Frajunolides E−K, Briarane Diterpenes from *Junceella fragilis*

Chia-Ching Liaw,[†] Ya-Ching Shen,*[,‡] Yun-Sheng Lin,[†] Tsong-Long Hwang,[§] Yao-Haur Kuo,[⊥] and Ashraf Taha Khalil[‡]

Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung 804, Taiwan, Republic of China, School of Pharmacy, College of Medicine, National Taiwan University, Jen-Ai Rd. Sec. 1, Taipei 100, Taiwan, Republic of China, National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China, and Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan, Republic of China

Received February 26, 2008

Chemical investigation of the gorgonian octocoral *Junceella fragilis*, collected in Taiwan, resulted in the isolation of seven new briarane-type diterpenes, frajunolides E−K (**1**−**7**), in addition to 14 known briaranes, praelolide, junceellin, junceellolides A−E, and K, 11a,20a-epoxy-4-deacetoxyjunceelolide D, umbraculolide A, junceellonoid A, and juncins Y, Z, and ZI, as well as ergosterol peroxide. The structures of **1**−**7** were determined by analysis of HRESIMS and 2D NMR spectroscopic data. Cytotoxicity and in vitro anti-inflammatory activities of compounds **1**−**7** were also evaluated.

Marine organisms are a fundamental part of Earth's environment and have been the focus of biological and chemical studies for their ecological impact and their remarkable ability to biosynthesize secondary metabolites of unique structures and potential medicinal and economic value.[1] The gorgonians of the genus *Junceella* grow in the western Pacific and other tropical regions as whip-shaped unbranched colonies with variable colors.[2] They are well-recognized for producing highly oxidized briarane-type diterpenoidal γ-lactones with bicyclic six- and 10-membered rings (3,8-cyclized cembranoids).[3,4] Many of these briaranes possess diverse biological activities such as cytotoxic,[5−7] anti-inflammatory,[8] immunomodulatory,[9] antiviral,[10] and insecticidal[11] effects. Several briarane diterpenes were previously isolated from *Junceella fragilis* (Cnidaria, Anthozoa, Octocoralia, Gorgonacea, Ellisellidae).[12−15] This species may produce novel and diverse structures of briaranes when collected in different seasons and at varying geographical locations. In the present study, chemical investigation of an acetone extract of this species collected in the eastern part of Taiwan led to the isolation of seven new briarane-type diterpene lactones, frajunolides E−K (**1**−**7**), in addition to 14 known briaranes, praelolide,[16,17] junceellin,[16] junceellolides A−E and K,[16] umbraculolide A,[18] 11a,20a-epoxy-4-deacetoxyjunceelolide D, junceellonoid A,[19] and juncins Y, Z, and ZI,[20] together with ergosterol peroxide.[21] The structures of **1**−**7** were determined by a detailed spectroscopic analysis, especially 2D NMR. Compounds **1**−**7** were evaluated biologically for their cytotoxic activities against cancer cell lines and in two in vitro anti-inflammatory assays.

Results and Discussion

The HRESIMS of **1** revealed a molecular ion peak at *m/z* 573.2310 $[M + Na]^+$, consistent with the molecular formula $C_{28}H_{38}O_{11}$ and 10 degrees of unsaturation ($\Omega = 10$). The IR spectrum demonstrated absorption bands diagnostic of hydroxyl (3505 cm^{-1}) and five-membered lactone and ester (1742 cm^{-1}) functionalities. Both the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated the presence of four acetate units, a lactone ring, a trisubstituted double bond, and an exomethylene double bond, which accounted for seven degrees of unsaturation and were suggestive of a tricyclic briarane bearing a lactone ring.[22,23] Four acetates resonated at δ_H 2.17, 1.95, 1.94, and 1.93 (each 3H, s) and δ_C 170.6, 170.5, 170.3, and 169.4 and 21.7, 21.2, and 20.9 (2C). The carbonyl signal at δ_C 176.0 (C-18) was assigned to a five-membered lactone ring together with the oxymethine at δ_C 78.3 (C-7) and the oxyquaternary carbon at δ_C 82.2 (C-8). The proton singlets at δ_H 5.17 and 5.08 (δ_C 115.6) were ascribed to an exocyclic methylene group and correlated to C-10, C-12, and C-11, suggesting the presence of a C-11/C-20 double bond (Figure 1). A tertiary methyl signal (H-15) correlated with a quaternary carbon (C-1), two oxymethines at δ_C 73.9 and 73.5, and the CH at δ_C 41.4 (C-10), implying oxygenation at C-2 and C-14. This was confirmed by HMBC correlations of H-10 to C-1, C-2, C-11, C-14, C-15, and C-20. The vinylic methyl signal (H-16) correlated with those of an olefinic CH (C-6), a CH_2 (C-4), and a quaternary olefinic carbon (C-5). The olefinic C<u>H</u> at δ_H 5.48 (H-6) was coupled to the oxymethine proton at δ_H 5.28 and correlated to CH_3-16 and C-4, supporting C-5/C-6 unsaturation. The large coupling constant $J_{6,7}$ (9.5 Hz) confirmed the antiparallel arrangement of H-6 and H-7 and the β-orientation of H-7.[17] The secondary methyl (H-19) correlated to a lactone carbonyl (C-18), an oxyquaternary carbon (C-8), and CH (C-17), confirming the presence of a 18,7-lactone ring. The low-field resonance of C-8 was explained by its attachment to an oxygen functionality. The oxymethine (H-9) was assigned from its HMBC correlations to C-7, C-8, C-10, and C-11. The three oxymethines observed at δ_H 5.00 (2H, br s, H-2 and H-13), and 4.87 (H-14) exhibited HMBC correlations to three acetyl carbonyls, proving acetyloxy substitution at these positions, and was confirmed by COSY correlations (Figure 1). The relative configuration of **1** was determined on the basis of the NOESY spectrum. Naturally occurring briaranes have the CH_3-15 in the β-orientation and H-10 in the α-orientation,[4,12] consistent with the absence of any NOE effect between H-10/H-15. The NOESY correlations between H-15/H-13, H-14 and H-7/H-6, H-17 implied the β-orientation of H-7, H-13, H-14, and H-17. Analogous correlations between H-10/H-2, H-9, H-19 and H-9/H-19 favored the α-orientation of H-2, H-9, and H-19. On the basis of these findings, the structure of **1** (frajunolide E) was determined as *rel*-(1*S**,2*S**,7*S**,8*S**,9*S**,10*S**,13*S**,14*R**,17*R**)-2,9,13,14-tetraacetoxy-8-hydroxybriaran-5(6)*Z*,11(20)-dien-18,7-olide.

The HRESIMS of **2** revealed this compound to a chlorinated diterpene having a molecular ion peak at *m/z* 621.1717 $[M + Na]^+$, consistent with the molecular formula $C_{28}H_{35}ClO_{12}$ ($\Omega = 11$). The presence of a chlorine atom was suggested from an isotope fragment ion at *m/z* 623.1685 $[M + Na + 2]^+$, with its typical relative intensity.[24] The NMR spectroscopic data revealed the basic features of a briarane ester with a lactone, four acetate esters, and two exomethylene double bonds (Tables 1 and 2). The four acetate units were assigned to C-2, C-9, C-13, and C-14 with the aid of HMBC correlations of the oxymethines at δ_H 5.24 (H-2), 6.01 (H-9), 4.93

* To whom correspondence should be addressed. Tel: 886-02-23123456, ext. 2226. Fax: 886-02-23919098. E-mail: ycshen@ntu.edu.tw.
[†] National Sun Yat-Sen University.
[‡] National Taiwan University.
[§] Chang Gung University.
[⊥] National Research Institute of Chinese Medicine.

10.1021/np800126f CCC: $40.75
Published on Web 09/06/2008

Table 1. ^{1}H NMR Spectroscopic Data (400 MHz, $CDCl_3$) for **1**−**7**

position	1	2	3[a]	4	5	6	7[a]
2	5.00, m	5.23, d (6.8)	6.51, d (9.5)	5.50, d (9.6)	5.48, d (9.6)	4.79, brs	6.26, d (9.4)
3	2.64, m 2.34, m	3.34, dd (16.4,7.2) 1.58, m	5.68, dd (11.0,9.5)	6.06, dd (16.0,9.6)	6.00, dd (15.9,9.6)	1.64, m	5.68, dd (11.5,9.4)
4	2.58, m 2.05, m		5.96, d (11.0)	6.84, d (16.0)	6.84, d (15.9)	2.56, m	6.00, brd (11.5)
6	5.48, d (9.6)	4.93, m	5.11, brd (2.5)	5.12, d (3.6)	5.08, d (3.9)	2.69, d (9.6)	5.14, brs
7	5.28, d (9.2)	4.41, d (2.8)	4.78, d (3.0)	4.24, d (3.6)	4.13, d (3.9)	5.23, d (10.0)	4.77, brs
9	5.53, d (4.4)	6.01, brs	4.86, d (3.5)	5.94, brs	5.12, brs	5.35, d (6.0)	5.35, d (4.5)
10	3.40, brd (4.0)	3.05, brs	3.26, d (3.5)	3.46, brs	3.21, s	3.53, d (6.0)	3.50, brs
12	2.23, m 1.80, m	2.54, m	2.22, m 1.15, m	2.48, d (8.8)	2.41, brt (11.7) 1.43, dd (12.0,6.4)	5.33, m	1.23, m
13	5.00, m	4.92, m	1.94, m 1.78, m	4.94, td (8.8,2.8)	5.14, m	2.54, m 1.62, m	1.84, m
14	4.87, brs	5.25, dd (8.4,2.0)	4.88, brs	5.20, d (2.4)	5.30, brs	4.61, d (4.4)	4.95, d (3.5)
15	0.85, s	1.20, s	1.06, s	1.16, s	1.25, s	1.10, s	1.10, s
16	1.98, s	5.96, d (2.0) 5.67s	5.94, brs 5.91, brs	5.40, s 5.36, s	5.36, s 5.32, s	2.07, s	5.87, s 5.81, s
17	2.49, q (6.5)	2.74, q (6.8)	2.61, q (7.5)	2.85, q (7.2)	2.89, q (7.3)	2.43, q (6.8)	2.61, q (7.5)
19	0.86, d (6.5)	1.28, d (6.9)	1.21, d (7.5)	1.11, d (7.2)	1.27, d (7.3)	1.09, d (6.8)	1.18, d (7.5)
20	5.17, s 5.08, s	5.24, s 4.94, s	3.06, brs 2.63, d (3.5)	5.25, s 5.06, s	2.69, brs 2.57, d (3.6)	5.17, s 5.03, s	4.97, s 4.82, s
Ac-2	1.95, s[b]	2.10, s		1.97, s	1.98, s	1.87, s	
Ac-9	2.17, s	2.26, s	2.17, s	2.11, s	2.18, s	2.17, s	2.15, s
Ac-13	1.94, s[b]	1.99, s		2.02, s	2.02, s		
Ac-14	1.93, s[b]	2.13, s	2.07, s	2.15, s	2.11, s	2.07, s	1.97, s
2′			4.58, d (15.5) 4.49, d (15.5)			2.26, q (7.4)	4.57, d (16) 4.48, d (16)
3′						1.06,t(7.2)	
4′			2.30, d (7.5) 2.28, d (7.5)				2.48, m
5′			2.20, m				2.08, m
6′			0.99, d (7.5)				0.99, d (7.7)
7′			0.99, d (7.5)				0.99, d (7.7)
OH-8			3.15, s	3.20, s	3.05, s	3.17, brs	2.92, s

[a] Measured at 500 MHz. [b] Exchangeble values.

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz, $CDCl_3$) of **1**−**7**

carbon	1	2	3[a]	4	5	6	7[a]
1	46.8, qC	47.2, qC	47.1, qC	48.6, qC	48.4, qC	46.8, qC	47.9, qC
2	73.9, CH	72.5, CH	72.8, CH	75.7, CH	75.5, CH	74.2, CH	73.9, CH
3	35.0, CH_2	40.4, CH_2	129.6 CH,	130.3, CH	129.3, CH	32.0, CH_2	130.4, CH
4	29.0, CH_2	97.2, qC	128.9, CH	132.9, CH	133.7, CH	29.1, CH_2	129.3, CH
5	145.4, qC	137.6, qC	136.9, qC	141.0, qC	141.5, qC	145.1, qC	138.0, qC
6	119.8, CH	55.3, CH	62.2, CH	63.5, CH	64.0, CH	120.3, CH	63.4, CH
7	78.3, CH	78.6, CH	78.6, CH	80.8, CH	81.0, CH	77.6, CH	78.9, CH
8	82.2, qC	81.2, qC	81.2, qC	82.9, qC	82.7, qC	83.1, qC	83.3, qC
9	71.7, CH	77.3, CH	70.3, CH	77.2, CH	72.8, CH	70.4, CH	77.0, CH
10	41.4, CH	43.2, CH	38.2, CH	42.0, CH	38.7, CH	42.1, CH	42.8, CH
11	146.8, qC	143.2, qC	59.2, qC	145.2, qC	55.5, qC	148.6, qC	148.8, qC
12	30.3, CH_2	37.6, CH_2	29.6, CH_2	37.8, CH_2	35.4, CH_2	67.2, CH	29.7, CH_2
13	68.7, CH	69.0, CH	25.0, CH_2	69.5, CH	67.5 CH	34.7 CH_2	25.6 CH_2
14	73.5, CH	73.7, CH	73.9, CH	73.4, CH	73.1, CH	73.9, CH	75.2, CH
15	15.1, CH_3	14.6, CH_3	14.5, CH_3	14.6, CH_3	15.1, CH_3	15.7, CH_3	15.2, CH_3
16	26.3, CH_3	118.2,CH_2	117.1, CH_2	116.7, CH_2	116.3, CH_2	28.0, CH_3	117.8, CH_2
17	43.2, CH	50.4, CH	47.7, CH	50.7, CH	50.6, CH	42.3, CH	46.1, CH
18	176.0, qC	174.0, qC	175.0, qC	174.8, qC	174.9, qC	176.0, qC	175.4, qC
19	6.6, CH_3	7.0, CH_3	7.6, CH_3	6.6, CH_3	7.7, CH_3	6.5, CH_3	8.0, CH_3
20	115.6, CH_2	115.3, CH_2	50.5, CH_2	115.2, CH_2	50.3, CH_2	110.1, CH_2	112.7, CH_2
Ac-2	170.6, qC[b]	173.8, qC		170.2, qC[b]	170.3, qC	169.9 qC	
	21.2, CH_3[c]	21.3, CH_3		20.9, CH_3	20.9, CH_3[b]	21.1, CH_3	
Ac-9	169.4, qC	169.4, qC	170.1, qC	170.2, qC[b]	170.3 qC	170.1 qC	169.9 qC
	21.7, CH_3	21.4, CH_3	21.2, CH_3	20.9, CH_3	21.0[b] CH_3	21.7CH_3	21.4CH_3
Ac-13	170.5, qC[b]	170.1, qC		170.3, qC[b]	170.3, qC		
	20.9, CH_3[c]	20.5, CH_3		21.2, CH_3	21.3, CH_3[b]		
Ac-14	170.3, qC[b]	170.0, qC	170.3, qC	170.4, qC[b]	170.3, qC	169.2 qC	170.5, qC
	20.9, CH_3[c]	20.9, CH_3	21.4, CH_3	21.3, CH_3	21.4, CH_3[b]	21.0CH_3	21.2, CH_3
1′			166.7, qC			173.8, qC	166.7, qC
2′			60.6, CH_2			27.5, CH_2	60.6, CH_2
3′			172.3, qC			8.8, CH_3	172.3, qC
4′			42.7, CH_2				42.7, CH_2
5′			25.6, CH				25.5, CH
6′			22.3, CH_3				22.3, CH_3
7′			22.3, CH_3				22.3, CH_3

[a] Measured at 125 MHz. [b] Exchangeable values in the same column. [c] Exchangeable values in the same column.

(H-13), and 5.25 (H-14) to their respective acetate carbonyls. The olefinic singlets at δ_H 5.24 and 4.93 (δ_C 115.6) that correlated to the quaternary carbon (C-11) and to CH (C-10) and CH_2 (C-12) were assigned to H-20 and were confirmed by correlations between H-9/C-11 and H-10/C-20. The methine proton (H-6) gave a COSY correlation to an oxymethine proton (H-7) and a HMBC correlation to an oxyquaternary carbon (C-8). The chemical shift of C-6 (δ_C 55.3) was more shielded than anticipated, suggesting its attachment

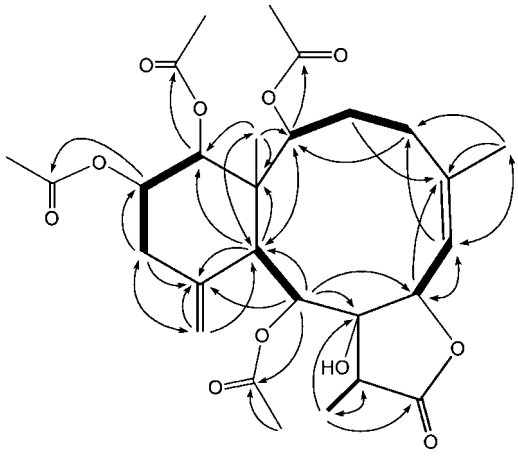


Figure 1. Selected HMBC (arrows) and COSY (bold lines) correlations for **1**.

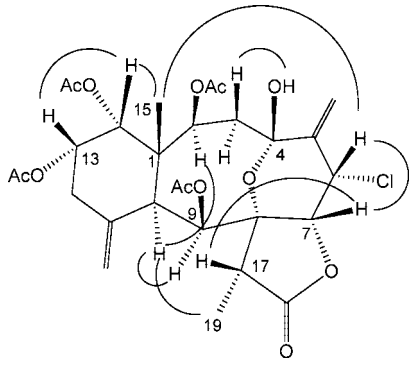


Figure 2. Key NOESY correlations for **2**.

to a chlorine atom.[24] A second set of exomethylene protons (CH_2-16) in the HMBC spectrum correlated to C-6 and the quaternary olefinic carbon (C-5), as well as the quaternary acetal carbon at δ_C 97.2 (C-4), suggesting that the latter is OH-bearing and connected to C-8 through an ether linkage. This was supported by considering the calculated 10 degrees of unsaturation in addition to the observed HMBC correlations between H-2/C-4, C-15, C-10; H-6/C-4; H-7/C-5, and H-19/C-8, C-18. The NOESY spectrum of **2** revealed correlations of H-2/H-10 and H-10/Me-19, indicating the α-orientation of H-2, H-10, and Me-19. However, NOESY correlations of H-3/OH-4, H-6/Me-15, H-6/H-7, H-7/H-17, and H-13/Me-15 suggested the β-disposition of H-3, 4-OH, H-6, H-7, H-13, H-17, and Me-15 (Figure 2). Therefore, the structure of **2** (frajunolide F) was deduced as *rel*-(1*S**,2*S**,4*S**,6*S**,7*R**,8*R**,9*S**,10*S**,13*S**,14*R**,17*R**)-2,9,13,14-tetraacetoxy-6-chloro-4-hydroxy-4,8-epoxybriaran-5(16),11(20)-dien-18,7-olide.

The molecular formula of **3** was established as $C_{31}H_{41}ClO_{12}$ (Ω = 11) from the HRESIMS molecular peak at *m*/*z* 663.2188 $[M + Na]^+$. The NMR spectroscopic data were also consistent with a briarane skeleton having a disubstituted double bond, and an exomethylene, corresponding to protons at δ_H 5.94 and 5.91 (each brs) that correlated to the chlorine-bearing CH (C-6) and the olefinic CH (C-4) carbon signal in the HMBC spectrum. This was supported by COSY correlations between H_2-16/H-6/H-7. A *Z*-double bond between C-4 and C-3 was evidenced by the coupling constant $J_{3,4}$ (11.0 Hz) between H-3 and H-4 as well as COSY correlations between H-4/H-3/H-2 and HMBC correlations of H-2 to C-4 and C-15 (Figure 3). The two *gem*-protons at δ_H 3.06 and 2.63 together with the corresponding CH_2 (δ_C 50.5) and quaternary carbon (δ_C 59.2) were attributed to an exocyclic epoxide (spirocyclic oxirane ring).[22] The epoxide ring was assigned to C-11/C-20 through the HMBC correlations between H-20/C-11, C-12 and H-9/C-11. In addition to a lactone carbonyl (δ_C 175.0), the ^{13}C NMR data

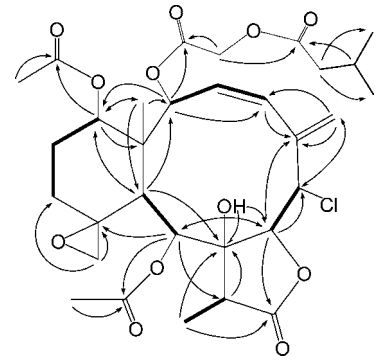


Figure 3. Selected HMBC (arrows) and COSY (bold lines) correlations for **3**.

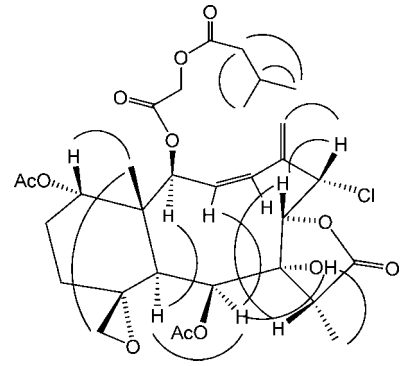


Figure 4. Key NOESY correlations for **3**.

exhibited four carbonyl signals, of which two (δ_C 170.3, 170.1) were attributed to two acetate moieties linked to C-9 and C-14, as indicated by HMBC correlations of H-9 and H-14 to the reportive carbonyls. The third acyl was assigned to C-2, as indicated by the HMBC correlation of H-2 to the carbonyl at δ_C 166.7 (C-1′). Two isolated oxymethylene protons that resonated at δ_H 4.58 and 4.49 correlated to an acyl carbonyl (C-1′) as well as to a carbonyl at δ_C 172.4 (C-3′). A multiplet (H-5′) correlated to the C-3′ carbonyl and gave COSY correlations to H_2-4′ and a two-methyl doublet (H-6′, H-7′). Furthermore, H_2-4′ correlated to two methyls (C-6′ and C-7′), suggesting that the third acyl is a 2-(3-methylbutanoyloxy)acetate unit, as supported by NOESY correlations between H-5′/H-6′, H-7′ and H-4′/H-6′, H-7′ (Figure 4). Closely related aliphatic esters were reported in some briaranes at position C-2.[25,26] The NOESY spectrum of **3** revealed correlations between H-10/H-2,H-9 and OH-8/H-9, H-19, indicating the α-orientation of H-2, H-9, and H-19 as well as OH-8. The NOESY correlations between H-15/H-14, H-20; H-20/H-14; and H-7/H-6, H-17 were in agreement with the β-orientation of H-6, H-7, H-17, and H-20.[12] The chemical shift values of C-11 and C-20 (ranging from δ_C 55 to 61 and 47to 52, respectively) confirmed the β-orientation of H-20, the 11*R* configuration of the epoxy ring, and the chair conformation of the cyclohexane ring.[27] Consequently, **3** (frajunolide F) was assigned as *rel*-(1*R**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,14*S**,17*R**)-9,14-diacetoxy-2[2-(3-methylbutanoyloxy)acetoxy]-6-chloro-8-hydroxy-11,20-epoxybriaran-3*Z*,5(16)-dien-18,7-olide.

The molecular formula of **4** was determined as $C_{28}H_{35}ClO_{11}$ (Ω = 11) from its HRESIMS. The NMR spectroscopic data (including HMBC and COSY) revealed a briarane skeleton with four oxymethine protons at δ_H 5.50, 5.94, 4.94, and 5.20 that were assigned to positions 2, 9, 13, and 14, to which four acetate units were attached (Table 2). The exomethylene proton singlets at δ_H 5.25 and 5.06 were assigned to H-20 (HMBC to C-1 and C-12), while the exomethylene singlets at δ_H 5.40 and 5.36 were located at C-16 (HMBC correlation to chlorinated C-6 at δ_C 63.5). The coupling constants of the olefinic protons at δ_H 6.06 (dd, J = 16.0, 9.6 Hz)

and δ_H 6.84 (d, J = 16.0 Hz) indicated a *trans*-disposed double bond and were assigned to H-3 and H-4, respectively, as proved by COSY correlations between H-2/H-3/H-4 and HMBC correlations between H-3/C-1 and C-2 and between H-4/C-2. HMBC correlations between H-10/C-1, C-2, C-11, C-15, C-20; OH-8/C-2; H-15/C-1, C-2, C-14, C-10; H-6/C-8; H-20/C-10, C-12; H-17/C-2, C-18, C-19; and H-4/C-2, C-16 confirmed frajunolide H as structure **4** (*rel*-(1*S**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,13*S**,14*R**,17*R**)-2,9,13,14-tetraacetoxy-6-chloro-8-hydroxybriaran-3*E*,5(16),11(20)-trien-18,7-olide).

The molecular formula $C_{28}H_{35}ClO_{12}$ (Ω = 11) was assigned to **5** from its HRESIMS and ^{13}C NMR data. The NMR spectroscopic values (Tables 1 and 2) revealed a briarane structure very similar to that of **4** but with one exoepoxy group. The only significant difference between **4** and **5** was the CH_2 signal at δ_C 50.3 with the attached *gem*-protons at δ_H 2.69 and 2.57 (d, J = 3.6 Hz), along with the quaternary carbon at δ_C 55.5, which indicated a 20,11-epoxy ring similar to compound **3**. Analysis of the NOESY spectrum supported the same relative stereochemistry as compound **4**. Accordingly, compound **5** was proposed as *rel*-(1*S**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,13*S**,14*R**,17*R**)-2,9,13,14-tetraacetoxy-6-chloro-8-hydroxy-11,20-epoxybriaran-3*E*,5(16)-dien-18,7-olide.

The HRESIMS and ^{13}C NMR data of **6** suggested this compound to have a molecular formula of $C_{29}H_{40}O_{11}$ (Ω = 10). The NMR spectra indicated a briarane skeleton very similar to **1** except for the absence of the acetyl group at C-13 and the presence of a propionyl group at C-12. This was confirmed through HMBC correlations between H-6/C-16, CH_3-16/C-6, and H_2-20/C-10. The HMBC spectrum was also used to locate three acetyloxy substitutions at C-2, C-9, and C-14, in a manner similar to that of **1**. The ^3J-correlation of CH_2-20 to C-12 suggested acylation at C-12 and was confirmed by correlation of H-12 to C-11 and C-13, as well as the relative upfield resonance of C-20 (δ_C 110.1) when compared to **1** (δ_C 115.6), **2** (δ_C 115.3), and **4** (δ_C 115.2). The upfield methyl signal at δ_H 1.06 (δ_C 8.9) was coupled to a methylene at δ_H 2.26 (δ_C 27.5), and both correlated to the acyl carbonyl (δ_C 173.8), thereby proving the presence of a propionyl ester group. Key NOESY correlations were observed between H-2/H-9, H-9/H-10, H-12/H-15, and H-14/H-15 and indicated the α-orientation of H-2, H-9, and H-10 and the β-orientation of H-12, H-14, H-17, and H-20. The structure of **6** was established as *rel*-(1*S**,2*S**,7*S**,8*S**,9*S**,10*S**,12*R**,14*S**,17*R**)-2,9,14-triacetoxy-12-propionyloxy-8-hydroxybriaran-5(6)*Z*,11(20)-dien-18,7-olide.

The HRESIMS of **7** displayed a molecular ion at *m*/*z* at 647.2232 ($[M + Na]^+$) corresponding to the molecular formula $C_{31}H_{41}ClO_{11}$ (Ω = 11). The ^1H NMR data displayed signals for a *cis*-arranged double bond and two sets of exomethylene double-bond singlets (H_2-16 and H_2-20). A chlorine atom was attached to C-6 (δ_C 63.4), and two acetyloxy groups were positioned at C-9 and C-14. This was demonstrated by the HMBC correlations. A careful analysis of the NMR data coupled with COSY, HMBC, and NOESY correlations proved that a C-11/C-20 double bond was present, instead of an epoxide as in **3** (Tables 1 and 2). Hence, the structure of **7** was identical to that of **3** except for the presence of an exomethylene group instead of an epoxy group at C-11. Compound **7** (frajunolide K) was assigned as *rel*-(1*R**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,14*S**,17*R**)-9,14-diacetoxy-2[2-(3-methylbutanoyloxy)acetoxy]-6-chloro-8-hydroxybriaran-3*Z*,5(16),11(20)-trien-18,7-olide.

Compounds **1**−**7** were evaluated for cytotoxicity against human human liver carcinoma (Hep2), medulloblastoma (Doay), colon adenocarcinoma (WiDr), and cervical epitheloid carcinoma (Hela) cells in vitro. However, all compounds tested were inactive (>20 μg/mL). In vitro antiinflammatory activities of **1**−**7** were evaluated for inhibition of elastase release and for the generation of superoxide anion, as tested on human neutrophils (Table 3). Compounds **1** and **6** exhibited weak inhibition of elastase release and superoxide anion at 10 μg/mL. Genistein was used as a positive control.

Table 3. Effect of Compounds **1**−**7** on Inhibition of Elastase Release and Superoxide Anion Generation, as Tested on Human Neutrophils in Response to FMLP/CB[a]

compound	elastase	superoxide anion
1	27.4 ± 4.27[b]	17.5 ± 4.23[b]
2	10.4 ± 4.55	7.4 ± 7.39
3	8.3 ± 0.64	−7.5 ± 5.89
4	0.1 ± 4.94	−1.9 ± 5.95
5	18.2 ± 1.38	−10.3 ± 4.63
6	19.9 ± 3.13	20.1 ± 6.2
7	8.0 ± 7.66	2.4 ± 6.32
genistein[c]	51.6 ± 5.89	65.0 ± 5.71

[a] Percentage of inhibition (Inh %) at 10 μg/mL. [b] Concentration necessary for 50% inhibition. Results are presented as mean + SEM (n = 3), $p < 0.05$. [c] Positive control.

Experimental Section

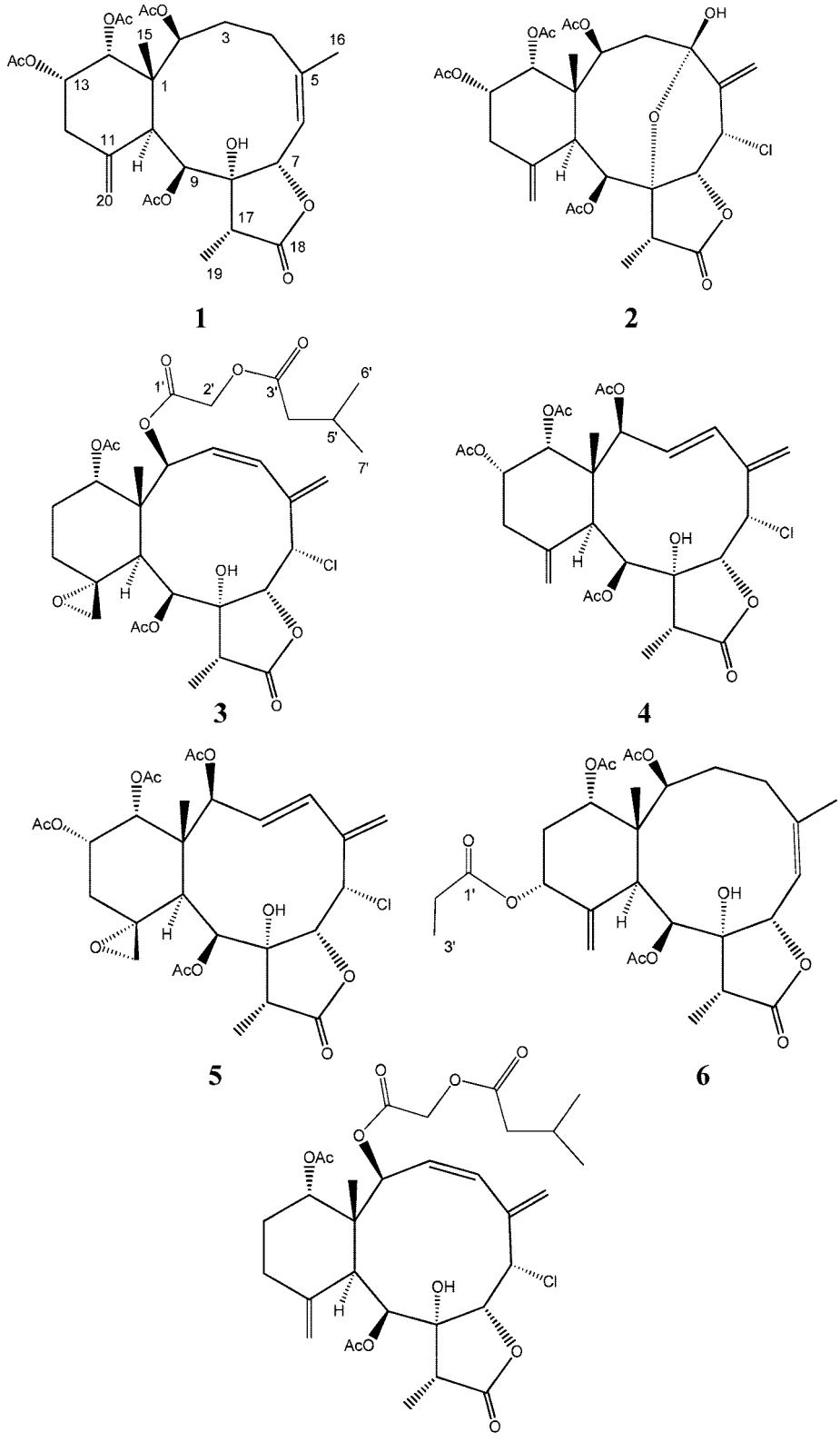
General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were measured on a Hitachi T-2001 spectrophotometer. The ^1H, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on Bruker AV-400 or AV-500 spectrometers, using TMS as internal standard. The chemical shifts are given in δ (ppm) and coupling constants in Hz. HRESIMS were run on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck) was utilized for column chromatography, and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) was used also for compound purification. LiChrospher Si 60 (5 μm, 250−10, Merck) and LiChrospher 100 RP-18e (5 μm, 250−10, Merck) were used for NP-HPLC and RP-HPLC (Hitachi), respectively.

Animal Material. The gorgonian *Junceella fragilis* Ridley (Ellisellidae) was collected in Tai-Tong County, Taiwan, by scuba diving at a depth of 15 m, in February 2006. The fresh gorgonian was immediately frozen after collection and kept at −20 °C until processed. A voucher specimen (WSG-5) was deposited in the School of Pharmacy, College of Medicine, National Taiwan University, Taiwan.

Extraction and Isolation. The gorgonian (wet weight, 3.9 kg) was minced and extracted with acetone (3 × 5 L) at rt, and the acetone extract was concentrated under vacuum. The crude extract (33 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble portion (24 g) was shaken with *n*-hexane−MeOH−H_2O (4:3:1) and the MeOH layer evaporated and separated on Sephadex LH-20 to give eight fractions (L1 to L8). Crystallization of L2 furnished praelolide (17 mg). Fraction L3 (3 g) was subjected to column chromatography using silica gel and a gradient of *n*-hexane−CH_2Cl_2−MeOH to obtain 33 fractions (L3-1 to L3-33). Fraction L3-14 (111 mg) was separated by NP-HPLC using *n*-hexane−CH_2Cl_2−MeOH (40:20:1) to yield junceellin (58 mg), junceelolide K, and ergosterol peroxide (21 mg). Fraction L3-16 (750 mg) was separated by RP-HPLC, using MeOH−H_2O (3:2), to afford **1** (5 mg) and **6** (6 mg) and a mixture that yielded junceellolides A (39 mg), B (35 mg), and E and 11a,20a-epoxy-4-deacetoxyjunceelolide D upon preparative TLC separation (silica gel) using *n*-hexane−CH_2Cl_2−MeOH (10:10:1). Fraction L3-17 (104 mg) was subjected to RP-HPLC using MeOH−H_2O−CH_3CN (70:25:5) to give umbraculolide A (18 mg), junceellolide A (7 mg), and junceellonoide A (8 mg). Crystallization of fraction L3-18 (454 mg) afforded praelolide (80 mg) and a mixture that yielded junceellolide D (70 mg), **4** (5 mg), and **7** (2.5 mg) after repeated separation by NP-HPLC ($CHCl_3$−MeOH, 200:1) and RP-HPLC (MeOH−H_2O, 65:35). Fraction L3-19 afforded junceellolide C. The MeOH-insoluble portion of fraction L3-20 (421 mg) was separated by RP-HPLC using MeOH−H_2O−CH_3CN (70:25:5) to obtain praelolide (20 mg) and **5** (12 mg). In turn, the MeOH-soluble portion was divided into two fractions (S1 and S2) after separation by NP-HPLC using *n*-hexane−CH_2Cl_2−MeOH (30:15:1). Fraction S1 (82 mg) was further separated by RP-HPLC using MeOH−H_2O−CH_3CN (65:30:5) to afford **3** (2.5 mg). Fraction S2 was repeatedly separated on RP-HPLC using CH_3CN−H_2O, 45:55, and MeOH−H_2O, 65:35, followed by preparative TLC purification (hexane−BuOH, 10:1, and *n*-hexane−CH_2Cl_2−MeOH, 20:20:1, to provide juncins Y (67 mg), Z (17 mg), and ZI (150 mg) and **2** (5 mg).

Frajunolide E (1): colorless, amorphous powder; $[\alpha]^{24}_D$ −242 (*c* 0.4, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3505 (OH), 2925, 2854 (CH), 1742 (lactone), 1457, 1376, 747 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Tables 1 and 2; ESIMS *m*/*z* 573 [M +

Chart 1



Na]$^+$, 513 [M + Na − AcOH]$^+$; HRESIMS *m/z* 573.2310 [M + Na]$^+$ (calcd for $C_{28}H_{38}O_{11}Na$, 573.2312).

Frajunolide F (2): colorless, amorphous powder; mp 188−194 °C; $[\alpha]^{24}_D$ −18.3 (*c* 0.6, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3450 (OH), 2925, 2854 (CH), 1742 (lactone, ester), 1457, 1376, 747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Tables 1 and 2; ESIMS *m/z* 621 [M]$^+$, 383 [M − 3×AcOH − Cl]$^+$; HRESIMS *m/z* 621.1717 [M + Na]$^+$, 623.1685 [M + Na + 2]$^+$ (calcd for $C_{28}H_{35}ClO_{12}Na$, 621.1715).

Frajunolide G (3): colorless, amorphous powder; $[\alpha]^{24}_D$ +32 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3446 (OH), 2927 (CH), 1771 (lactone), 1741 (ester), 1374, 1254, 1220, 1042 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Tables 1 and 2; ESIMS *m/z* 640 [M]$^+$; HRESIMS *m/z* 663.2188 [M + Na]$^+$, 665.2166 [M + Na + 2]$^+$ (calcd for $C_{31}H_{41}ClO_{12}Na$, 663.2184).

Frajunolide H (4): colorless, amorphous powder; mp 153−156 °C; $[\alpha]^{24}_D$ +9.6 (*c* 0.5, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3453 (OH), 1784 (lactone), 1739 (ester), 1456, 1370, 1228, 1043, 985, 739 cm^{-1}; ^{1}H

NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Tables 1 and 2; ESIMS *m/z* 605 $[M + Na]^+$; HRESIMS *m/z* 605.1768 $[M + Na]^+$, 607.1778 $[M + Na + 2]^+$ (calcd for $C_{28}H_{35}ClO_{11}Na$, 605.1765).

Frajunolide I (5): colorless, amorphous powder; mp 172−175 °C; $[\alpha]^{24}_D$ −1.6 (*c* 0.1, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3453 (OH), 1784 (lactone), 1739 (ester), 1456, 1370, 1228, 1043, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Tables 1 and 2; ESIMS *m/z* 621 $[M + Na]^+$; HRESIMS *m/z* 621.1716 $[M + Na]^+$, 623.1690 $[M + Na + 2]^+$ (calcd for $C_{28}H_{35}ClO_{12}Na$, 621.1715).

Frajunolide J (6): colorless, amorphous powder; $[\alpha]^{24}_D$ −96.6 (*c* 0.3, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3446 (OH), 1772 (lactone), 1734 (ester), 1221, 960 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 587.2472 $[M + Na]^+$ (calcd for $C_{29}H_{40}O_{11}Na$, 587.2468).

Frajunolide K (7): colorless, amorphous powder; $[\alpha]^{24}_D$ +50 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3378 (OH), 2925 (CH), 1792 (lactone), 1746 (ester), 1456, 1375, 1244, 1216 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Tables 1 and 2; ESIMS *m/z* 624 $[M]^+$; HRESIMS *m/z* 647.2232 $[M + Na]^+$ (calcd for $C_{31}H_{41}ClO_{11}Na$, 647.2232).

Cytotoxicity Assay. Cytotoxicity was tested against Hep2 (liver carcinoma), WiDr (colon adenocarcinoma), Daoy (medulloblastoma), and HeLa (cervical epitheloid carcinoma) human tumor cells, using a MTT assay method. The assay procedure was carried out as previously described.[28] Camptothecin was used as positive control and gave IC_{50} values for Hep2, WiDR, Daoy, and HeLa cells of 0.02, 0.07, 0.08, and 0.19 μg/mL, respectively.

Anti-inflammatory Assays. Measurement of Elastase Release. Degranulation of azurophilic granules in human neutrophils was determined by elastase release as described previously.[29] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. After supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with each test compound for 5 min. Cells were activated by FMLP (100 nM)/CB (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously for elastase release. The results were expressed as the percentage of the initial rate of elastase release in the FMLP/CB-activated, test-compound-free (DMSO) control system. Genistein was used as a standard compound.

Human Neutrophil Superoxide Generation. Huamn neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.[30] The assay of $O_2^{\bullet-}$ generation was based on the SOD-inhibitable reduction of ferricytochrome *c*.[31] In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated with 100 nM FMLP for 10 min. When FMLP was used as a stimulant, CB (1 μg/mL) was incubated for 3 min before activation by the peptide (FMLP/CB). Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* ($\epsilon = 21.1$/mM/10 mm). Genistein was used as a positive control.

Acknowledgment. The authors are grateful to the National Science Council, Taipei, Taiwan, for financial support (grant no. NSC 96-2320-B-110-009).

References and Notes

(1) Bhakuni, D. S.; Rawat, D. S. *Bioactive Marine Natural Products*; Springer: New York, 2005.
(2) Fabricius, K.; Alderslade, P. *Soft Corals and Sea Fans*; Australian Institute of Marine Science: Townsville, Queensland, Australia, 2001; p 230.
(3) Rodriguez, A. D.; Ramirez, C.; Cobar, O. M. *J. Nat. Prod.* **1996**, *59*, 15–22, and references therein.
(4) Sung, P. J.; Sheu, J. H.; Xu, J. P. *Heterocycles* **2002**, *57*, 535–579, and references therein.
(5) Rodriguez, J.; Nieto, R. M.; Jimenez, C. *J. Nat. Prod.* **1998**, *61*, 313–317.
(6) Sung, P. J.; Su, J. H.; Wang, G. H.; Lin, S. F.; Duh, C. Y.; Sheu, J. H. *J. Nat. Prod.* **1999**, *62*, 457–463.
(7) Wu, S. L.; Sung, P. J.; Chiang, M. Y.; Wu, J. Y.; Sheu, J. H. *J. Nat. Prod.* **2001**, *64*, 1415–1420.
(8) Shin, J.; Park, M.; Fenical, W. *Tetrahedron* **1989**, *45*, 1633–1638.
(9) Hamann, M. T.; Harrison, K. N.; Carroll, A. R.; Scheuer, P. J. *Heterocycles* **1996**, *42*, 325–331.
(10) Rodriguez, A. D. *Tetrahedron* **1995**, *51*, 4571–4618.
(11) El Sayed, K. A.; Dunbar, D. C.; Perry, T. L.; Wilkins, S. P.; Hamann, M. T.; Greenplate, J. T.; Wideman, M. A. *J. Agric. Food Chem.* **1997**, *45*, 2735–2739.
(12) Garcia, M.; Rodriguez, J.; Jimenez, C. *J. Nat. Prod.* **1999**, *62*, 257–260.
(13) Sung, P. J.; Wu, S. L.; Fang, H. J.; Chiang, M. Y.; Wu, J. Y.; Fang, L. S.; Sheu, J. H. *J. Nat. Prod.* **2000**, *63*, 1483–1487.
(14) Sung, P. J.; Fan, T. Y.; Fang, L. S.; Sheu, J. H.; Wu, S. W.; Wang, G. H.; Lin, M. R. *Chem. Pharm. Bull.* **2003**, *51*, 1429–1431.
(15) Sung, P. J.; Lin, M. R.; Fang, L. S. *Chem. Pharm. Bull.* **2004**, *52*, 1504–1506.
(16) Shin, J.; Park, M.; Fenical, W. *Tetrahedron* **1989**, *45*, 1633–1638.
(17) Subrahmanyam, C.; Kulatheeswaran, R.; Ward, R. S. *J. Nat Prod.* **1998**, *61*, 1120–1122.
(18) Subrahmanyam, C.; Ratnakumar, S.; Ward, R. S. *Tetrahedron* **2000**, *56*, 4585–4588.
(19) Zhang, W.; Guo, Y. W.; Mollo, E.; Cimino, G. *Helv. Chim. Acta* **2004**, *87*, 2341–2345.
(20) Qi, S. H.; Zhang, S.; Qian, P. Y.; Xiao, Z. H.; Li, M. Y. *Tetrahedron* **2006**, *62*, 9123–9130.
(21) Jinming, G.; Lin, H.; Jikai, L. *Steroids* **2001**, *66*, 771–776.
(22) He, H. Y.; Faulkner, D. J. *Tetrahedron* **1991**, *47*, 3271–3280.
(23) Hamann, M. T.; Harrison, K. N.; Carroll, A. R.; Scheuer, P. J. *Heterocycles* **1996**, *42*, 325–331.
(24) Pordesimo, E. O.; Schmitz, F. J.; Ciereszko, L. S.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1991**, *56*, 2344–2357.
(25) Maharaj, D.; Pascoe, K. O.; Tinto, W. F. *J. Nat. Prod.* **1999**, *62*, 313–314.
(26) Dookran, R.; Maharaj, D.; Mootoo, B. S.; Ramsewak, R.; McLean, S.; Reynolds, W. F.; Tinto, W. F. *J. Nat. Prod.* **1993**, *56*, 1051–1056.
(27) Sheu, J. H.; Chen, Y. P.; Hwang, T. L.; Chiang, M. Y.; Fang, L. S.; Sung, P. J. *J. Nat. Prod.* **2006**, *69*, 269–273.
(28) Shen, Y. C.; Wang, S. S.; Pan, Y. L.; Lo, K. L.; Chakraborty, R.; Chien, C. T.; Kuo, Y. H.; Lin, Y. C. *J. Nat. Prod.* **2002**, *65*, 1848–1852.
(29) Sklar, L. A.; McNeil, V. M.; Jesaitis, A. J.; Painter, R. G.; Cochrane, C. G. *J. Biol. Chem.* **1982**, *257*, 5471–5475.
(30) Hwang, T. L.; Leu, Y. L.; Kao, S. H.; Tang, M. C.; Chang, H. L. *Free Radical Biol. Med.* **2006**, *41*, 1433–1441.
(31) Babior, B. M.; Kipnes, R. S.; Curnutte, J. T. *J. Clin. Invest.* **1973**, *52*, 741–744.

NP800126F