclooctadiene-type lignan with an *S*-biphenyl configuration. The oxygenated substituents on the biphenyl system were the same as those of **5**, as determined by HMBC experiment. NOESY cross-peaks were found between H-4, H-6, and H-7 and between H-17eq, H-18ax, and H-11, suggesting a β-orientation for the two methyl groups. Thus, the chiral centers of arisanschinin G (**6**) were identified as 7*S** and 8*R**.

The molecular formula of **7** was deduced as $C_{27}H_{32}O_9$ from its HRESIMS and DEPT NMR spectra. The UV and CD [254.4 (−20.7), 210.1 (+48.7) nm] absorption bands suggested that **7** had a C_{18}-dibenzocyclooctadiene system with an *S*-biphenyl configuration, the same as **6**. IR absorptions at 3521 and 1716 cm^{-1} revealed the presence of OH and ester functionalities. In the ^{1}H NMR spectrum of **7**, the substituents were identified as two CH_3 groups (δ 1.16, 1.35), three OCH_3 groups (δ 3.84, 3.86, 3.88), a methylenedioxy group (δ 5.98, 6.01), an OH (δ 5.49), and an angeloyl group (δ 5.90, 1.83, 1.34). The substituents on biphenyl rings were assigned by HMBC correlations of δ 3.84/C-2, δ 3.86/C-14, δ 3.88/C-3, δ 6.01/C-12/C-13, and δ 5.49/C-1. An oxymethine signal resonating at δ 5.49 (H-9) showed HMBC correlations to δ 165.7 (C-1′), δ 72.3 (C-8), δ 129.8 (C-10), δ 120.3 (C-15), and δ 106.1 (C-11), suggesting that the angeloyl group was located at C-9. It was noted that the Me-18 (s) should be attached to an oxygenated quaternary carbon. This was confirmed by HMBC correlations of the methyl with δ 42.3 (C-7), δ 72.3 (C-8), and δ 84.6 (C-9). NOESY correlations between H-11/H-9β, H-4/H6α/H-7, and H-7/H-17/H-18 indicated that the relative configuration at C-7, C-8, and C-9 in **7** was *S*. From the above interpretation, arisanschinin H was assigned structure **7**.

The molecular formula $C_{27}H_{32}O_9$ of **8** was deduced from HRESIMS. The CD, UV, IR, and NMR data of **8** were similar to those of **7** and suggested an *S*-biphenyl dibenzocyclooctadiene lignan with OH, OCH_3, and angeloyl substituents. The location of substituents on the biphenyl rings was the same as **7**, as indicated by their identical HMBC correlations. The differences between **8** and **7** on the cyclooctadiene ring were revealed by analyses of HMBC and NOESY spectra. HMBC correlations of the oxymethine at δ 5.64 with δ 166.2 (C-1′), δ 106.6 (C-4), δ 132.0 (C-5), and δ 115.0 (C-16) indicated that the angeloyl group was attached at C-6. An OH group on C-7 (δ 75.4) was confirmed by its chemical shift and HMBC correlations with H-17 (δ 1.11) and H-6 (δ 5.64) with C-7. NOESY correlations between H-4/H-6α/H-17ax and H-11/H9β/H-18ax, as well as the absence of an NOE interaction between H-17ax and H-18ax, indicated that the relative configurations of C-6, C-7, and C-8 in **8** were *S**, *R**, and *R**, respectively. Compound **8** was named arisanschinin I.

Compound **9** had the molecular formula $C_{28}H_{36}O_9$. Comparison of the CD, UV, and IR data of **9** with those of **8** revealed that they were very similar analogues. The ^{1}H and ^{13}C NMR spectra of **9** showed the presence of five OCH_3 groups and no methylenedioxy group, indicating that the difference between **9** and **8** was the substituents on the biphenyl rings. The five OCH_3 groups on the biphenyl rings were assigned by HMBC correlations. The remaining OH group was attached to C-12, as confirmed by its downfield chemical shift to δ 149.0 and HMBC correlations of H-11 (δ 6.62) with C-10 (δ 137.1), C-12, and C-15 (δ 121.1). Other HMBC and NOESY correlations of **9** were identical to those of **8**. Compound **9** was named arisanschinin J.

Arisanschinin K (**10**) had the molecular formula $C_{30}H_{32}O_9$. The CD, UV, IR, and NMR data were closely related to those of gomisin C,[21] suggesting that **10** was an analogue. All the substituents in **10** were assigned by COSY and HMBC experiments. The NOESY correlations of H-4/H-6α/H-17ax/H-18eq revealed that the relative

Table 4. ^{13}C NMR Data (δ) (100 MHz) of Compounds **5**−**11**[a]

position	5	6	7	8	9	10	11
1	146.7 s	146.6 s	146.5 s	147.3 s	152.0 s	152.2 s	152.1 s
2	133.8 s	133.4 s	133.4 s	134.9 s	141.8 s	141.8 s	140.8 s
3	150.5 s	151.8 s	151.8 s	150.4 s	151.9 s	151.9 s	151.8 s
4	107.3 d	103.9 d	103.9 d	106.6 d	110.3 d	110.0 d	110.1 d
5	134.4 s	139.8 s	136.9 s	132.0 s	131.2 s	131.3 s	131.7 s
6	39.2 t	35.5 t	36.5 t	86.5 d	86.1 d	86.8 d	86.2 d
7	33.7 d	40.9 d	42.3 d	75.4 s	75.6 s	75.5 s	75.5 s
8	41.0 d	33.7 d	72.3 s	43.0 d	42.6 d	43.4 d	43.0 d
9	35.3 t	38.7 t	84.6 d	37.2 t	37.1 t	37.2 t	37.2 t
10	140.6 s	135.3 s	129.8 s	135.6 s	137.1 s	135.0 s	135.2 s
11	110.6 d	113.7 d	106.1 d	103.2 d	109.8 d	102.3 d	102.5 d
12	149.0 s	147.7 s	148.3 s	148.9 s	149.0 s	148.8 s	148.6 s
13	137.4 s	137.6 s	137.1 s	134.8 s	137.6 s	134.4 s	134.5 s
14	150.1 s	150.2 s	141.3 s	140.2 s	149.7 s	140.5 s	139.0 s
15	120.4 s	121.5 s	120.3 s	120.0 s	121.1 s	121.0 s	121.3 s
16	116.7 s	115.8 s	115.8 s	115.0 s	121.8 s	121.8 s	121.8 s
17	12.8 q	21.8 q	19.0 q	19.6 q	19.1 q	19.2 q	19.2 q
18	21.6 q	12.4 q	28.4 q	18.8 q	18.9 q	18.8 q	18.8 q
19			101.4 t	100.7 t		100.5 t	100.5 t
1′			165.7 s	166.2 s	166.2 s	165.2 s	166.7 s
2′			126.9 s	127.1 s	127.1 s	129.5 s	127.7 s
3′			139.6 d	140.2 d	141.0 d	129.6 d	137.6 d
4′			15.6 q	15.8 q	15.6 q	127.9 d	14.2 q
5′			19.9 q	19.2 q	19.8 q	132.9 d	11.5 q
1-OMe					60.8 q	60.7 q	60.7 q
2-OMe	61.1 q	61.1 q	59.7 q	61.0 q	61.0 q	60.8 q	60.9 q
3-OMe	55.7 q	55.7 q	55.8 q	55.7 q	56.0 q	56.0 q	55.9 q
13-OMe	61.1 q	61.0 q			60.6 q		
14-OMe	60.4 q	60.4 q	60.4 q	59.3 q	59.6 q	58.7 q	59.0 q

[a] Assignments were made using HMQC and HMBC techniques. [b] Multiplicities, s = C, d = CH, t = CH_2, q = CH_3.

configuration at C-7 was different from that of gomisin C. Thus, the configuration of **10** was identified as 6*S**, 7*R**, and 8*S** and the name arisanschinin K was given.

Arisanschinin L (**11**) had the molecular formula $C_{28}H_{34}O_9$, as established from HRESIMS and DEPT spectra. The UV, IR, and CD spectra were similar to those of **10**. The ^1H and ^{13}C NMR spectra of **11** were also quite similar to those of **10**, except that the benzoyl group in **10** was replaced by a tigloyl group in **11**. The position of the tigloyl group at C-6 (δ 86.2) was confirmed by HMBC correlations of H-6 (δ 5.68) with C-1′ (δ 166.7), C-4 (δ 110.1), C-5 (δ 131.7), C-7 (δ 75.5), and C-16 (δ 121.8). The NOESY correlations of **11** showed results similar to those of **10**, suggesting that the configuration of C-6, C-7, and C-8 in **11** was *S**, *R**, and *S**, respectively. Thus the structure of arisanschinin L was established.

The isolated lignans **1**−**17** were tested in vitro on resting cells and on cells activated with PHA at 100 μM. Inhibition of cell proliferation was determined by tritiated thymidine uptake. As indicated in Table 5, compound **4** exhibited significant inhibition of proliferation of peripheral blood mononuclear cells (PBMC) induced by phytohemaglutinin (PHA).

Table 5. Inhibition of Isolated Lignans on Human PBMC Proliferation

compound (100 μM)	inhibitory activity (%) resting cells	PHA (0.2 μg/mL)	PHA (5 μg/mL)
1	59.7 ± 0.8	78.1 ± 2.1	91.9 ± 0.9
2	56.9 ± 0.7	75.3 ± 4.5	90.8 ± 1.1
3	54.1 ± 3.0	76.3 ± 2.4	89.3 ± 1.1
4	34.1 ± 1.6	70.1 ± 6.0	83.9 ± 0.9
5	58.5 ± 2.8	81.7 ± 2.1	92.9 ± 1.2
6	54.7 ± 3.2	76.3 ± 1.6	92.5 ± 1.5
7	12.7 ± 3.6	16.4 ± 2.0	14.6 ± 7.9
8	47.0 ± 6.7	74.0 ± 2.4	66.6 ± 1.7
9	44.5 ± 4.4	67.0 ± 2.2	44.3 ± 2.9
10	33.3 ± 2.5	57.1 ± 2.1	25.3 ± 7.3
11	47.4 ± 2.4	70.0 ± 5.1	37.8 ± 0.2
(−)-holostyligone	61.0 ± 2.9	82.7 ± 1.9	95.6 ± 1.0
pregomisin	63.9 ± 4.6	83.0 ± 2.2	95.6 ± 0.8
gomisin F	36.1 ± 4.5	75.2 ± 3.3	58.9 ± 2.1
gomisin G	46.2 ± 1.1	75.8 ± 1.1	88.6 ± 0.7
epigomisin O	61.3 ± 1.5	81.3 ± 2.9	86.6 ± 0.6
(+)-gomisin K_3	60.8 ± 2.3	80.1 ± 1.6	76.0 ± 0.9
cyclosporin A[a]	25.5 ± 7.3	68.0 ± 8.5	89.9 ± 4.5

[a] Positive control (5 μM).

Experimental Section

General Experimental Procedures. Melting points were measured on a Buchi melting point B-540 apparatus and are uncorrected. Optical rotations were recorded on a Jasco DIP-1000 polarimeter. The UV and IR spectra were taken with a Hitachi U-2001 and a Horiba FT-720 spectrophotometer, respectively. The ^1H and ^{13}C NMR spectra as well as 2D NMR spectra (COSY, HMQC, HMBC, and NOESY) were recorded in $CDCl_3$ using a Bruker AVX NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C using $CDCl_3$ as an internal standard (δ_H 7.265, δ_C 77.0 ppm). Low-resolution EIMS spectra were recorded on a VG Quattro 5022 mass spectrometer, and high-resolution ESIMS spectra were measured on a JEOL HX 110 mass spectrometer. LiChrospher Si 60 (5 mm, 250−10, Merck) and LiChrospher 100 RP-18e (5 mm, 250−10, Merck) were used for HPLC and RP-HPLC (Hitachi, L-6250, flow rate 2 mL/min, UV detection at 254 nm), respectively.

Plant Material. The fruits of *Schisandra arisanensis* Hay. were collected from Nan-Tou County, Taiwan, in May 2006. This species was identified by one of the authors (C.T.C.). A voucher specimen (code no. TP 93-3) was deposited in the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. Freeze-dried fruits (460 g) of *S. arisanensis* were ground and extracted thrice with acetone (3 L) at room temperature. The filtrates were combined and concentrated under vacuum to afford a crude extract (60 g), which was partitioned between H_2O/EtOAc (1:1) to yield an EtOAc-soluble portion (32 g). This portion was chromatographed on a silica gel column (300 g) eluted with an *n*-hexane/EtOAc gradient to give fractions A−I. Fractions G (3.9 g) and H (1.4 g) were further separated on a Sephadex LH-20 column eluted with MeOH to obtain fractions G1−G3 and fractions H1−H4, respectively. Fraction G2 (2.6 g) was further separated on a silica gel column eluted with *n*-hexane/CH_2Cl_2/MeOH (10:10:1 to 3:3:1) to yield **17** (800 mg) and fractions G2A−G2C. Separation of fraction G2A (100 mg) by NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (50:10:1) afforded **15** (3.4 mg) and **16** (1.9 mg). Fraction G2B (200 mg) was separated by HPLC eluting with *n*-hexane/CH_2Cl_2/MeOH (50:10:1) to furnish **12** (6.5 mg), **13** (20.1 mg), **14** (55.7 mg), and fractions G2B1−G2B2. Fraction G2B1 (9.1 mg) was separated by RP-HPLC (MeOH/H_2O, 7:3) to give **5** (3.4 mg) and **7** (1.3 mg). Fraction G2B2 (7.5 mg) was also separated by a RP-HPLC with the same solvent system to give **6** (4.6 mg). Fraction G2C (53 mg) was fractionated by NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (50:10:1) to yield **8** (5.8 mg), **9** (1.3 mg), and **10** (1.5 mg). Fraction H2 (422.4 mg) was subjected to RP-HPLC using MeOH/H_2O (65:35) to give subfraction H2A (8.1 mg), which was further purified by HPLC (*n*-hexane/EtOAc, 1:1) to yield **11** (2.4 mg). Fraction H4 (231.9 mg) was separated by RP-HPLC using MeOH/H_2O (7:3) to furnish **1** (37.6 mg), **2** (5.2 mg), **3** (43.3 mg), and **4** (5.1 mg).

Arisantetralone A (1): pale yellow prisms; mp 186−187 °C; $[\alpha]^{25}_D$ −35 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 317 (3.02), 277 (3.36), 233 (3.65), 209 (3.71) nm; IR (neat) ν_{max} 3512, 3060, 2925, 1743, 1666, 1600, 1373, 1281, 1070, 904 cm^{-1}; CD (*c* 0.1, MeOH) 343.3 (−22.5), 327.2 (+9.0), 266.1 (−10.0), 214.6 (+10.7) nm; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2, respectively; HRESIMS *m/z* 365.1368 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_5Na$, 365.1365).

Arisantetralone B (2): white, amorphous powder; $[\alpha]^{25}_D$ −35 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 281 (3.08), 234 (3.32), 204 (3.69) nm; IR (CH_2Cl_2) ν_{max} 3410, 3026, 2850, 1662, 1601, 1377, 1281, 1070, 906 cm^{-1}; CD (*c* 0.1, MeOH) 290.8 (−21.9), 274.4 (+10.9), 215.0 (+40.3) nm; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2, respectively; HRESIMS *m/z* 365.1366 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_5Na$, 365.1365).

Arisantetralone C (3): pale yellow needles; mp 180−181 °C; $[\alpha]^{25}_D$ −18.5 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 317 (3.53), 276 (3.75), 240 (3.82) nm; IR (CH_2Cl_2) ν_{max} 3451, 3060, 2850, 1668, 1601, 1373,

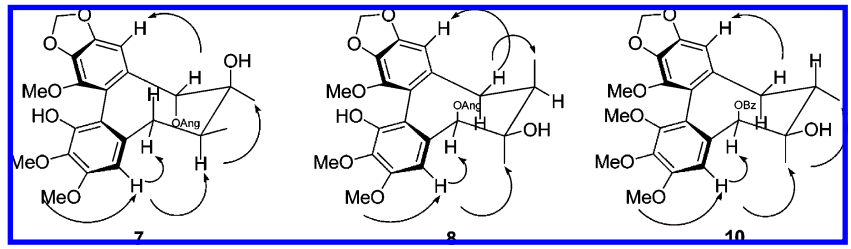
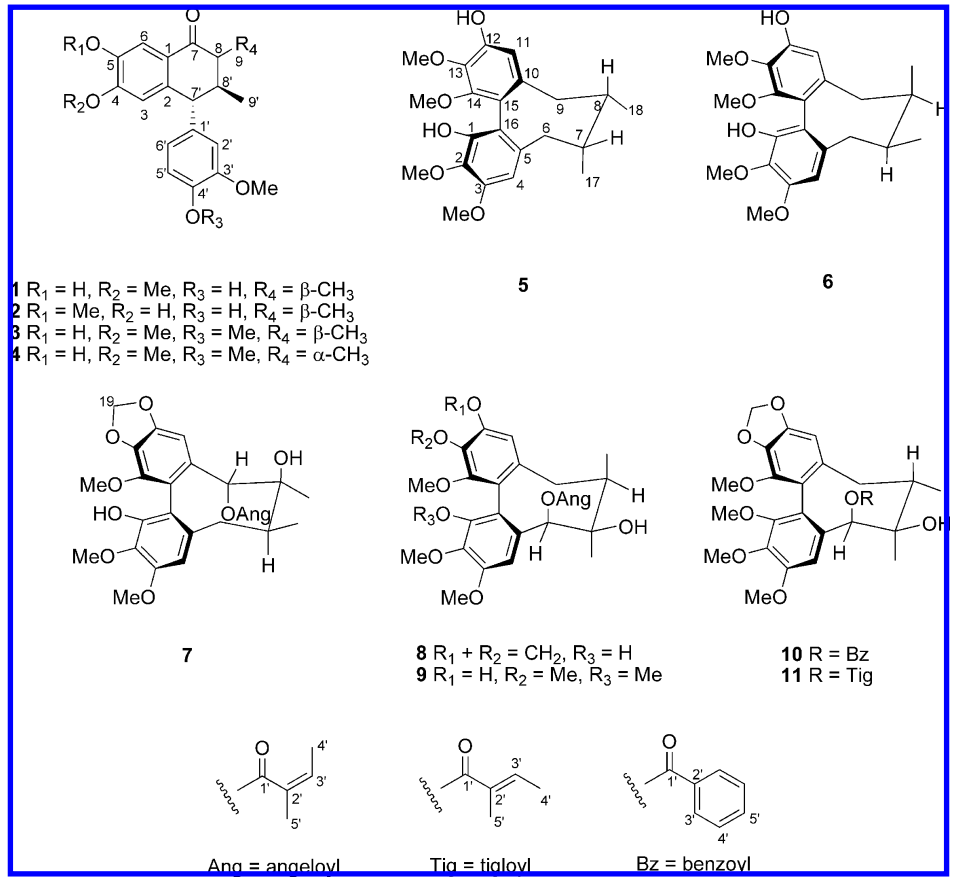


Figure 6. Selected NOESY correlations of **7**, **8**, and **10**.

Chart 1



1277, 1070, 906 cm^{-1}; CD (*c* 0.1, MeOH) 327.4 (−40.0), 299.9 (−28.5), 250.3 (+18.9) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2, respectively; HRESIMS *m/z* 379.1523 [M + Na]$^+$ (calcd for $C_{21}H_{24}O_5Na$, 379.1521).

Arisantetralone D (4): pale yellow oil; $[\alpha]^{25}_D$ +17.5 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 276 (3.24), 209 (3.75) nm; IR (CH_2Cl_2) ν_{max} 3468, 3060, 2850, 1668, 1601, 1373, 1070, 906 cm^{-1}; CD (*c* 0.1, MeOH) 334.4 (+6.3), 288.0 (−10.5), 212.9 (+19.5), 202.9 (−34.1) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 379.1524 [M + Na]$^+$ (calcd for $C_{21}H_{24}O_5Na$, 379.1521).

Arisanschinin F (5): white, amorphous powder; $[\alpha]^{25}_D$ +13.3 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 278 (3.65), 242 (3.85) nm; IR (CH_2Cl_2) ν_{max} 3427, 2933, 1583, 1456, 1124, 1003, 849 cm^{-1}; CD (*c* 0.1, MeOH) 250.2 (+7.5), 239.0 (+5.0), 231.3 (−6.3), 226.1 (+27.7) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m/z* 411.1785 [M + Na]$^+$ (calcd for $C_{22}H_{28}O_6Na$, 411.1783).

Arisanschinin G (6): pale yellow oil; $[\alpha]^{25}_D$ −86.7 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 240 (3.84) nm; IR (CH_2Cl_2) ν_{max} 3474, 2929, 1612, 1454, 1124, 1005 cm^{-1}; CD (*c* 0.1, MeOH) 249.4 (−25.7), 240.8 (−28.4), 235.2 (+15.5), 228.6 (+19.5) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m/z* 411.1786 [M + Na]$^+$ (calcd for $C_{22}H_{28}O_6Na$, 411.1783).

Arisanschinin H (7): white, amorphous powder; $[\alpha]^{25}_D$ +16.7 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 238 (3.96) nm; IR (CH_2Cl_2) ν_{max} 3521, 2931, 1716, 1614, 1232, 1107 cm^{-1}; CD (*c* 0.1, MeOH) 254.4 (−20.7), 210.1 (+48.7), 203.5 (+54.2) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m/z* 523.1940 [M + Na]$^+$ (calcd for $C_{27}H_{32}O_9Na$, 523.1944).

Arisanschinin I (8): pale yellow oil; $[\alpha]^{23}_D$ −25 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 220 (3.96) nm; IR (CH_2Cl_2) ν_{max} 3508, 3026, 2925, 1714, 1601, 1373, 843 cm^{-1}; CD (*c* 0.1, MeOH) 253.4 (−4.8), 229.7 (+4.6), 223.8 (−19.9), 214.1 (+16.4) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m/z* 523.1947 [M + Na]$^+$ (calcd for $C_{27}H_{32}O_9Na$, 523.1944).

Arisanschinin J (9): pale yellow oil; $[\alpha]^{23}_D$ −30 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 226 (3.95) nm; IR (CH_2Cl_2) ν_{max} 3452, 3026, 2922, 1714, 1601, 843 cm^{-1}; CD (*c* 0.1, MeOH) 255.3 (−26.2), 234.0 (+4.6), 218.7 (+25.7) nm;^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m/z* 539.2260 [M + Na]$^+$ (calcd for $C_{28}H_{36}O_9Na$, 539.2257).

Arisanschinin K (10): white, amorphous powder; $[\alpha]^{23}_D$ −43 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 216 (3.87) nm; IR (CH_2Cl_2) ν_{max} 3471, 3026, 2924, 1718, 1601, 1373, 1153, 906 cm^{-1}; CD (*c* 0.1, MeOH) 256.7 (−24.5), 242.6 (−36.0), 226.4 (+11.7) nm; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m/z* 559.1947 [M + Na]$^+$ (calcd for $C_{30}H_{32}O_9Na$, 559.1944).

Arisanschinin L (11): white, amorphous powder; $[\alpha]^{23}_D$ −73 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 219 (3.98) nm; IR (CH_2Cl_2) ν_{max} 3459, 3026, 2924, 1705, 1601, 1269, 1107, 906 cm^{-1}; CD (*c* 0.1, MeOH) 253.4 (−33.0), 244.0 (−37.2), 230.2 (+9.2) nm; ^{1}H NMR

($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 3 and 4, respectively; HRESIMS *m*/*z* 537.2097 $[M + Na]^+$ (calcd for $C_{28}H_{34}O_9Na$, 537.2101).

Single Crystal X-ray Structure Determination of Arisantetralone A (1). A single crystal of arisantetralone A (**1**) was obtained by evaporation from CH_2Cl_2. Crystal data: $C_{20}H_{22}O_5$, $M = 342.38$, orthorhombic system, space group $P2(1)2(1)2(1)$, $a = 9.2700(3)$ Å, $b = 11.3223(3)$ Å, $c = 17.3105(6)$ Å, $V = 1816.87(10)$ Å^3, $Z = 2$, $d = 1.252$ Mg/m^3. A crystal of dimensions $0.25 \times 0.20 \times 0.15$ mm was used for measurements on a Siemens SMART CCD XRD diffractometer. The total number of independent reflections measured was 9285, of which 3282 were observed [R(int) = 0.0245]. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure. The final X-ray structural model is shown in Figure 3.

Lymphoproliferation Test. The lymphoproliferation test was modified from that previously described.[22,23] The density of PBMC was adjusted to 2×10^6 cells/mL before use. A cell suspension (100 μL) was applied to each well of a 96-well flat-bottomed plate (Nunc 167008, Nunclon, Raskilde, Denmark) with or without PHA (Sigma). Compounds were added to the cells at 100 μM. The plates were incubated in 5% CO_2−air humidified atmosphere at 37 °C for 3 days. Subsequently, tritiated thymidine (1 μCi/well, NEN) was added into each well. After 16 h of incubation, the cells were harvested on glass fiber filters by an automatic harvester (Dynatech, Multimash 2000, Billingshurst, U.K.). Radioactivity in the filters was measured by a scintillation counter. Cyclosporine A was used as a standard compound.

Acknowledgment. This work was supported by the National Science Council of Republic of China (NSC98-2320-B002-027-MY3). We are grateful to S.-C. Yu of NSC Southern MS Instrument Center for measurement of MS data.

Note Added after ASAP Publication: Errors in Figure 1 and in the caption of Figure 4 were corrected in the version posted on Aug 18, 2009.

Supporting Information Available: Structures of compounds **12**−**17**, NMR spectra of **1**−**11**, and cif file of X-ray data of **1**. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Yao, D. M.; Zhang, J. B. *A Coloured Altas of the Chinese Materia Medica Specified in Pharmacopoeia of the People's Republic of China (1995 edition)*; Guangdong Science & Technology Press: Guangdong, 1996; pp 78−79.
(2) Kuo, Y. H.; Li, S. Y.; Huang, R. L.; Wu, M. D.; Huang, H. C.; Lee, K. H. *J. Nat. Prod.* **2001**, *64*, 487–490.
(3) Shen, Y. C.; Lin, Y. C.; Michael, Y. C.; Sheau, F. Y.; Cheng, Y. B.; Liao, C. C. *Org. Lett.* **2005**, *7*, 3307–3310.
(4) Xiao, W. L.; Li, R. T.; Huang, S. X.; Pu, J. X.; Sun, H. D. *Nat. Prod. Rep.* **2008**, *25*, 871–891.
(5) Tang, M. H.; Chiu, P. Y.; Ko, K. M. *Biofactors* **2003**, *19*, 33–42.
(6) Hausott, B.; Greger, H.; Marian, B. *J. Cancer Res. Clin. Oncol.* **2003**, *129*, 569–576.
(7) Chen, D. F.; Zhang, S. X.; Chen, K.; Zhou, B. N.; Wang, P.; Cosentino, L. M.; Lee, K. H. *J. Nat. Prod.* **1996**, *59*, 1066–1068.
(8) Lu, H.; Liu, G. T. *Planta Med.* **1991**, *58*, 311–313.
(9) Charlton, J. L. *J. Nat. Prod.* **1998**, *61*, 1447–1451.
(10) Shen, Y. C.; Lin, Y. C.; Cheng, Y. B.; Kuo, Y. H.; Liaw, C. C. *Org. Lett.* **2005**, *7*, 5297–5300.
(11) Shen, Y. C.; Cheng, Y. B.; Lam, T. W.; Liaw, C. C.; Liou, S. S.; Kuo, Y. H.; Khalil, A. S. *J. Nat. Prod.* **2007**, *70*, 1139–1145.
(12) Shen, Y. C.; Lin, Y. C.; Cheng, Y. B.; Chang, C. J.; Lan, T. W.; Liou, S. S.; Chien, C. T.; Liaw, C. C.; Khalil, A. S. *Helv. Chim. Acta* **2008**, *91*, 483–494.
(13) Silva, T. D.; Lopes, L. M. X. *Phytochemistry* **2006**, *67*, 929–937.
(14) Ikeya, Y.; Taguchi, H.; Yosioka, I.; Kobayashi, H. *Chem. Pharm. Bull.* **1979**, *27*, 1583–1588.
(15) Taguchi, H.; Ikeya, Y. *Chem. Pharm. Bull.* **1977**, *25*, 364–366.
(16) Ikeya, Y.; Taguchi, H.; Sasaki, H.; Nakajima, K.; Yosioka, I. *Chem. Pharm. Bull.* **1980**, *28*, 2414–2421.
(17) Ikeya, Y.; Taguchi, H.; Yosioka, I. *Chem. Pharm. Bull.* **1980**, *28*, 2422–2427.
(18) Lopes, L. M. X.; Yoshida, M.; Gottlieb, O. R. *Phytochemistry* **1982**, *21*, 751–755.
(19) Silva, T. D.; Lopes, L. M. X. *Phytochemistry* **2004**, *65*, 751–759.
(20) Kuo, Y. H.; Chen, C. H.; Chiang, Y. M. *Tetrahedron letters* **2001**, *42*, 6731–6735.
(21) Ikeya, Y.; Taguchi, H.; Yosioka, I.; Kobayashi, H. *Chem. Pharm. Bull.* **1979**, *27*, 1383–1394.
(22) Kuo, Y. C.; Yang, N. S.; Chou, C. J.; Lin, L. C.; Tsai, W. J. *Mol. Pharmacol.* **2000**, *58*, 1057–1066.
(23) Kuo, Y. C.; Lu, C. K.; Huang, L. W.; Kuo, Y. H.; Chang, C.; Hsu, F. L.; Lee, T. H. *Planta Med.* **2005**, *71*, 412–415.

NP9003678