JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

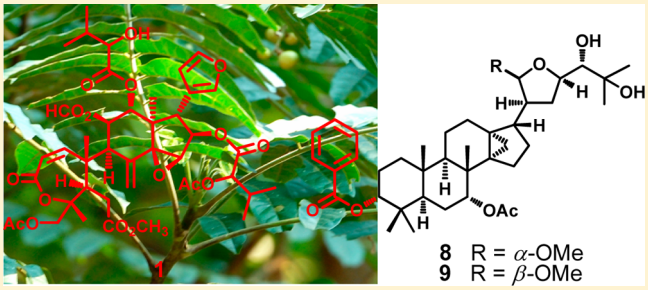
Limonoids and Triterpenoids from *Dysoxylum mollissimum* var. *glaberrimum*

Mei-Ling Han,† Jin-Xin Zhao,† Hong-Chun Liu, Gang Ni, Jian Ding, Sheng-Ping Yang,* and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai, 201203, People's Republic of China

Supporting Information

ABSTRACT: Seven new limonoids, dysomollides A–G (**1**–**7**), and two new cycloapotirucallane-type triterpenoids, dysomollins A and B (**8** and **9**), together with three known compounds, dysoxylumin A (**10**) and toonapubesins A (**11**) and B (**12**), were isolated from the twigs of *Dysoxylum mollissimum* var. *glaberrimum*. The structures of **1**–**9** were elucidated on the basis of spectroscopic methods. Compound **10** showed inhibitory activity against A549 cells with an IC_{50} value of 2.1 μM, and compound **11** exhibited activity against P388 cells with an IC_{50} value of 6.7 μM.



The plant genus *Dysoxylum* (family Meliaceae) comprises about 75 species in the tropical and subtropical zones globally, of which about 15 species and varieties grow in mainland China.[1] Previous phytochemical investigations on this plant genus have led to the isolation of a series of structurally diverse compounds, including triterpenoids,[2−5] limonoids,[6−8] diterpenoids,[9] sesquiterpenoids,[10−12] and alkaloids,[13,14] and some of them have been found to exhibit significant bioactivities, such as cytotoxic, antiviral, and insect antifeedant activities. The plant *Dysoxylum mollissimum* var. *glaberrimum* P. Y. Chen, which is a tall tree, is distributed in the southern areas of China,[1] and its chemical components have not been reported hitherto. The present investigation on this plant has led to the isolation of seven new limonoids, dysomollides A–H (**1**–**7**), and two new cycloapotirucallane-type triterpenoids, dysomollins A and B (**8** and **9**), along with three known compounds, dysoxylumin A[8a] and toonapubesins A and B.[15] Presented herein are the isolation and structural elucidation of these compounds and their inhibitory activity against three tumor cell lines.

RESULTS AND DISCUSSION

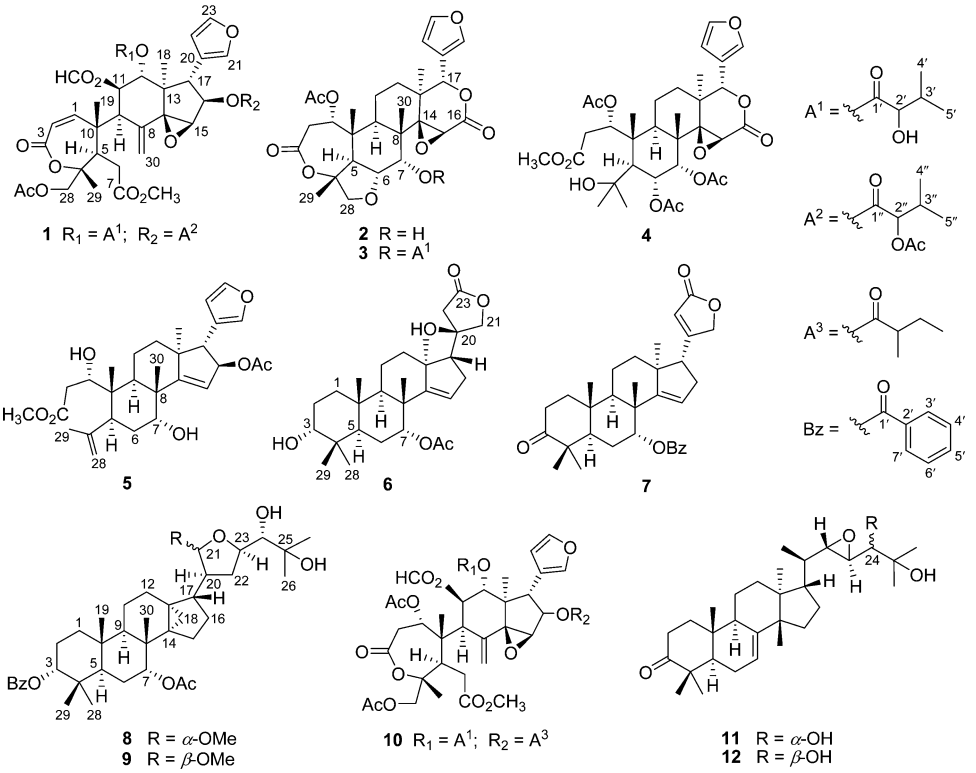
The (+) HREISMS of **1** showed a molecular ion $[M + Na]^+$ at m/z 853.3267 (calcd 853.3259) corresponding to a molecular formula of $C_{42}H_{54}O_{17}$ with 16 indices of hydrogen deficiency. The IR absorptions at 3437 and 1741 cm^{-1} indicated the presence of hydroxy and ester carbonyl groups, respectively. Except for the readily distinguishable resonances of two acetyls (δ_H 2.06 and 2.10, each 3H, s) and a methoxy group (δ_H 3.70, 3H, s), the ^1H NMR data (Table 1) also indicated the presence of three tertiary methyls (δ_H 1.01, 1.06, and 1.32, each 3H, s), four secondary methyls (δ_H 0.71, 0.87, 0.88, and 0.89, each 3H, d, J = 6.8 Hz), and a β-substituted furan ring (δ_H 6.33, 7.26, and 7.40, each 1H, s). All 42 carbons in the molecular formula were resolved as individual carbon resonances (Table 1) in the ^{13}C NMR spectrum and classified with the aid of DEPT and HSQC experiments as 10 methyls (one methoxy), two sp^3 methylenes (one oxygenated), 11 sp^3 methines (six oxygenated), four quaternary carbons (two oxygenated), seven ester carbonyls, and four double bonds (one exocyclic). The aforementioned data indicated that **1** is a ring B-*seco* limonoid bearing a typical $\Delta^{8(30)}$ double bond.[8a]

Comparison of the NMR data of **1** with those of dysoxylumin B[8a] revealed that they are structural congeners, with the differences being the absence of an acetoxy group at C-1 and the concomitant presence of a Δ^1 double bond in **1**. This deduction was confirmed by the key HMBC correlation (Figure 1A) from H-1 (δ_H 7.02, d, J = 13.0 Hz) to C-3 (δ_C 168.9). The presence of an exocyclic $\Delta^{8(30)}$ double bond was verified by the HMBC correlations of H_2-30/C-8, C-9, and C-14. The chemical shifts of C-14 (δ_C 71.4) and C-15 (δ_C 61.1) suggested the presence of an 14,15-epoxy group, which was confirmed by the multiple HMBC correlations of H-15/C-16 and C-17, H_3-18/C-14, and H_2-30/C-14. The HMBC correlations of H_3-19/C-1 and H-1/C-3 and C-5, as well as a ^4J HMBC correlation of Me-29/C-3 provided evidence of an α,β-unsaturated lactone formed between C-3 and C-4. The ROESY correlations of H-9/H-5, H-9/H-11, H_3-18/H-11, H_3-18/H-16, H-16/H-15, and H-12/H-17 (Figure 1B), as well as the very similar NMR patterns to those of dysoxylumin B,[8a] suggested that they share the same relative configurations in the limonoid core. The structure of **1**, named dysomollide A, was thus assigned as depicted.

Received: December 2, 2014

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

Chart 1



Compound **2** gave the molecular formula $C_{28}H_{34}O_{10}$ as determined by the (−) HRESIMS ion at *m/z* 575.2129 $[M + HCO_2]^-$ (calcd 575.2129) and from the ^{13}C NMR data. The ^1H and ^{13}C NMR data (Table 1) of dysomollide B (**2**) showed high similarities to those of dysoxylin[6] except for the major changes of the chemical shifts around C-1 and C-2. The proton and carbon signals for a methylene (δ_H 3.19, 3.12; δ_C 36.1), an oxygenated methine (δ_H 4.84; δ_C 71.6), and an acetyl group (δ_H 2.07; δ_C 20.9, 169.6) were observed in **2** instead of those of the Δ^1 double bond in dysoxylin.[6] The presence of an acetoxy group at C-1 was confirmed by the HMBC correlations (Figure 2A) from H-1 (δ_H 4.84) to C-2 (δ_C 36.1), C-3 (δ_C 168.8), and the carbonyl carbon (δ_C 169.6) of the acetyl group. The AcO-1 group was established as being α-oriented on the basis of the key ROESY correlations (Figure 2B) of H-1/H-2β and H_3-19. The structure of **2** (dysomollide B) was thus assigned as depicted and further verified by a comprehensive analysis of the 2D NMR data including the HSQC, HMBC, and ROESY spectra.

Compound **3** was found to possess a molecular formula of $C_{33}H_{42}O_{12}$ based on the (+) HREISMS ion at *m/z* 653.2575 $[M + Na]^+$ (calcd 653.2574) and the ^{13}C NMR data. Comparison of its NMR data (Table 1) with those of **2** revealed that **3** possesses an additional 2-hydroxy-3-methylbutyryl group, which was confirmed by the related HMBC correlations (Figure S27, Supporting Information) within this moiety. The H-7 resonance (δ_H 5.03) of **3** was deshielded (ca. $\Delta\delta$ 1.46) when compared with **2**, indicating that the 2-hydroxy-3-methylbutyryloxy group is located at C-7, which was verified by the key HMBC correlation of H-7/C-1′. The structure of **3** (dysomollide C) was thus assigned as shown.

Compound **4** gave the molecular formula $C_{33}H_{44}O_{13}$, as determined by the ^{13}C NMR data and the (+) HREISMS ion peak at *m/z* 671.2674 $[M + Na]^+$ (calcd 671.2680). Comprehensive analysis of the ^1H and ^{13}C NMR data (Table 2) of **4** suggested that it is an isomer of odoralide,[16] with the difference being that an OAc-6 group in **4** replaced the OAc-11 of the latter. This assignment was corroborated by analysis of the HMBC spectrum (Figure S35, Supporting Information), in which the correlations from H-6 (δ_H 5.13) to C-5 (δ_C 48.0), C-7 (δ_C 72.0), and the carbonyl carbon (δ_C 172.0) of the acetyl group were observed. The OAc-6 group was then assigned in an α-configuration from the ROESY cross-peaks of H-6 with H-7, H_3-19, and H_3-30. Thus, the structure of **4** (dysomollide D) was elucidated unequivocally as shown.

Compound **5** gave the molecular formula $C_{29}H_{40}O_7$ as deduced from the ^{13}C NMR data and the (+) HREISMS ion at *m/z* 523.2678 $[M + Na]^+$ (cacld 523.2672). Its IR absorptions at 3512, 3460, 1741, and 1724 cm^{-1} indicated the presence of hydroxy and ester carbonyl groups. The ^1H NMR data (Table 2) of **5** revealed the presence of four tertiary methyls (δ_H 0.90, 0.99, 1.22, and 1.82, each 3H, s), an acetyl (δ_H 1.98, 3H, s), a methoxy group (δ_H 3.59, 3H, s), and a typical β-furan ring (δ_H 6.40, 7.41, and 7.46, each 1H, s). Its ^{13}C NMR data (Table 2), with the aid of a DEPT experiment, displayed 29 carbon signals including six methyls, four sp^3 methylenes, six sp^3 methines (three oxygenated), three sp^3 quaternary carbons, four double bonds (one terminal), and two ester carbonyls. The aforementioned analysis suggested that **5** is a ring A-*seco* limonoid.[17]

Four structural fragments in **5** drawn in bold bonds (Figure 3A) in the limonoid core were established by analysis of its HSQC and $^1H-^1H$ COSY spectra, which were connected with the quaternary carbons, oxygen atoms, and the other structural pieces by analysis of the HMBC spectrum (Figure 3A). In the HMBC spectrum, a methoxycarbonyl group was identified and located at C-2 by the correlations from OCH_3 (δ_H 3.59) and H_2-2 to the carbonyl carbon C-3 (δ_C 174.2). Also a $\Delta^{4(28)}$

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of 1−3

	1[a]		2[b]		3[b]	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	150.4	7.02 (d, 13.0)	71.6	4.84 (d, 6.5)	71.4	4.87 (d, 6.7)
2	124.0	6.27 (d, 13.0)	36.1	α 3.19 (dd, 14.6, 6.5)	36.2	α 3.21 (dd, 14.9, 6.7)
				β 3.12 (d, 14.6)		β 3.15 (d, 14.9)
3	168.9		168.8		168.5	
4	86.8		83.1		82.6	
5	52.1	3.50 (m)	46.4	2.97 (d, 12.1)	47.6	2.83 (d, 12.4)
6	35.7	2.53 (2H, m)	77.9	4.12 (dd, 12.1, 2.5)	76.3	4.21 (dd, 12.4, 2.3)
7	175.9		70.0	3.57 (br s)	72.6	5.03 (d, 2.3)
8	136.3		43.8		43.7	
9	54.7	3.23 (d, 7.0)	34.3	2.83 (m)	35.4	2.84 (m)
10	47.8		42.9		42.9	
11	71.2	5.76 (dd, 11.0, 7.0)	14.3	α 1.42 (m)	14.4	α 1.47 (m)
				β 1.64 (m)		β 1.72 (m)
12	75.7	6.05 (d, 11.0)	26.1	α 1.64 (m)	25.4	α 1.65 (m)
				β 1.44 (m)		β 1.49 (m)
13	46.7		38.3		39.0	
14	71.4		69.5		69.4	
15	61.1	4.21 (s)	57.7	3.91 (s)	56.7	3.76 (s)
16	80.1	5.46 (d, 9.0)	167.6		167.2	
17	44.2	3.08 (d, 9.0)	78.2	5.56 (s)	78.0	5.56 (s)
18	15.8	1.06 (3H, s)	17.3	1.19 (3H, s)	16.8	1.18 (3H, s)
19	24.9	1.01 (3H, s)	15.6	1.17 (3H, s)	15.5	1.23 (3H, s)
20	121.2		120.5		120.1	
21	144.8	7.40 (s)	141.1	7.39 (s)	141.2	7.38 (s)
22	112.8	6.33 (s)	109.9	6.31 (d, 0.8)	109.8	6.29 (s)
23	143.6	7.26 (s)	143.0	7.40 (s)	141.2	7.40 (s)
28	64.9	4.22 (d, 12.1)	81.8	α 4.05 (d, 8.5)	81.7	α 3.88 (d, 8.5)
		5.04 (d, 12.1)		β 3.89 (d, 8.5)		β 3.81 (d, 8.5)
29	25.2	1.32 (3H, s)	22.3	1.72 (3H, s)	22.3	1.71 (3H, s)
30	124.8	5.53 (s)	18.0	1.05 (3H, s)	18.9	1.18 (3H, s)
		5.56 (s)				
1′	175.5				173.4	
2′	76.5	3.40 (d, 2.2)			76.8	4.08 (dd, 5.1, 3.1)
3′	32.8	1.71 (m)			31.5	2.13 (m)
4′	16.4	0.71 (3H, d, 6.8)			16.6	0.99 (3H, d, 6.8)
5′	20.2	0.88 (3H, d, 6.8)			18.9	1.07 (3H, d, 6.8)
1″	171.3					
2″	78.8	4.70 (d, 4.7)				
3″	31.8	2.15 (m)				
4″	18.1	0.87 (3H, d, 6.8)				
5″	19.1	0.89 (3H, d, 6.8)				
OAc-1			169.6		169.2	
			20.9	2.07 (3H, s)	20.6	2.06 (3H, s)
OAc-28	172.9					
	20.9	2.10 (3H, s)				
OAc-2″	172.7					
	21.0	2.06 (3H, s)				
HCO_2-11	162.9	8.09 (s)				
OMe-7	53.3	3.70 (3H, s)				

[a]Spectra were recorded in methanol-d_4. [b]Spectra were recorded in $CDCl_3$.

double bond was established by the mutual correlations of H_3-29/C-4, C-5, and C-28 and of H_2-28/C-4 and C-5, which is indicative of a ring A-*seco* limonoid for **5**.[17] The presence of a Δ^{14} double bond was assigned by the HMBC correlations from H-15 (δ_H 5.49), H-16 (δ_H 4.97), H_3-18, and H_3-30 to C-14 (δ_C 162.4) and from H-16 to C-15 (δ_C 126.1). An acetoxy group was placed at C-16 by the key HMBC correlation from H-16 to its carbonyl carbon (δ_C 172.9). The β-furan ring was attached to C-17 by the HMBC correlations from H-17 to C-20 and C-21. The chemical shifts of CH-1 (δ_H 5.45, δ_C 79.2) and CH-7 (δ_H 3.93, δ_C 73.6) indicated these to be oxygenated methines both bearing a hydroxy group.

In the ROESY spectrum (Figure 3B), the correlation networks of H_3-19/H-6β, H-6β/H_3-30, H_3-30/H-12β, H_3-30/H-7, and H-12β/H-17 indicated that H_3-19, H_3-30, H-7, and H-17 are spatially close, and these were randomly proposed in a β-

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

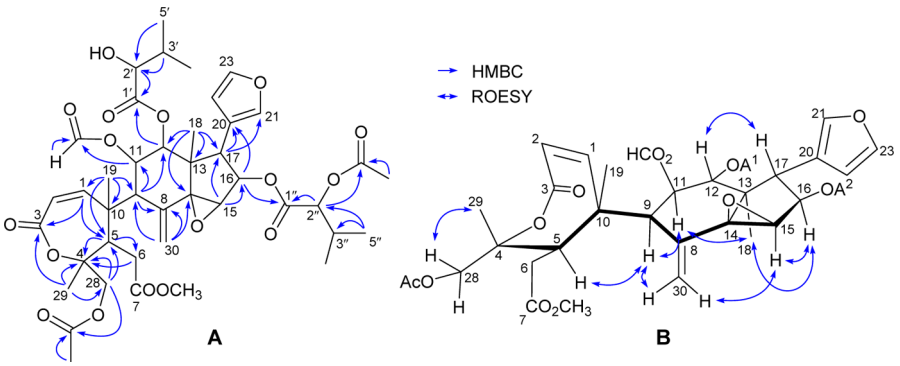


Figure 1. Selected HMBC (A) and ROESY (B) correlations of **1**.

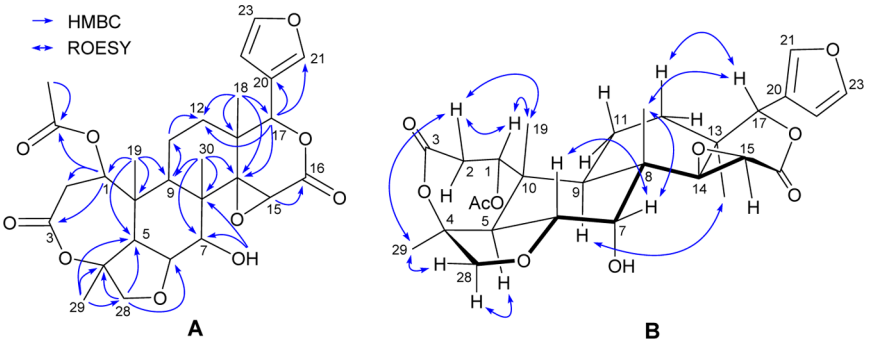


Figure 2. Selected HMBC (A) and ROESY (B) correlations of **2**.

configuration. Next, the ROESY cross-peaks of H-5/H-9 and H-21/H-16 indicated that H-5, H-9, and H-16 are α-oriented. The ROESY correlations of H_3-19/H-1 and H-2β suggested that the H-1, H-2β, and CH_3-19 are cofacial and β-oriented, which was consistent with the ROESY correlation of H-1/H-11β. The structure of **5** (dysomollide E) was thus established as depicted.

Compound **6** was assigned the molecular formula $C_{28}H_{42}O_6$ according to the ^{13}C NMR data and a (−) HRESIMS ion at m/z 519.2961 $[M + HCO_2]^-$ (calcd 519.2958). The ^1H and ^{13}C NMR data (Table 2) of **6** showed many similarities to those of lenticellatumin,[18] suggesting their structures to be closely related, with the only difference being a hydroxy group attached at C-3 (δ_C 75.9) in **6** replacing the C-3 keto group of the latter. This was confirmed by the HMBC correlations from H_2-1, H_3-28, and H_3-29 to C-3 (Figure S54, Supporting Information). The small coupling constant of H-3 (dd, J = 2.7, 2.7 Hz) indicated that it is equatorially bonded toward the β-orientation (Figure S3, Supporting Information). The only acetoxy group (δ_H 1.98, 3H, s; δ_C 170.4, 21.4) was confirmed to be located at C-7 by the HMBC correlation from H-7 (δ_H 5.18) to the carbonyl carbon of the acetyl group. The other stereocenters of **6** were established as being identical with those of lenticellatumin from the NMR data and ROESY spectrum (Figure S55, Supporting Information). Thus, the structure of **6** (dysomollide F) was elucidated as shown.

Compound **7** gave the molecular formula $C_{33}H_{40}O_5$ on the basis of the ^{13}C NMR data and the (−) HRESIMS ion peak at m/z 561.2838 $[M + HCO_2]^-$ (calcd for $C_{34}H_{41}O_7$, 561.2852). Initial analysis of the ^1H and ^{13}C NMR data of **7** (Table 3) revealed that it possesses the same tetranortriterpenoid skeleton as that of **6**. Further comparison of its NMR data with those of meliatoosenin B[19] indicated that they are structural analogues except for the presence of one more trisubstituted double bond (δ_H 5.79, d, J = 1.5 Hz; δ_C 117.0, 169.7) and an additional benzoyl group in **7**. The trisubstituted $\Delta^{20(22)}$ double bond was located by the chemical shifts of C-20 (δ_C 169.7) and C-22 (δ_C 117.0) and the HMBC correlations from H_2-21 to C-22 and C-23 and from H-17 to C-20 and C-21(Figure S63, Supporting Information). The H-7 (δ_H 5.53) signal was deshielded as compared with that of meliatoosenin B, indicating that the benzoyloxy group could be placed at C-7, which was confirmed by the key HMBC correlations from H-7 and H-3′ (or H-7′) (δ_H 7.95) to C-1′ (δ_C 165.5). The relative configurations of the tetracyclic core were assigned from the ROESY spectrum (Figure S64, Supporting Information) to be the same as those of meliatoosenin B. The structure of **7** (dysomollide G) was thus elucidated as shown.

Compound **8** was assigned a molecular formula of $C_{40}H_{58}O_8$ as determined by the ^{13}C NMR data and the (+) HREISMS ion peak at m/z 689.4034 $[M + Na]^+$ (calcd 689.4029). Analysis of the ^1H and ^{13}C NMR data (Table 3) of **8** revealed this molecule to have an apotirucallane scaffold, and its structure proved to be very similar to that of 3α-(3-methyl-2-butenoyloxy)-21,23β-epoxy-21α-methoxyapotirucallane-7α,24α,25-triol.[20] The major structural differences between these compounds were found to be the substitutions at C-3 and C-7. A benzoyloxy group was located at C-3 by the HMBC correlation from H-3 (δ_H 4.86) to C-1′ (δ_C 165.6), and an acetoxy group was located at C-7 by the HMBC correlations from H-7 (δ_H 5.03) to its acetyl carbonyl carbon (δ_C 169.9) (Figure S72, Supporting Information). The ROESY spectrum verified that the relative configuration of **8** is consistent with that of 3α-(3-methyl-2-butenoyloxy)-21,23β-epoxy-21α-methoxyapotirucallane-7α,24α,25-triol.[20] The structure of compound **8** was thus assigned as shown, and this compound has been named dysomollin A.

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of 4−6

	4[a]		5[a]		6[b]	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	78.4	7.00 (br s)	79.2	5.45 (d, 10.3)	32.6	1.38 (2H, m)
2	37.2	α 2.85 (d, 13.4)	36.7	α 2.93 (d, 14.3)	25.0	α 1.58 (m)
		β 2.42 (dd, 13.4, 9.9)		β 2.36 (dd, 14.3, 10.5)		β 1.98 (m)
3	174.1		174.2		75.9	3.42 (dd, 2.7, 2.7)
4	74.1		148.1		36.9	
5	48.0	2.16 (d, 11.8)	44.7	2.67 (dd, 13.9, 2.7)	41.8	1.84 (dd, 13.0, 2.3)
6	73.9	5.13 (dd, 11.8, 2.0)	32.8	α 1.60 (m)	23.2	1.68 (2H, m)
				β 2.27[d]		
7	72.0	4.80 (d, 2.0)	73.6	3.93 (br s)	75.3	5.18 (dd, 2.6, 2.6)
8	43.5		45.4		42.4	
9	36.9	2.63 (m)	34.9	2.23[d]	42.6	2.07 (m)
10	48.3		46.1		37.4	
11	18.3	α 2.08 (m)	19.8	α 1.77 (m)	16.3	α 1.78 (m)
		β 1.93 (m)		β 1.88 (m)		β 1.53 (m)
12	27.4	α 1.50 (m)	35.0	α 1.65 (m)	34.6	α 1.96 (m)
		β 1.75 (m)		β 1.78 (m)		β 1.52 (m)
13	39.6		49.7[c]		47.8	
14	71.0		162.4		158.9	
15	57.1	3.59 (s)	126.1	5.49 (s)	117.7	5.28 (d, 2.1)
16	169.6		79.7	4.97[e]	31.6	α 2.34 (m)
						β 2.12 (m)
17	79.9	5.62 (s)	63.0	2.63 (d, 8.6)	60.3	1.94 (m)
18	18.0	1.25 (3H, s)	23.6	0.90 (3H, s)	20.9	1.18 (3H, s)
19	16.1	1.31 (3H, s)	16.0	0.99 (3H, s)	15.4	0.90 (3H, s)
20	121.9		124.3		79.3	
21	142.8	7.51 (s)	142.1	7.46 (s)	78.4	4.25 (2H, s)
22	111.0	6.43 (s)	112.9	6.40 (s)	42.4	α 2.57 (d, 17.5)
						β 2.67 (d, 17.5)
23	144.5	7.52 (s)	144.4	7.41 (s)	175.5	
28	36.9	1.43 (3H, s)	116.8	a 4.98[e]	28.0	0.85 (3H, s)
				b 4.92[c]		
29	28.1	1.19 (3H, s)	23.9	1.82 (3H, s)	21.9	0.83 (3H, s)
30	18.4	1.25 (3H, s)	28.0	1.22 (3H, s)	27.7	1.09 (3H, s)
OAc-1	171.7					
	21.3	1.96 (3H, s)				
OAc-6	172.0					
	21.0	2.00 (3H, s)				
OAc-7	172.0				170.4	
	21.1	2.15 (3H, s)			21.4	1.98 (3H, s)
OAc-16			172.9			
			21.5	1.98 (3H, s)		
OMe-3	52.6	3.64 (3H, s)	52.9	3.59 (3H, s)		

[a]Spectra were recorded in methanol-d_4. [b]Spectra were recorded in $CDCl_3$. [c]Overlapping signals with the solvent peaks. [d,e]Overlapping signals.

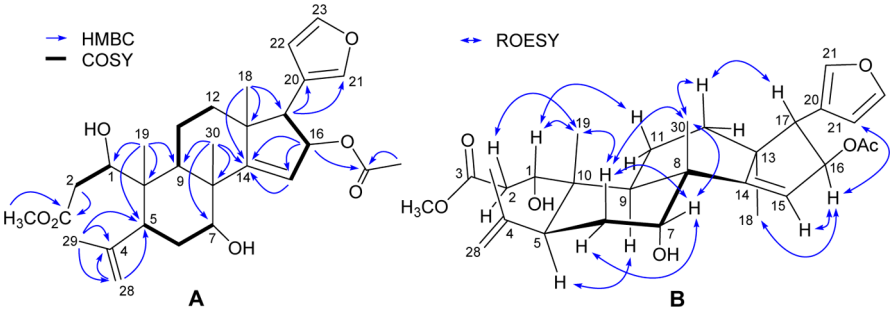


Figure 3. ^{1}H−^{1}H COSY (A), key HMBC (A), and ROESY (B) correlations of **5**.

Compound **9** shared the same molecular formula of $C_{40}H_{58}O_8$ with **8** as determined by the ^{13}C NMR data and the (+) HREISMS ion peak at *m/z* 689.4027 $[M + Na]^+$ (calcd 689.4029). Comparison of the ^{1}H and ^{13}C NMR data (Table 3) of **9** with those of **8** revealed that they are C-21 stereoisomers. As a result, the chemical shifts of C-17 and C-22 of **9** were shifted upfield as compared with those of **8** due largely to the γ-gauche effects of OCH_3-21β.[21,22] Furthermore, the ^{13}C NMR data of C-20 to C-23 of **9** matched well those of 3α-(3-methyl-2-butenoyloxy)-21,23β-epoxy-21β-methoxyapotirucallane-7α,24α,25-triol,[19] indicating that OMe-21 adopts a β-configuration. The structure of **9** (dysomollin B) was therefore defined as shown.

Three known compounds, dysoxylumin A (**10**)[8a] and toonapubesins A (**11**) and B (**12**),[15] were also isolated. Their structures were identified by NMR and MS analysis as well as by comparison with reported data.

The cytotoxic activities of the isolated compounds (**1**−**12**) against HL-60, P388, and A549 cells were tested by an MTT method[23] and the SRB protein staining method,[24] and doxorubicin[25] was used as a positive control. Compounds **10** and **11** displayed inhibitory activities against A549 and P388

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of 7−9 (in $CDCl_3$)

	7		8		9	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.8	α 1.99 (m)[a]	34.4	α 1.28 (m)	34.5	α 1.28 (m)
		β 1.60 (m)		β 1.47 (m)		β 1.47 (m)
2	33.9	α 2.43 (m)	22.6	α 1.76 (m)	22.7	α 1.78 (m)
		β 2.61 (m)		β 1.98 (m)		β 1.98 (m)
3	216.3		78.8	4.86 (s)	78.9	4.88 (br s)
4	46.9		36.7		36.7	
5	48.7	1.98[a]	42.4	2.10 (dd, 11.8, 3.2)	42.4	2.10 (m)
6	24.4	α 2.02 (m)	22.9	1.63 (2H, m)	23.0	1.66 (2H, m)
		β 1.90 (m)				
7	75.0	5.53 (br s)	76.2	5.03 (br s)	76.3	5.05 (br s)
8	42.4		38.1		38.1	
9	43.2	2.18 (m)	45.4	1.41[a]	44.5	2.12 (m)
10	37.1		37.2		37.2	
11	16.5	1.57 (2H, m)	16.6	1.29 (2H, m)	16.8	1.30 (2H, m)
12	33.1	α 1.87 (m)	26.1	1.84 (2H, m)	25.8	α 1.65 (m)
		β 1.63 (m)				β 1.95 (m)
13	48.1		28.4		28.9	
14	157.9		36.5		37.2	
15	118.8	5.42 (s)	26.1	α 1.42 (m)[a]	26.4	α 1.43 (m)
				β 1.77 (m)		β 1.85 (m)
16	33.0	2.29 (2H, m)	26.3	1.58 (2H, m)	27.6	1.59 (2H, m)
17	54.4	2.67 (t, 8.6)	48.3	1.96 (m)	45.5	1.39 (m)
18	20.4	0.89 (3H, s)	15.0	a 0.73 (d, 5.4)	14.8	a 0.66 (d, 5.4)
				b 0.41 (d, 5.4)		b 0.37 (d, 5.4)
19	15.0	1.12 (3H, s)	15.9	0.95 (3H, s)	15.9	0.96 (3H, s)
20	169.7		49.1	2.03 (m)	48.7	1.81 (m)
21	73.3	α 4.75 (dd, 17.1, 1.5)	109.1	4.85 (d, 3.8)	105.3	4.77 (d, 4.0)
		β 4.64 (d, 17.1)				
22	117.0	5.79 (d, 1.5)	32.1	1.80 (2H, m)	30.8	1.85 (2H, m)
23	173.8		77.0	4.23 (dd, 8.7, 5.3)	78.9	4.41 (td, 7.9, 2.0)
24			75.5	3.22 (d, 8.2)	76.6	3.15 (dd, 8.0, 2.0)
25			73.1		72.9	
26			26.3	1.25 (3H, s)	26.3	1.26 (3H, s)
27			26.4	1.28 (3H, s)	26.4	1.26 (3H, s)
28	25.9	1.05 (3H, s)	27.9	0.82 (3H, s)	28.0	0.84 (3H, s)
29	21.1	0.94 (3H, s)	21.4	0.93 (3H, s)	21.5	0.94 (3H, s)
30	27.5	1.26 (3H, s)	19.5	1.10 (3H, s)	19.6	0.96 (3H, s)
1′	165.5		165.6		165.6	
2′	130.2		130.9		131.0	
3′+7′	129.3	7.95 (d, 7.1)	129.3	8.05 (d, 7.3)	129.4	8.07 (d, 7.1)
4′+6′	128.5	7.43 (t, 7.7)	128.4	7.44 (t, 7.7)	128.4	7.45 (t, 7.7)
5′	133.1	7.57 (t, 7.4)	132.8	7.57 (t, 7.4)	132.8	7.58 (t, 7.4)
OAc-7			169.9		169.8	
			21.3	2.06 (3H, s)	21.3	2.09 (3H, s)
OMe-21			55.7	3.33 (3H, s)	55.1	3.38 (3H, s)

[a]Overlapping signals.

cells with IC_{50} values of 2.1 and 6.7 μM, respectively. The other compounds exhibited inhibition rates lower than 50% at 10 μM against the three tested cell lines and were inactive. The positive control doxorubicin showed IC_{50} values of 1.2, 0.058, and 0.20 μM against A549, HL60, and P388 cells, respectively.

EXPERIMENTAL SECTION

General Experimental Producedures. Optical rotations were run on a PerkinElmer 341 polarimeter. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. IR spectra were made on a PerkinElmer 577 IR spectrometer with KBr disks. NMR spectra were acquired on a Bruker AM-400 spectrometer. ESIMS and HRESIMS were obtained on an Esquire 3000 Plus (Bruker Daltonics) and a Waters-Micromass Q-TOF Ultima Global electrospray mass spectrometer, respectively. Semipreparative HPLC was performed on a Waters 1525 pump equipped with a Waters 2489 detector (210 nm) and a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). Silica gel (200−300 mesh) (Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China), C_{18} reverse-phased silica gel (20−45 μM, Fuji Silysia Chemical Ltd.), MCI gel (CHP20P, 75−150 μm, Mitsubishi Chemical Corporation), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography (CC). Precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd.) were used for TLC. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Company, Ltd., Shanghai, People's Republic of China), and those used for HPLC were of HPLC grade (J&K Scientific Ltd.).

Plant Material. The twigs of *Dysoxylum mollissimum* var. *glaberrimum* were collected in June 2008 from Xishuangbanna in Yunnan Province, People's Republic of China, and were authenticated by Prof. Y.-K. Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (CAS). A voucher specimen (accession number Dymo-2008YN-1Y) has been deposited at the Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The air-dried, powdered twigs of *D. mollissimum* (6 kg) were percolated with 95% ethanol (25 L) five times. After removal of the solvent under reduced pressure, the EtOH extract (280 g) was partitioned between H_2O and EtOAc to give an EtOAc-soluble fraction (130 g), which was fractionated via a column of MCI gel eluted with mixtures of MeOH/H_2O (4:6 to 9:1) to obtain six fractions (A−F). Fraction B was subjected to passage over a silica gel column eluted with petroleum ether/acetone mixtures (50:1 to 1:3) to yield 17 fractions (B1−B17). Fraction B13 was separated over a column of RP-18 silica gel (CH_3OH/H_2O, 11:10 to 3:1) to give five subfractions (B13a−B13f). Fraction B13b was purified by semipreparative HPLC (3.0 mL/min, 55% CH_3CN in H_2O isocratic elution) to yield compounds **4** (30 mg, 0.00050% dry wt) and **5** (10 mg, 0.00017% dry wt). Fraction B14 was separated over a column of RP-18 silica gel (CH_3OH/H_2O, 1:1 to 3:1), and each of the major components was purified by semipreparative HPLC (CH_3OH/H_2O, 1:1 to 3:1) to furnish **1** (4 mg, 0.000067% dry wt), **2** (23 mg,

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

0.00038% dry wt), **3** (12 mg, 0.00020% dry wt), **7** (3 mg, 0.000050% dry wt), and **10** (25 mg, 0.00042% dry wt). Fraction C was chromatographed on a column of silica gel (ether/acetone, 50:1 to 1:3, v/v) to give five major subfractions, with each of these purified by semipreparative HPLC to yield compounds **7** (3 mg, 0.000050% dry wt), **8** (15 mg, 0.00025% dry wt), **9** (12 mg, 0.00020% dry wt), **11** (3 mg, 0.000050% dry wt), and **12** (5 mg, 0.000083% dry wt).

*Dysomollide A (**1**):* white powder; $[\alpha]^{20}_{D}$ +103 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.99) nm; IR (KBr) ν_{max} 3437, 2966, 2935, 1741, 1637, 1466, 1375, 1234, 1132, 1034, 604, 557 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+) EISMS *m*/*z* 831.4 $[M + H]^+$; (−) ESIMS *m*/*z* 875.7 $[M + HCO_2]^-$; (+) HREISMS *m*/*z* 853.3267 $[M + Na]^+$ (calcd for $C_{42}H_{54}O_{17}Na$, 853.3259).

*Dysomollide B (**2**):* white powder; $[\alpha]^{20}_{D}$ +23 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.05), 215 (3.68) nm; IR (KBr) ν_{max} 3456, 2952, 2893, 1738, 1466, 1398, 1375, 1279, 1227, 1200, 1165, 1024, 876, 820, 602 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+) EISMS *m*/*z* 531.2 $[M + H]^+$, 1083.4 $[2 M + Na]^+$; (−) ESIMS *m*/*z* 529.6 $[M + H]^-$; (−) HRESIMS *m*/*z* 575.2129 $[M + HCO_2]^-$ (calcd for $C_{29}H_{35}O_{12}$, 575.2129).

*Dysomollide C (**3**):* white powder; $[\alpha]^{20}_{D}$ +8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.99) nm; IR (KBr) ν_{max} 3440, 2962, 1738, 1631, 1466, 1396, 1373, 1230, 1200, 1124, 1061, 1026, 876, 820, 604, 480 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+) EISMS *m*/*z* 631.3 $[M + H]^+$, 653.3 $[M + Na]^+$, 1283.5 $[2 M + Na]^+$; (−) ESIMS *m*/*z* 675.8 $[M + HCO_2]^-$; (+) HREISMS *m*/*z* 653.2575 $[M + Na]^+$ (calcd for $C_{33}H_{42}O_{12}Na$, 653.2574).

*Dysomollide D (**4**):* white powder; $[\alpha]^{22}_{D}$ +19 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.09) nm; IR (KBr) ν_{max} 3502, 2958, 1745, 1437, 1367, 1232, 1174, 1026, 876, 604 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; (+) EISMS *m*/*z* 671.3 $[M + Na]^+$, 1319.5 $[2 M + Na]^+$; (−) ESIMS *m*/*z* 693.7 $[M + HCO_2]^-$; (+) HREISMS *m*/*z* 671.2674 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{13}Na$, 671.2680).

*Dysomollide E (**5**):* white powder; $[\alpha]^{20}_{D}$ −18 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.07) nm; IR (KBr) ν_{max} 3512, 3460, 2952, 2881, 1741, 1724, 1633, 1441, 1385, 1300, 1232, 1026, 876, 604, 471 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; (+) EISMS *m*/*z* 523.7 $[M + Na]^+$, 1023.9 $[2 M + Na]^+$; (−) ESIMS *m*/*z* 545.8 $[M + HCO_2]^-$; (+) HREISMS *m*/*z* 523.2678 $[M + Na]^+$ (calcd for $C_{29}H_{40}O_7Na$, 523.2672).

*Dysomollide F (**6**):* white powder; $[\alpha]^{20}_{D}$ +5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.97) nm; IR (KBr) ν_{max} 3429, 2922, 2852, 2131, 1770, 1728, 1633, 1379, 1254, 1165, 1032, 561 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; (+) EISMS *m*/*z* 497.3 $[M + Na]^+$, 971.5 $[2 M + Na]^+$; (−) ESIMS *m*/*z* 519.6 $[M + HCO_2]^-$; (−) HRESIMS *m*/*z* 519.2961 $[M + HCO_2]^-$ (calcd for $C_{29}H_{43}O_8$, 519.2958).

*Dysomollide G (**7**):* white powder; $[\alpha]^{20}_{D}$ +20 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.24), 270 (3.01) nm; IR (KBr) ν_{max} 3433, 2920, 2850, 1782, 1751, 1707, 1628, 1464, 1275, 1113, 1028, 715, 565 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; (+) EISMS *m*/*z* 517.3 $[M + H]^+$, 1033.7 $[2 M + H]^+$, 1055.7 $[2 M + Na]^+$; (−) HRESIMS *m*/*z* 561.2838 $[M + HCO_2]^-$ (calcd for $C_{34}H_{41}O_7$, 561.2852).

*Dysomollin A (**8**):* white powder; $[\alpha]^{20}_{D}$ −93 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.15), 273 (3.62) nm; IR (KBr) ν_{max} 3450, 2949, 2873, 1718, 1637, 1452, 1375, 1300, 1275, 1252, 1117, 1028, 889, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; (+) EISMS *m*/*z* 689.4 $[M + Na]^+$, 1355.7 $[2 M + Na]^+$; (−) ESIMS *m*/*z* 711.7 $[M + HCO_2]^-$; (+) HREISMS *m*/*z* 689.4034 $[M + Na]^+$ (calcd for $C_{40}H_{58}O_8Na$, 689.4029).

*Dysomollin B (**9**):* white powder; $[\alpha]^{20}_{D}$ −36 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.11) nm; IR (KBr) ν_{max} 3444, 2949, 2873, 1734, 1718, 1633, 1452, 1375, 1275, 1252, 1174, 1117, 1028, 712 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; (+) EISMS *m*/*z* 689.4 $[M + Na]^+$; (+) HREISMS *m*/*z* 689.4027 $[M + Na]^+$ (calcd for $C_{40}H_{58}O_8Na$, 689.4029).

Cytotoxicity Assays. Compounds **1**−**12** were evaluated for cytotoxicity against three tumor cell lines, with the MTT method[23] used for HL-60 (human premyelocytic leukemia) and P-388 (murine leukemia) cells and the SRB protein staining method[24] used for A-549 (human lung adenocarcinoma) cells, according to reported protocols in triplicate independent experiments. Doxorubicin[25] was used as a positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Key HMBC and ROESY correlations of compounds **2**, **4**, **6**, **7**, and **8** and IR, ESIMS, HRESIMS, and 1D and 2D NMR spectra of compounds **1**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: spyang@whu.edu.cn (S.-P. Yang).

*Tel: +86-21-50806718. Fax: +86-21-50806718. E-mail: jmyue@simm.ac.cn (J.-M. Yue).

Author Contributions

†M.-L. Han and J.-X. Zhao contributed to this work equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the National Natural Science Foundation (21072203) of P. R. China is gratefully acknowledged. We thank Prof. Y.-K. Xu of Xishuangbanna Tropical Botanical Garden, CAS, for the identification of the plant material.

■ REFERENCES

(1) Chen, S. K.; Li, H.; Chen, P. Y. In *Chinese Flora (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, 1997; Vol. *43* (3), pp 87−97.

(2) Liu, H. M.; Heilmann, J.; Rali, T.; Sticher, O. *J. Nat. Prod.* **2001**, *64*, 159−163.

(3) Huang, H. L.; Wang, C. M.; Wang, Z. H.; Yao, M. J.; Han, G. T.; Yuan, J. C.; Gao, K.; Yuan, C. S. *J. Nat. Prod.* **2011**, *74*, 2235−2242.

(4) Hu, J.; Wang, X.; Shi, X. D. *Eur. J. Org. Chem.* **2011**, 7215−7223.

(5) Wang, F.; Guan, Y. J. *Fitoterapia* **2012**, *83*, 13−17.

(6) Jogia, M. K.; Andersen, R. J. *Phytochemistry* **1987**, *26*, 3309−3311.

(7) Chen, J. L.; Kernan, M. R.; Jolad, S. D.; Stoddart, C. A.; Cooper, R. *J. Nat. Prod* **2007**, *70*, 312−315.

(8) (a) Luo, X. D.; Wu, S. H.; Yang, X. S.; Teng, R. W.; Ma, Y. B.; Wu, D. G.; Hao, X. J.; Lu, Y.; Liu, X. N.; Zheng, Q. T. *Heterocycles* **2000**, *53*, 2225−2232. (b) Luo, X. D.; Wu, S. H.; Wu, D. G.; Ma, Y. B.; Qi, S. H. *Tetrahedron* **2002**, *58*, 7797−7804.

(9) (a) Fujioka, T.; Yamamoto, M.; Kashiwada, Y.; Fujii, H.; Mihashi, K.; Ikeshiro, Y.; Chen, I. S.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3479−3482. (b) Duh, C. Y.; Wang, S. K.; Chen, I. S. *J. Nat. Prod.* **2000**, *63*, 1546−1547.

(10) Xie, B. J.; Yang, S. P.; Yue, J. M. *Phytochemistry* **2008**, *69*, 2993−2997.

(11) Nishizawa, M.; Inoue, A.; Sastrapradja, S.; Hayashi, Y. *Phytochemistry* **1983**, *22*, 2083−2085.

(12) Liu, H. B.; Zhang, C. R.; Dong, S. H.; Yang, S. P.; Geng, M. Y.; Yue, J. M. *J. Asian Nat. Prod. Res.* **2012**, *14*, 224−232.

(13) (a) Aladesanmi, A. J.; Kelley, C. J.; Leary, J. D. *J. Nat. Prod.* **1983**, *46*, 127−131. (b) Aladesanmi, A. J.; Adewunmi, C. A.; Kelley, C. J.; Leary, J. D.; Bischoff, T. A.; Zhang, X. L.; Snyder, J. K. *Phytochemistry* **1988**, *27*, 3789−3792. (c) Aladesanmi, A. J.; Hoffmann, J. J. *Phytochemistry* **1994**, *35*, 1361−1362.

(14) Zhang, X. Y.; Li, Y.; Wang, Y. Y.; Cai, X. H.; Feng, T.; Luo, X. D. *J. Nat. Prod.* **2010**, *73*, 1385−1388.

(15) Wang, J. R.; Liu, H. L.; Kurtan, T.; Mandi, A.; Antus, A.; Li, J.; Zhang, H. Y.; Guo, Y. W. *Org. Biomol. Chem.* **2011**, *9*, 7685−7696.

(16) Kipassa, N. T.; Iwagawa, T.; Okamura, H.; Doe, M.; Morimoto, Y.; Nakatani, M. *Phytochemistry* **2008**, *69*, 1782−1787.

(17) Maclachlan, L. K.; Taylor, D. A. H. *Phytochemistry* **1982**, *21*, 1701−1703.

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX

(18) Qi, S. H.; Wu, D. G.; Zhang, S.; Luo, X. D. *Z. Naturforsch. B* **2003**, *58*, 1128−1132.
(19) Zhang, Y.; Tang, C. P.; Ke, C. Q.; Yao, S.; Ye, Y. *J. Nat. Prod.* **2010**, *73*, 664−668.
(20) Mitsui, K.; Maejima, M.; Saito, H.; Fukaya, H.; Hitotsuyanagi, Y.; Takeya, K. *Tetrahedron* **2005**, *61*, 10569−10582.
(21) Chen, H. D.; Yang, S. P.; Wu, Y.; Dong, L.; Yue, J. M. *J. Nat. Prod.* **2009**, *72*, 685−689.
(22) Xie, B. J.; Yang, S. P.; Chen, H. D.; Yue, J. M. *J. Nat. Prod.* **2007**, *70*, 1532−1535.
(23) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589−601.
(24) Xiao, D.; Zhu, S. P.; Gu, Z. L. *Acta Pharmacol. Sin.* **1997**, *18*, 280−283.
(25) Laginha, K. M.; Verwoert, S.; Charrois, G. J. R.; Allen, T. M. *Clin. Cancer Res.* **2005**, *11*, 6944−6949.

DOI: 10.1021/np500967k
J. Nat. Prod. XXXX, XXX, XXX−XXX