Cytotoxic Polyisoprenyl Benzophenonoids from *Garcinia subelliptica*

Li-Jie Zhang,[†] Chun-Tang Chiou,[†] Jing-Jy Cheng,[†] Hui-Chi Huang,[‡] Li-Ming Yang Kuo,[†] Chia-Chin Liao,[†] Kenneth F. Bastow,[§] Kuo-Hsiung Lee,*[,§] and Yao-Haur Kuo*[,†,⊥]

National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China, School of Chinese Medicine Resources, China Medical University, Taichung, 404, Taiwan, Republic of China, Natural Products Research Laboratories, Division of Medicinal Chemistry and Natural Products, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360, and Graduate Institute of Integrated Medicine, China Medical University, Taichuang 404, Taiwan, Republic of China

Received October 2, 2009

Six new polyisoprenyl benzophenonoids, (±)-garcinialiptone A (**1**, **2**), garcinialiptone B (**3**), (−)-cycloxanthochymol (**4**), garcinialiptone C (**5**), and garcinialiptone D (**6**), along with three known compounds, xanthochymol (**7**), isoxanthochymol (**8**), and cycloxanthochymol (**9**), were isolated from the fruits of *Garcinia subelliptica*. The structures of **1**−**6** were elucidated by spectroscopic analysis. Biological evaluation showed that all compounds **1**−**9** exhibited cytotoxic activity against a small panel of human tumor cell lines (A549, DU145, KB, vincristine-resistant KB).

Garcinia subelliptica Merr. (Guttiferae) is a tree that serves as a dye source in tropical and subtropical Asia. Several xanthones and phloroglucinol derivatives have been isolated from this plant[1–14] and found to exhibit various biological activities, including inhibitory activity against DNA topoisomerases I and II[13] and antioxidant,[2] anti-inflammatory,[9,10] and cytotoxic[12,15] effects. Polyisoprenylated benzophenones isolated from the Guttiferae family feature a bicyclo[3.3.1]nonane-2,4,9-trione skeleton substituted with a benzoyl group and prenyl or geranyl groups. These polyprenylated benzoylphloroglucinol derivatives are classified into three types according to the relative position of the benzoyl group.[16] Type A, with the benzoyl group linked at C-1, is exemplified by nemorosone[16] and garcinielliptone FB;[12] type B, with the benzoyl group at C-3, is represented by xanthochymol, the main component of *G. subelliptica*; and type C, with the benzoyl group at C-5, is typified by garcinielliptone K.[11] Many polycyclic polyprenylated acylphloroglucinol derivatives, also isolated from plants of the family Guttiferae, undergo secondary cyclizations involving the β-diketone and olefinic groups to afford adamantanes, homoadamantanes, dihydrofuro-fused structures, and related structures.[17] The occurrence of cyclized secondary metabolites is also documented for polyprenylated benzoylphloroglucinol derivatives.

In a continuing search for novel plant-derived antitumor agents, it was found that an acetone extract of the fruits of *G. subelliptica* showed moderate cytotoxicity against HeLa and WiDr cells. Bioassay-directed fractionation of *G. subelliptica* resulted in the isolation of six new cytotoxic polyisoprenyl benzophenonoids, (±)-garcinialiptone A (**1**, **2**, type B), garcinialiptone B (**3**, type C), (−)-cycloxanthochymol (**4**, type B), garcinialiptone C (**5**, type A), and garcinialiptone D (**6**, type A), along with the three known compounds xanthochymol (**7**, type B), isoxanthochymol (**8**, type B), and cycloxanthochymol (**9**, type B).

Results and Discussion

Extraction of the fruits of *G. subelliptica* with acetone, followed by chromatography, led to the isolation of nine compounds, including the known compounds **7**−**9**, as well as six new polyprenylated benzoylphloroglucinol compounds (**1**−**6**).

Compounds **1** and **2** [the (+) and (−) isomers of garcinialiptone A], obtained as yellow, amorphous solids, had almost identical HRESIMS, UV, IR, and NMR spectra. Compound **1** was assigned the molecular formula $C_{38}H_{48}O_6$ (corresponding to 15 degrees of unsaturation) on the basis of positive-ion HRESIMS of the peak at *m*/*z* 623.3377 $[M + Na]^+$. Signals for hydroxy (3403 cm^{-1}), carbonyl (1742, 1700 cm^{-1}), and aromatic (1599, 1549, 1440 cm^{-1}) groups were found in the IR spectrum of **1**, and its UV spectrum showed peak maxima at 314, 280, and 232 nm. The molecular formula, together with NMR evidence (Tables 1 and 2), indicated that **1** has a trioxygenated benzophenone-derived skeleton. On the basis of ^{13}C NMR signals at δ 203.9, 202.4, and 202.0 ppm,[18] the trioxygenated ring is nonaromatic and has three nonconjugated ketones. In addition to a pendant dioxygenated benzoyl group, the NMR data supported the presence of five additional five-carbon units: a 2-isopropenyl-5-methylhex-5-enyl group made up of two 3-methyl-3-butenyl units with one (C-22 to C-26) linked to the C-2 (C-18) position of the other (C-17 to C-21), two individual 3-methyl-2-butenyl groups (C-27 to C-31 and C-34 to C-38), and *gem*-dimethyl groups (C-32 and C-33) attached to a quaternary center (C-8), in turn attached to a methine (C-7) and methylene (C-6). The foregoing data account for 12 of the 15 required degrees of unsaturation. The absence of additional signals for sp^2 carbons suggested that **1** contains a tricyclic moiety. Its structure and the location of the attached groups were fully determined from $^1H-^1H$ COSY, HSQC, and HMBC data (Figure 1).

In the $^1H-^1H$ COSY spectrum, cross-peaks were found between H-34/H-35 and H-7 and between H-6/H-7. Moreover, in the HMBC spectrum, long-range correlations were observed between H-34/C-3 (δ_C 79.7), C-4 (δ_C 202.0), C-6 (δ_C 44.4), and C-7 (δ_C 47.6) and between H-7/C-1 (δ_C 77.1), C-3, and C-5 (δ_C 68.6). These facts indicated that the methine carbon (C-34) was connected to three carbons (C-35, C-7, and C-3). Furthermore, long-range correlations between H_2-27 (δ_H 2.68, dd, J = 13.5, 6.5 Hz and 2.53, dd, J = 13.5, 6.0 Hz)/C-1, C-2, and C-9 showed that one 3-methyl-2-butenyl unit was located at C-1, while correlations between H_2-17 (δ_H 2.17, dd, J = 14.0, 9.0 Hz and 1.95, m)/C-9 (δ_C 203.9), C-4, C-5, and C-6 permitted the assignment of the 2-isopropenylhex-5-enyl group at C-5. The linkages from C-1 to C-8 to C-7 were determined on the basis of ^3J HMBC correlations between the *gem*-dimethyl groups (δ_H 1.11 and 1.12, each 3H, s) at C-8 with C-1 and C-7. Also, on the basis of ^2J and ^3J HMBC correlations (Figure 1), the 3,4-dihydroxybenzoyl group was attached at C-3 of the main skeleton. Thus, the main tricyclic core skeleton of **1** and locations of the pendant residues were established as shown.

* To whom correspondence should be addressed. (Y.H.K.) Tel: 886-02-28201999, ext. 7051. E-mail: kuoyh@nricm.edu.tw. (K.H.L.) Tel: 919-962-0066. E-mail: khlee@unc.edu.

[†] National Research Institute of Chinese Medicine.

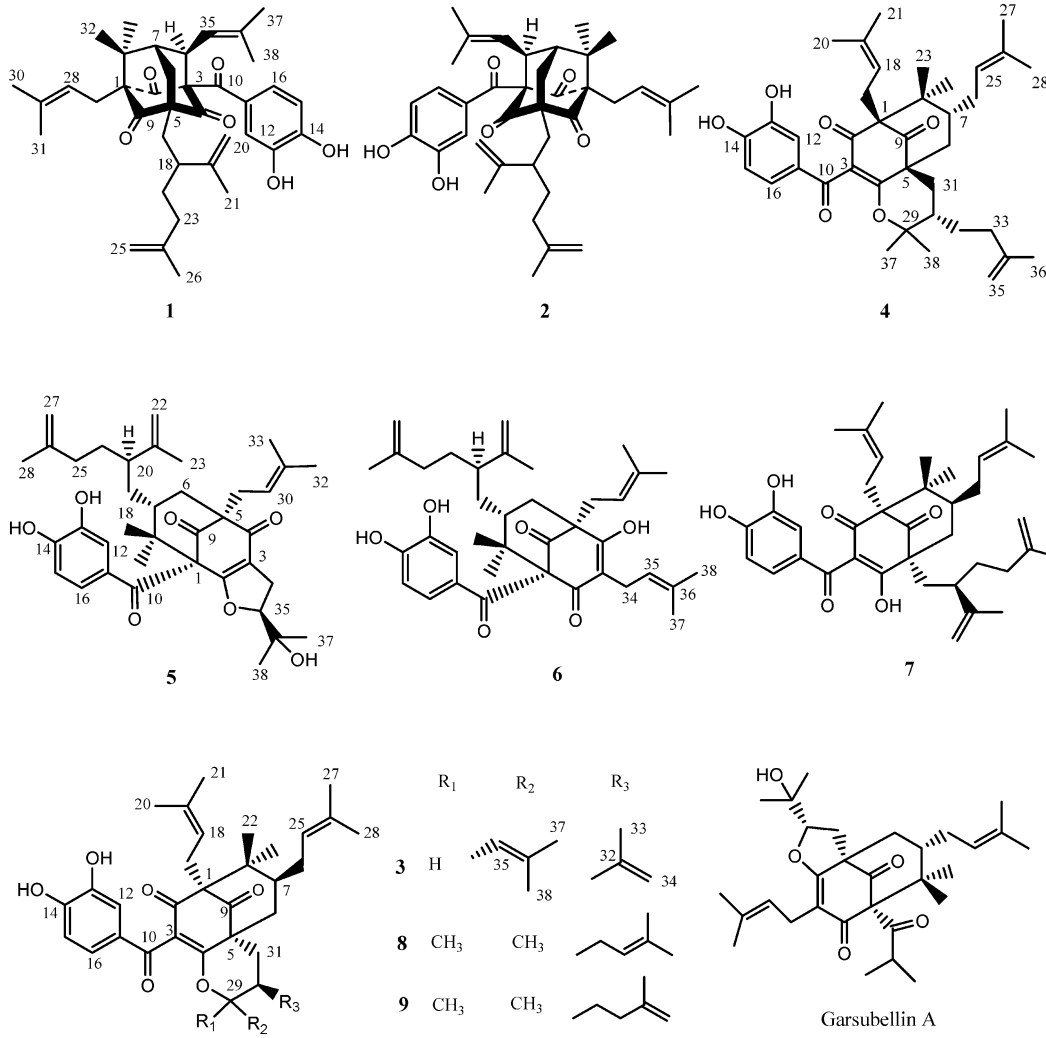
[‡] School of Chinese Medicine Resources, China Medical University.

[§] Eshelman School of Pharmacy, University of North Carolina.

[⊥] Graduate Institute of Integrated Medicine, China Medical University.

10.1021/np900620y
Published on Web 03/16/2010

Chart 1



Regarding the relative configuration at H-34, this proton showed *W*-coupling in the ^{1}H NMR spectrum to H-6, a strong HMBC cross-peak with C-4, and a weak peak with C-2, together with a NOE interaction with the C-33 methyl protons, indicating an *anti*-arrangement of C-4 and H-34. Thus, the stereochemistry of H-34 was determined and was also consistent with a literature report for plukenetione A.[18]

Compound **1** and hyperibone K[19] gave similar ^{1}H and ^{13}C NMR spectra, except for signals for 2-isopropenylhex-5-enyl and 3,4-dihydroxybenzoyl groups in the former rather than a prenyl moiety and unsubstituted benzoyl group in the latter. The specific rotation $[\alpha]_D^{25}$ +12.1 (*c* 3.40, MeOH) of **1** was similar to those of hyperibone K[19] [+22.3 (*c* 0.3, $CHCl_3$)] and plukenetione A[18] [+1 (*c* 0.77, $CHCl_3$)]. As a result of this information, the structure of **1** was deduced completely, and **1** is an analogue of hyperibone K and plukenetione A.

Although **1** and **2** have very similar UV, IR, and NMR data, as well as the same molecular ion in the HRESIMS, they were found to have opposite specific rotations. The specific rotation of **2** was −17.3 (*c* 3.36, MeOH). These findings verified that **1** and **2** are enantiomers. Thus, **1** and **2** have been named (+)-garcinialiptone A and (−)-garcinialiptone A, respectively.

Garcinialiptone B (**3**) was isolated as an optically active, yellow, amorphous solid, $[\alpha]_D^{25}$ +84.8 (*c* 5.40, MeOH). The molecular formula was established as $C_{38}H_{48}O_6$ from the HRESIMS (*m/z* 623.3386 $[M + Na]^+$). The IR spectrum of **3** displayed bands for hydroxy (3300 cm^{-1}), carbonyl (1726, 1670, 1640 cm^{-1}), and aromatic (1594, 1519, 1439 cm^{-1}) groups. Although **1**−**3** have the same molecular formula and, thus, degrees of unsaturation, the ^{1}H and ^{13}C NMR data of **3** (Tables 1 and 2) indicated a structural variation from the caged structures of **1** and **2**. The ^{13}C NMR spectrum of **3** (Table 2) showed resonances for six aromatic carbons, a conjugated carbonyl group at δ_C 192.0, and a bicyclic [3.3.1]nonane-2,4,9-trione moiety[16] with three quaternary carbons (δ_C 69.6, 48.3, and 46.6), one methine (δ_C 46.7), one methylene (38.1), a nonconjugated ketone (δ_C 208.9), and an enolized 1,3-diketone (δ_C 194.5, 124.3, and 170.3). [If the enol and ketone interconverted, two carbon signals would be at ca. δ_C 194 with one quaternary carbon at ca. δ_C 119; however, if an enol ether is present, one carbon signal is shifted upfield to ca. δ_C 172.] These data together with the proton NMR data (Table 1) suggested **3** contains a bicyclic [3.3.1]nonane-2,4,9-trione moiety attached to four prenyl units and a 3,4-disubstituted benzoic acid moiety. The ^{1}H−^{1}H COSY correlations of H-29/H-30, H-29/H-35, and H-30/H-31 (Figure 1), along with HMBC correlations of H-29/C-4, C-35, C-36 and H-30/C-5, C-32, C-34, were used to establish a 1-isobutenyl-2-propenyltetrahydropyran ring moiety fused at C-4 and C-5 of the nonane system. On the basis of the ^{1}H and ^{13}C NMR spectra, **3** has a similar structure to that of isoxanthochymol (**8**),[20] except for the substitution pattern on the tetrahydropyran ring.

The relative configurations at C-1, C-5, C-7, C-29, and C-30 in **3** were determined on the basis of the NOESY spectrum (Figure 2b) and coupling constant analysis, while assignment of the methylene protons at C-6, C-17, C-24, and C-31 as pro*R* or pro*S* relied on NOE data and the dihedral angular dependence of three-bond C−H correlations.[21] Like the relative stereochemistry of

Table 1. ^{1}H NMR Spectroscopic Data of **1**−**6** (*J* in Hz) (**1**−**4** in pyridine-d_5, and **5**−**6** in methanol-d_4)

position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[b]
6	2.62, dd, 13.5, 2.0; 2.49, brd, 13.0	2.64, dd, 13.2, 1.6; 2.50, brd, 14.0	2.80 pro*R*, d, 14.0; 2.00 pro*S*, dd, 13.5, 5.5	2.40, d, 14.0; 2.10, dd, 14.0, 8.0	1.88, m; 1.40, m	1.91, brd 10.4, 1.42, m
7	1.70, o	1.70, o	1.66, o	1.57, m	1.67, o	1.68, o
12	7.77, d, 2.0	7.77, br s	8.03, br s	8.06, d, 3.0	7.29, br s	7.31, d, 1.6
15	7.10, d, 8.5	7.09, d, 8.4	7.13, d, 8.5	7.22, d, 8.5	6.60, d, 8.4	6.54, d, 8.4
16	6.93, dd 8.5, 2.0	6.96, d, 8.4	7.65, d, 8.5	7.57, o	6.91, brd, 8.4	6.84, dd, 8.4, 1.6
17	2.17, dd, 14.0, 9.0; 1.95, m	2.18, dd, 14.4, 8.8 1.96, dd, 14.0, 8.8	2.92 pro*R*, dd, 13.5, 8.0; 2.79 pro*S*, o	2.92, dd, 13.0, 6.0; 2.75, dd, 13.5, 6.5	1.34, s	1.30, s
18	2.97, m	2.99, m	5.39, t, 6.0	5.41, t-like	1.14, s	1.08, s
19					2.11, m; 1.76, m	2.07, m; 1.76, m
20	4.93, s; 4.87, s	4.95,s; 4.90, s	1.66, s	1.63, s	2.48, m	2.41, m
21	1.60, s	1.60, s	1.75, s	1.70, s		
22	1.54, m	1.57, m	1.31, s	1.28, s	4.61, s; 4.64, s	4.58, s; 4.63, s
23	1.94, o, 2H	1.94, o, 2H	1.10, s	1.07, s	1.59, s	1.56, s
24			3.05 pro*S*, m; 2.51 pro*R*, brd, 14.5	3.16, m; 2.40, d, 14.0	1.46, 2H, m	1.50, m; 1.46, m
25	4.78, s; 4.75, s	4.80, s; 4.77, s	5.12, t, 6.5	5.05, t, 7.0	1.88, o	1.87, m
26	1.63, s	1.68, s				
27	2.68, dd, 13.5, 6.5; 2.53, dd, 13.5, 6.0	2.70, dd, 14.0, 6.8; 2.55, dd, 14.4, 5.6	1.68, s	1.71, s	4.68, s; 4.73, s	4.68, s; 4.66, s
28	5.26, t, 6.0	5.30, t, 6.0	1.85, s	1.87, s	1.71, s	1.71, s
29			4.59, t-like, 9.0		2.15, m; 1.75, o	2.11, m; 1.70, o
30	1.72, s	1.74, s	2.71, t-like, 10.5	1.55,o	4.98, d, 6.4	4.98, d (6.4)
31	1.60, s	1.63, s	2.59 pro*S*, t, 13.5; 1.88 pro*R*, m	3.18, d, 11.0, m		
32	1.12, s	1.16, s		1.40, m; 1.10, o	1.67, s	1.66, s
33	1.11, s	1.13, s	1.57, s	2.20, m; 1.90, m	1.58, s	1.57, s
34	4.37, d, 8.0	4.39, d, 7.6	4.87, s; 4.86, s		3.01, dd, 10.0, 14.8; 2.75, dd, 10.0, 15.2	3.15, dd, 10.0, 14.8; 3.11, dd, 10.0, 14.4
35	5.32, brd, 7.0	5.35, brd, 8.0		4.84, s, 2H	4.80, t, 10.0	5.09, t-like
36			5.27, d, 8.0	1.65, s		
37	1.56, s	1.60, s	1.41, s	1.18, s	0.89, s	1.66, s
38	1.68, s	1.71, s	1.30, s	0.99, s	0.77, s	1.56, s

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

Table 2. ^{13}C NMR Spectroscopic Data of **1**−**6** (**1**−**4** in pyridine-d_5 and **5**, **6** in methanol-d_4)

C	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[b]	C	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[b]
1	77.1	77.1	69.6	69.1	71.3	77.1	20	113.5	113.5	26.1	26.6	44.9	45.1
2	202.4	202.4	194.5	195.0	175.0		21	17.7	17.8	18.2	18.7	149.0	148.6
3	79.7	79.7	124.3	127.3	119.0	120.7	22	32.2	32.4	22.3	22.9	113.7	113.6
4	202.0	202.1	170.3	171.4	193.5		23	35.7	35.8	27.4	27.0	18.0	18.0
5	68.6	68.8	48.3	52.3	65.1	61.8	24	145.9	146.1	30.0	30.3	33.2	32.9
6	44.4	44.5	38.1	39.4	45.2	44.7	25	109.8	109.9	126.1	126.3	36.6	36.8
7	47.6	47.7	46.7	46.7	44.2	44.1	26	22.6	22.7	132.6	133.4	147.2	147.1
8	53.8	53.9	46.6	46.8	48.3	48.2	27	23.4	23.5	25.9	26.4	110.2	110.2
9	203.9	204.0	208.9	207.8	207.8	208.8	28	120.8	120.9	18.4	18.9	22.9	22.9
10	192.1	192.2	192.0	192.9	192.1	194.0	29	133.6	133.6	80. 0	87.4	28.7	28.2
11	127.5	127.7	130.3	130.7	130.5	130.8	30	18.2	18.3	43.0	42.6	123.8	124.0
12	117.1	117.2	115.8	116.3	117.0	116.9	31	25.8	25.9	33.8	28.5	134.4	134.1
13	146.8	146.9	147.2	147.7	146.5	146.0	32	22.1	22.2	144.3	29.0	25.3	26.0
14	152.2	152.3	153.1	153.6	151.6	150.7	33	22.6	22.7	20.1	35.7	17.9	17.9
15	115.0	115.0	115.8	116.2	114.9	114.6	34	51.5	51.6	113.8	145.5	27.7	22.9
16	123.7	123.9	123.7	124.2	123.5	123.3	35	121.8	121.9	122.4	111.3	95.1	122.1
17	33.8	33.9	25.8	26.5	24.7	24.6	36	133.7	133.7	141.3	22.7	71.9	133.4
18	42.6	42.7	121.2	121.5	16.1	16.3	37	25.7	25.8	25.3	21.5	25.3	26.0
19	148.2	148.4	133.9	134.4	36.3	36.6	38	18.1	18.2	17.8	28.9	24.4	18.0

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

isoxanthochymol,[20] the correlations of H_3-22/H-17pro*R*, H_3-22/H-24pro*R*, H_3-23/H-17pro*S*, H_3-23/H-6pro*S*, H_3-23/H-7, H-7/H-30, and H-30/H-31pro*R* indicated that CH_2-17, CH_3-22, H-7, H-30, and CH_2-31 all have an α-orientation. NOESY correlations between H-29 and H-33 suggested that C-35 and C-32 are *anti* to each other. The coupling patterns of H-29 (δ_H 4.59, t-like, $J_{29,35}$ = 9.0, Hz $J_{29,30}$ = 10.0 Hz, in pyridine-d_5), H-30 (δ_H 2.71, t-like, $J_{29,30}$ = 10.5 Hz, $J_{30,31S}$ = 13.0 Hz, in pyridine-d_5), and H-31pro*S* (δ_H 2.59, t, $J_{31proS,31proR} = J_{31proS,30}$ = 13.5 Hz, in pyridine-d_5) further confirmed axial orientations for H-29, H-30, and H-31pro*S* and equatorial orientations for C-35 and C-31. Therefore, the structure of garcinialiptone B (**3**) was determined as shown.

(−)-Cycloxanthochymol (**4**) was obtained as a yellow solid. The HRESIMS indicated a molecular formula of $C_{38}H_{50}O_6$ (*m/z* 625.3545 [M + Na]$^+$). Compound **4** and cycloxanthochymol (**9**, isolated from the same plant source in the present investigation) showed a striking resemblance in their spectroscopic data, indicating a close similarity between the two molecules. However, like compounds **1** and **2**, one main difference between **4** and **9** was their opposite specific rotations. The specific rotation $[\alpha]_D^{25}$ of cycloxanthochymol (**9**) is +104.0 (*c* 3.00, MeOH), whereas that of **4** is $[\alpha]_D^{25}$ −80.9 (*c* 2.20, MeOH). Thus, **4** was concluded to be the enantiomer of cycloxanthochymol and has been named (−)-cycloxanthochymol.

The molecular formula of **5**, $C_{38}H_{50}O_7$ (corresponding to 14 units of unsaturation), was established by HRESIMS (*m/z* 641.3494 [M + Na]$^+$). Analysis of the spectroscopic data showed **5** to be a polyprenylated benzoylphloroglucinol derivative. Its ^{1}H and ^{13}C

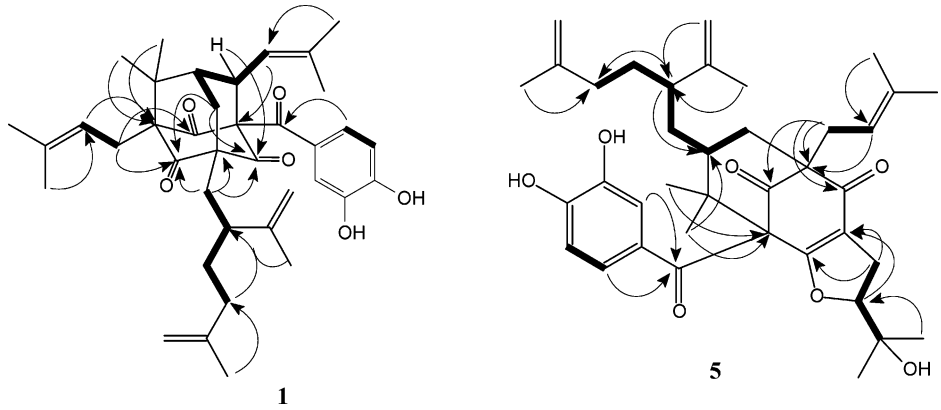


Figure 1. Key HMBC (→) and COSY (—) correlations of compounds **1** and **5**.

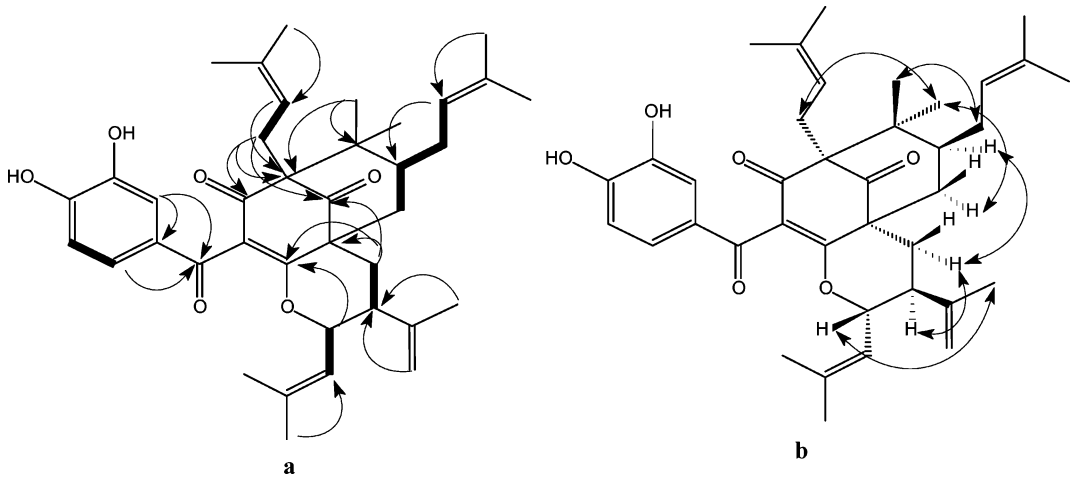


Figure 2. Key HMBC (→ a), COSY (— a), and NOE (b) correlations of **3**.

NMR (Tables 1, 2) spectra showed the presence of a bicyclic [3.3.1]nonane-2,4,9-trione basic skeleton and a 3,4-dihydroxybenzoyl group. The other pendant residues were assigned as *gem*-dimethyl groups (C-17 and C-18), a 2-isopropenyl-5-methylhex-5-enyl group (C-19 to C-28), a prenyl group (C-29 to C-32), and a (2-hydroxyisopropyl)dihydrofuran ring moiety (C-34 to C-38) on the basis of 1D and 2D NMR results. Inspection of the HMBC spectrum of **5** showed long-range correlations between H_2-34/C-2 and C-3 and between H_2-29/C-4, C-5, C-6, and C-9, and thus the (2-hydroxyisopropyl)dihydrofuran ring was established at C-2 and C-3, with the prenyl group connected at C-5. $^1H-^1H$ COSY correlations of H-20/H-19, H-19/H-7, and H-7/H-6 were observed, indicating that the 2-isopropenylhex-5-enyl group was located at C-7. Therefore, the 3,4-dihydroxybenzoyl group must be located at C-1. In order to determine the relative stereochemistry of **5**, a NOESY experiment was performed. Cross-peaks of H_3-17/H-19 (δ_H 2.11) and H-29 (δ_H 2.15), H_3-18/H-7 (δ_H 1.67), and H-6 (δ_H 1.40)/H-19 (δ_H 2.11) and H-29 were observed, indicating that CH_3-17, CH_2-19, and CH_2-29 are all located on the α-face. For benzophenone types A and C, Grossman et al.[17] have clarified the saturated ring conformation (chair or boat) and the C-7 prenyl group orientation (exo or endo), on the basis of NMR chemical shift analysis for the ring CH_2-6 (^1H), geminal methyls (^{13}C), and C-7 (^{13}C). With a chair conformation and an exo C-7 prenyl, the following values are usually found: $\Delta\delta_H$ ca. 0.5 ppm (ring CH_2-6), $\Delta\delta_C$ ca. 7.7 ppm (geminal methyls), and the C-7 chemical shift at ca. 43 ppm. In contrast, with a boat conformation and endo C-7 prenyl, the values would be $\Delta\delta_H$ ca. 0.2 ppm (ring CH_2-6), $\Delta\delta_C$ ca. 4.0 ppm (geminal methyls), and the C-7 chemical shift at ca. 48 ppm. For compound **5**, the diastereotopic ring CH_2-6 proton signals resonated 0.48 ppm apart, the diastereotopic Me groups (Me-17 and Me-18) resonated 8.6 ppm apart, and the C-7 chemical shift was found at δ_H 44.2 ppm. Thus, the structure of **5** was deduced to have a chair conformation in the more saturated ring (C-5, C-6, C-7, C-8, C-1, and C-9). Also, like nemorosone II,[17] the 2-isopropenylhex-5-enyl group was linked exo at C-7, and the benzophenone and prenyl units were located equatorially at C-1 and C-5, respectively. The structure of **5** is closely similar to that of garcinielliptone FB,[12] except for changes at the C-2 and C-4 positions. Thus, the complete structure of **5** was determined as shown, and this compound has been named garcinialiptone C.

The molecular formula for **6**, $C_{38}H_{50}O_6$, was deduced by HRESIMS (625.3545 $[M + Na]^+$). The ^1H and ^{13}C NMR spectra and molecular formula suggested it to also be a polyprenylated benzoylphloroglucinol derivative and to possess the same pendant residues as **5** at the C-1, C-5, and C-7 positions. On the basis of the HMBC spectrum, the last substituted prenyl group was located at C-3, and the planar structure of **6** was established. However, it still remained to determine the relative positions of the enol and ketone between C-2 and C-4. Intensive study of ^{13}C NMR literature data for type A benzophenone compounds showed that these positions can be decided by the $\Delta\delta_C$ value between C-1 and C-5. As shown in Table 3, C-1 and C-5 would resonate 21 ppm apart if the enolized 1,3-diketone is present as a C-2 ketone and C-4 enol, and in contrast, C-1 and C-5 would differ by only $\Delta\delta_C$ 7 ppm with a C-2 enol and C-4 ketone. Consequently, the enolized 1,3-diketone in **6** is present as a 2-ketone and 4-enol, because the actual $\Delta\delta_C$ value of 15.3 ppm is closer to $\Delta\delta_C$ 21 ppm. The relative configuration of **6** was confirmed as described for **5**; accordingly, the structure of **6** was assigned unambiguously as shown.

Moreover, the three known compounds, xanthochymol (**7**),[27] isoxanthochymol (**8**),[20] and cycloxanthochymol (**9**),[27] also isolated

Table 3. C-2 and C-4 Data of Enolized 1,3-Diketone Units of Benzophenone Type-A Compounds

	C-2 ketone, C-4 enol, ppm				C-2 enol, C-4 ketone, ppm		
compound	C-1	C-5	Δδ	compound	C-1	C-5	Δδ
plukenetione E[22]	77.7	55.9	21.8	plukenetione D[22]	71.8	64.3	7.5
plukenetione G[22]	77.7	55.9	21.8	plukenetione F[22]	70.8	63.8	7.0
sampsonione K[23]	77.1	58.7	18.4	sampsonione M[23]	68.0	63.0	5.0
sampsonione L[23]	77.1	59.8	18.3	*O*-methyl-chamone I[25]	74.4	63.8	10.6
hyperibone A[24]	79.2	55.6	23.6	chamone II[25]	72.5	64.3	8.2
scrobiculatone A[26]	79.3	57.6	21.7	insignone[26]	73.0	63.3	9.7
garcinielliptone FA[12]	78.9	55.1	23.8	scrobiculatone B[26]	71.9	65.3	6.6
nemorosone 4a[16]	78.0	57.1	20.9	nemorosone 4b[16]	71.4	64.8	5.6
average			21.3				7.5

Table 4. Cytotoxicity Data of **1**−**9** against Four Human Tumor Cells

	cell line (IC_{50}, μg/mL)[a]			
compound	A549[b]	DU145[b]	KB[b]	KBvin[b]
1	4.2	4.1	5.7	5.6
2	4.2	4.2	4.4	5.3
3	6.7	7.3	5.7	6.6
4	4.5	4.7	4.9	5.2
5	4.3	4.3	3.4	4.9
6	4.4	3.3	3.9	4.6
7	4.0	4.0	5.0	4.6
8	4.4	4.2	4.5	5.2
9	4.5	3.7	5.0	4.9
paclitaxel	0.002	0.002	0.002	>0.085

[a] IC_{50} = concentration that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay. [b] The cell lines are described in the Experimental Section.

were confirmed by comparing their NMR and MS data with analytical data reported in the literature.

All isolates (**1**−**9**) and garsubellin A (**10**, isolated by Lin and co-workers from the seeds of *G. subelliptica*)[9] were evaluated for anticancer activity against human tumor cell lines, including non-small-cell lung carcinoma (A549), prostate carcinoma (DU145), and nasopharyngeal carcinoma (KB). Also used were vincristine-resistant KB (KBvin) cells, which exhibit the multi-drug-resistant (MDR) phenotype associated with P-glycoprotein overexpression. The potent antitumor drug paclitaxel was used as a positive control. Cytotoxicity data (Table 4) showed that all the polyisoprenyl benzophenonoid compounds (**1**−**9**) had significant but borderline activity (IC_{50} 4−5 μg/mL range), whereas the polyprenylated acylphloroglucinol derivative, garsubellin A (**10**), was inactive (IC_{50} >5 μg/mL). Although the new compounds were about 2000-fold less active than paclitaxel, in contrast to the positive control, all were equally active against the MDR subline (KBvin), suggesting they can bypass a clinically important P-glycoprotein mechanism of cancer drug resistance. The results were also consistent with literature reports for the bioactivities of garcinielliptone FB,[12] garcinielliptones A and B, and garsubellin A.[9] The current results suggest that the benzoyl group in the phloroglucinol skeleton played a crucial role in the in vitro activity that was observed.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO P-1020 polarimeter. UV spectra were measured with a GBC 918 spectrophotometer. IR spectra were recorded as KBr disks, using an IR-FT Mattson Genesis II spectrometer. NMR spectra were recorded using Bruker UltraShield 400 MHz and Varian Inova 500 MHz spectrometers. High-resolution ESIMS were determined using a Finingan MAT 95S mass spectrometer. For column chromatography, silica gel 60 (70−230, 230−400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used. Precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with 5% H_2SO_4 and then heating at 110 °C. MPLC was performed on a system equipped with a Buchi B-688 pump, Buchi B-684 fraction collector, and Buchi columns. HPLC separations were performed on a Shimadzu LC-8A series apparatus with a SPD-20A UV detector, equipped with a 250 × 20 mm i.d. preparative Cosmosil 5C_{18} AR-II column (Nacalai Tesque, Inc.).

Plant Material. The fruits of *G. subelliptica* were collected in the northern mountains of Taiwan, in June 2005 and June 2007, and identified by one of us (Y.H.K.). A voucher specimen (no. NRICM20070614A) has been deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. The pericarp of *G. subelliptica* (12.5 kg dried; obtained in 2007) was extracted three times with acetone at 45 °C. The acetone extract was concentrated under reduced pressure, and the residue (1.53 kg) was coated on 1.3 kg silica gel and separated by passage over a MPLC silica gel column eluting with *n*-hexane/$CHCl_3$/acetone (1:0:0, 2:1:0, 1:2:0, 0:1:0, 0:10:1, 0:3:1, 0:1:1, 0:0:1) to yield eight fractions (Fr 1 to Fr 8). Fraction 4 ($CHCl_3$/acetone, 1:2, 25 g) was then submitted to Sephadex LH-20 CC, eluting with $CHCl_3$/MeOH/acetone (1:1:1), to afford four fractions (Fr 4.1 to Fr 4.4). Fr 4.2 (20 g) was subjected to a silica gel MPLC column, eluting with *n*-hexane/$CHCl_3$/acetone from 1:0:0 to 5:5:2, to give eight fractions (Fr 4.2.1 to Fr 4.2.8). In turn, Fr 4.2.2 was subjected to Sephadex LH-20 CC eluting with $CHCl_3$/MeOH (1:1) to give six fractions (Fr 4.2.2.1 to Fr 4.2.2.6). Fr 4.2.2.4 (4.80 g) was subjected to a reversed-phase HPLC ODS column eluting with 90% MeCN (10 mL/min, Cosmosil 250 × 20 mm i.d.) to afford six fractions (Fr 4.2.2.4.1 to Fr 4.2.2.4.6). Fr 4.2.2.4.3 was purified repeatedly by recycle preparative HPLC (250 × 20 mm i.d., Cosmosil 5C_{18} AR-II column, MeCN/H_2O, 80:20, repeated five times) to afford compound **3** (54.1 mg). Fr 4.2.2.4.4 and Fr 4.2.2.4.5 were purified by the same method to afford compounds **2** (62.6 mg), **4** (22.4 mg), **5** (60.1 mg), **8** (49.6 mg), and **9** (12.0 mg). Fr 5 ($CHCl_3$ 100%, 8.0 g) was chromatographed by repeated silica gel, LH-20, and recycle HPLC to afford compound **6** (66.2 mg).

The pericarp of *G. subelliptica* (5.5 kg dried, collected in 2005) was extracted three times with 95% EtOH at 50 °C. The EtOH extract was concentrated under reduced pressure, the residue (400 g) was suspended in water, and this suspension was successively extracted with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc layer provided 80 g of extract and was separated using a MPLC silica gel column eluting with *n*-hexane/$CHCl_3$/MeOH (1:0:0, 2:1:0, 1:2:0, 0:1:0, 0:20:1, 0:10:1, 0:5:1, 0:0:1), to yield 13 fractions (Fr E1 to Fr E13). Fraction E6 ($CHCl_3$ 100%, 5.8 g) was then submitted to Sephadex LH-20 CC, eluting with $CHCl_3$/MeOH/acetone (2:1:1), to afford five fractions (Fr E6.1 to Fr E6.5). Fr E6.3 (3.3 g) was subjected to passage over a silica gel MPLC column, eluting with $CHCl_3$/acetone/MeOH from 1:0:0 to 9:1:1, to give seven subfractions (Fr E6.3.1 to Fr E6.3.7). Fr E6.3.1 (1.1 g) was separated using a ODS MPLC column (Vercopak, C_{18}, cartridge, 40 × 150 mm i.d.), eluting with MeCN/H_2O, 70:30, to give six fractions (Fr E6.3.1.1 to Fr E6.3.1.6). Fr E6.3.1.4 (90 mg) was purified using a LH-20 open column, eluting with pure MeOH, to afford compound **1** (69.7 mg). Fr E6.3.1.6 was purified also using a LH-20 open column eluting with pure MeOH to afford compound **7** (150 mg).

(+)-Garcinialiptone A (1): yellow solid, mp 106 °C; $[\alpha]_D^{25}$ +12.1 (*c* 3.40, MeOH); UV (MeOH) λ_{max} (log ε) 314 (3.84), 280 (3.91), and 232 (sh, 4.16) nm; IR ν_{max} (KBr) 3403, 3074, 2970, 2927, 1742, 1700, 1599, 1549, 1518, 1440, 1289, 763 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRESIMS *m/z* 623.3377 $[M + Na]^+$ (calcd for $C_{38}H_{48}O_6Na$, 623.3349).

(−)-Garcinialiptone A (2): yellow solid, mp 109 °C; $[\alpha]_D^{25}$ −17.3 (*c* 3.36, MeOH); UV (MeOH) λ_{max} (log ε) 313 (3.85), 279 (3.94), and 231 (4.20) nm; IR ν_{max} (KBr) 3400, 3073, 2970, 2923, 1739, 1695,

1592, 1552, 1521, 1438, 1291, 756 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRESIMS *m/z* 623.3391 $[M + Na]^+$ (calcd for $C_{38}H_{48}O_6Na$, 623.3349).

Garcinialiptone B (3): yellow solid, mp 111 °C (dec); $[\alpha]_D^{25}$ +84.8 (*c* 5.40, MeOH); UV (MeOH) λ_{max} (log ε) 313 (3.84), 276 (4.13), and 229 (4.19) nm; IR ν_{max} (KBr) 3344, 3082, 2973, 2934, 1727, 1668, 1645, 1598, 1555, 1516, 1445, 1297, 758 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRESIMS *m/z* 623.3386 $[M + Na]^+$ (calcd for $C_{38}H_{48}O_6Na$, 623.3349).

(−)-Cycloxanthochymol (4): yellow solid, mp 225 °C; $[\alpha]_D^{25}$ −80.9 (*c* 2.20, MeOH); UV (MeOH) λ_{max} (log ε) 313 (3.97), 277 (4.23), and 230 (4.29) nm; IR ν_{max} (KBr) 3400, 2975, 2927, 1728, 1666, 1592, 1522, 1441, 1287, 760 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRESIMS *m/z* 625.3545 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_6Na$, 625.3505).

Garcinialiptone C (5): yellow solid, mp 209 °C; $[\alpha]_D^{25}$ −94.0 (*c* 0.86, MeOH); UV (MeOH) λ_{max} (log ε) 320 (sh, 3.88), 284 (4.20), and 235 (sh, 4.20) nm; IR ν_{max} (KBr) 3400, 3068, 2976, 2930, 1724, 1686, 1600, 1594, 1524, 1443, 1278, 758 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRESIMS *m/z* 641.3494 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_7Na$, 641.3454).

Garcinialiptone D (6): yellowish solid, mp 118 °C; $[\alpha]_D^{25}$ −79.1 (*c* 7.83, MeOH); UV (MeOH) λ_{max} (log ε) 313 (3.84), 280 (4.23), and 232 (4.21) nm; IR ν_{max} (KBr) 3300, 3067, 2968, 2930, 1723, 1688, 1630, 1597, 1516, 1440, 1291, 756 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRESIMS *m/z* 625.3545 $[M + Na]^+$ (calcd for $C_{38}H_{50}O_6Na$, 625.3505).

Cytotoxicity Assay. All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well with compounds added from DMSO-diluted stock. After 3 days in culture, attached cells were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: A549 (human lung carcinoma), DU145 (prostate cancer), KB (nasopharyngeal carcinoma), and KB-vin (vincristine-resistant KB subline). All cell lines were obtained from Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD), except KB-vin, which was a generous gift from Professor Y.-C. Cheng, Yale University. Cells were cultured in RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin.

Acknowledgment. The authors would like to thank Dr. M. J. Don, National Research Institute of Chinese Medicine, for preliminary EIMS measurements, and the Instrument Center of National Taiwan University, for the HRESIMS measurements. This work was supported by grants from the National Science Council (93-2323-B-077-002) and the National Research Institute of Chinese Medicine (NRICM-95-DHM-02), Taiwan, Republic of China, and NIH CA-17625 awarded to K.H.L.

Supporting Information Available: ^1H and ^{13}C NMR spectra of new compounds **1**−**6**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Fukuyama, Y.; Kamiyama, A.; Mima, Y.; Kodama, M. *Phytochemistry* **1991**, *30*, 3433–3436.
(2) Minami, H.; Kinoshita, M.; Fukuyama, Y.; Kodama, M.; Yoshizawa, T.; Sugiura, M.; Nakagawa, K.; Tago, H. *Phytochemistry* **1994**, *36*, 501–506.
(3) Minami, H.; Takahashi, E.; Fukuyama, Y.; Kodama, M.; Yoshizawa, T.; Nakagawa, K. *Chem. Pharm. Bull.* **1995**, *43*, 347–349.
(4) Minami, H.; Kuwayama, A.; Yoshizawa, T.; Fukuyama, Y. *Chem. Pharm. Bull.* **1996**, *44*, 2103–2106.
(5) Minami, H.; Hamaguchi, K.; Kubo, M.; Fukuyama, Y. *Phytochemistry* **1998**, *49*, 1783–1785.
(6) Iinuma, M.; Tosa, H.; Tanaka, T.; Shimano, R.; Asai, F.; Yonemori, S. *Phytochemistry* **1994**, *35*, 1355–1360.
(7) Lin, C. N.; Kiang, C. W.; Lu, C. M.; Wu, R. R.; Lee, K. H. *Chem. Commun.* **1996**, 1315–1316.
(8) Chung, M. I.; Su, H. J.; Lin, C. N. *J. Nat. Prod.* **1998**, *61*, 1015–1016.
(9) Weng, J. R.; Tsao, L. T.; Wang, J. P.; Lin, C. N. *Eur. J. Chem.* **2003**, *9*, 1958–1963.
(10) Weng, J. R.; Lin, C. N.; Tsao, L. T.; Wang, J. P. *Eur. J. Chem.* **2003**, *9*, 5520–5527.
(11) Weng, J. R.; Tsao, L. T.; Wang, J. P.; Wu, R. R.; Lin, C. N. *J. Nat. Prod.* **2004**, *67*, 1796–1799.
(12) Wu, C. C.; Weng, J. R.; Won, S. J.; Lin, C. N. *J. Nat. Prod.* **2005**, *68*, 1125–1127.
(13) Tosa, H.; Iinuma, M.; Tanaki, T.; Zozaki, H.; Ikeda, S.; Tsutsui, K.; Tsutsui, K.; Yamada, M.; Fujimori, S. *Chem. Pharm. Bull.* **1997**, *45*, 418–420.
(14) Wu, C. C.; Lu, Y. H.; Wei, B. L.; Yang, S. C.; Won, S. J.; Lin, C. N. *J. Nat. Prod.* **2008**, *71*, 246–250.
(15) Protiva, P.; Hopkins, M. E.; Baggett, S.; Yang, H.; Lipkin, M.; Holt, P. R.; Kennelly, E. J.; Bernard, W. I. *Int. J. Cancer* **2008**, *123*, 687–694.
(16) Cuesta-Rubio, O.; Velez-Castro, H.; Frontana-Uribe, B. A.; Càrdenas, J. *Phytochemistry* **2001**, *57*, 279–283.
(17) Grossman, R. B.; Jacobs, H. *Tetrahedron Lett.* **2000**, *41*, 5165–5169.
(18) Henry, G. E.; Jacobs, H.; Plukenetione, A. *Tetrahedron Lett.* **1996**, *37*, 8663–8666.
(19) Tanaka, N.; Takaishi, Y.; Shikishima, Y.; Nakanishi, Y.; Bastow, K.; Lee, K. H.; Honda, G.; Ito, M.; Takeda, Y.; Kodzhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod.* **2004**, *67*, 1870–1875.
(20) Gustafson, K. R.; Blunt, J. W.; Munro, M. H. G.; Fuller, R. W.; Mckee, T. C.; Cardellina, J. H., II; McMahon, J. B.; Cragg, G. M.; Boyd, M. R. *Tetrahedron* **1992**, *48*, 10093–10102.
(21) Marshall, J. L. *Carbon-Carbon and Carbon-Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis*; Weinheim: Verlag Chemie International, Deerfield Beach, FL, 1983; p 22.
(22) Henry, G. E.; Jacobs, H.; Sean Carrington, C. M.; Mclean, S.; Reynolds, W. F. *Tetrahedron* **1999**, *55*, 1581–1596.
(23) Hu, L. H.; Sim, K. Y. *Tetrahedron* **2000**, *56*, 1379–1386.
(24) Matsuhisa, M.; Shikishima, Y.; Takaishi, Y.; Honda, G.; Ito, M.; Takeda, Y.; Shibata, H.; Higuti, T.; Kodzhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod.* **2002**, *65*, 290–294.
(25) Lokwam, J.; Braddock, J. F.; Reichardt, P. B.; Clausen, T. P. *Phytochemistry* **2000**, *55*, 29–34.
(26) Porto, A. L. M.; Machado, S. M. F.; de Oliveira, C. M. A.; Bittrich, W.; Amaral, M. do C. E.; Marsaioli, A. J. *Phytochemistry* **2000**, *55*, 755–768.
(27) Iinuma, M.; Tosa, H.; Tanaka, T.; Kanamaru, S.; Asai, F.; Kobayashi, Y.; Miyauchi, K.; Shimano, R. *Biol. Pharm. Bull.* **1996**, *19*, 311–314.

NP900620Y