Cytotoxic Activity of Capnellene-8β,10α-diol Derivatives from a Taiwanese Soft Coral *Capnella* sp.

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The sesquiterpene capnellene-8β,10α-diol (**1**) was isolated from non-polar extract of the soft coral *Capnella* sp. Ten acylation products of capnellene-8β,10α-diol were prepared: 10α-hydroxy-8β-*O*-benzoylcapnellene (**2**), 10α-hydroxy-8β-*O*-*p*-toluoylcapnellene (**3**), 10α-hydroxy-8β-*O*-4-chlorobenzoylcapnellene (**4**), 10α-hydroxy-8β-*O*-2-furoylcapnellene (**5**), 10α-hydroxy-8β-*O*-2-thiophenoylcapnellene (**6**), 10α-hydroxy-8β-*O*-4-fluorobenzoylcapnellene (**7**), 10α-hydroxy-8β-*O*-4-propylbenzoylcapnellene (**8**), 10α-hydroxy-8β-*O*-cinnamoylcapnellene (**9**), 10α-hydroxy-8β-*O*-4-nitrobenzoylcapnellene (**10**), and 10α-hydroxy-8β-*O*-4-anisoylcapnellene (**11**). The structures of capnellene-8β,10α-diol as well as its derivatives were established through standard spectroscopic analysis. The *in vitro* cytotoxic activities of the eleven compounds were evaluated against Hela, KB, Daoy, and WiDr human tumor cell lines.

Keywords: *Capnella* sp.; Capnellene-8β,10α-diol; Acyl derivatives; Cytotoxicity.

INTRODUCTION

Soft corals of the order Alcyonacea are the major contributors to the biomass of many tropical reefs. Most of them live in symbiotic relationship with intracellular algae that play a major role in the biology of the colonies.[1-3] These corals have been widely studied as they produce a huge array of skeletal classes of terpenes with unique substitution patterns and functionalities.[4,5] *Capnella* (Nephtheidae) is a widely distributed genus of Alcyonacea that provide non-isoprenoid sesquiterpenes with a 5-membered tricyclic skeleton named capnellane, few xenicane-type diterpenes, and no sterols.[2,4] Some of the soft coral sesquiterpenoids have demonstrated interesting biological activities but little has been reported about the biological activities of capnellene sesquiterpenes which have fused tricyclic rings with an exo-double bond at C_9.[3] A chemical investigation of *Capnella* sp. led to isolation of a capnellene derivative, capnellene-8β,10α-diol (**1**), previously isolated from *Capnella imbricate*,[1,2,6] with reported cytotoxic activity against K562 leukemia (IC_{50} = 0.7 μM).[7] This major compound was acylated to yield ten derivatives, 10α-hydroxy-8β-*O*-benzoyl-capnellene (**2**), 10α-hydroxy-8β-*O*-*p*-toluoyl-capnellene (**3**), 10α-hydroxy-8β-*O*-4-chlorobenzoyl-capnellene (**4**), 10α-hydroxy-8β-*O*-2-furoyl-capnellene (**5**), 10α-hydroxy-8β-*O*-2-thiophenoyl-capnellene (**6**), 10α-hydroxy-8β-*O*-4-fluorobenzoyl-capnellene (**7**), 10α-hydroxy-8β-*O*-4-propylbenzoyl-capnellene (**8**), 10α-hydroxy-8β-*O*-cinnamoyl-capnellene (**9**), 10α-hydroxy-8β-*O*-4-nitrobenzoyl-capnellene (**10**), 10α-hydroxy-8β-*O*-4-anisoyl-capnellene (**11**). The structure of **1** and its derivatives was elucidated based on spectroscopic analysis, including HRESIMS and 2D NMR. All compounds were tested against a panel of human tumor cell lines. The cytotoxic activity of capnellene-8β,10α-diol as well as its ten derivatives was evaluated against a panel of Hela, KB, Daoy, and WiDr human tumor cell lines.

RESULTS AND DISCUSSION

Extraction of *Capnella* sp. and subsequent chromatographic fractionation of the CH_2Cl_2 extract yielded capnellene-8β,10α-diol (**1**) that was characterized through spectroscopic analysis and by comparison of its spectral

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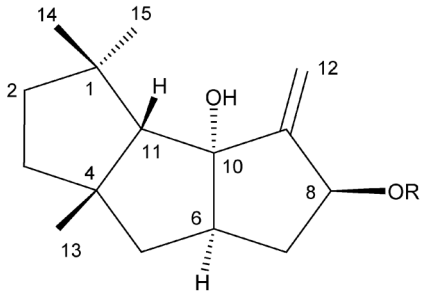

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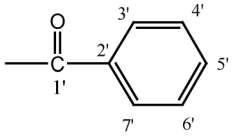
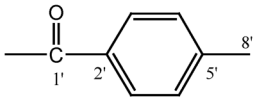
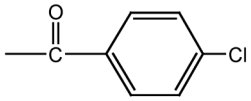
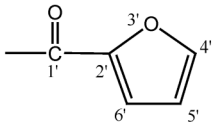
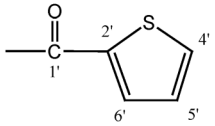
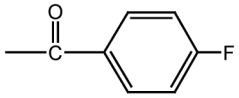
2: R = 

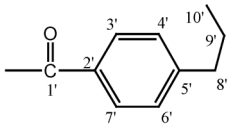
3: R = 

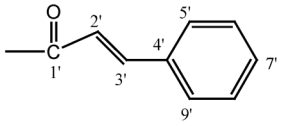
4: R = 

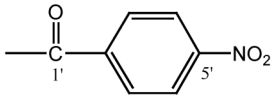
5: R = 

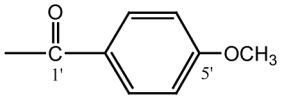
7: R = 

6: R = 

8: R = 

9: R = 

10: R = 

11: R = 

data with published values.[1,8-10] The acylation products **2**-**11** were prepared from **1** using different acid chlorides: benzoyl chloride, toluoylchloride, 4-chlorobenzoyl chloride, 2-furoylcarbonyl chloride, 2-thienoylcarbonyl chloride, 4-fluorobenzoyl chloride, 4-propylbenzoyl chloride, cinnamoyl chloride, 4-nitrobenzoyl chloride, and anisoyl chloride, respectively. The structures of **2**-**11** were established through analyses of their spectroscopic data, particularly HRESIMS, ^{1}H- and ^{13}C NMR, DEPT, and 2 D NMR (COSY, HMQC and HMBC). The spectral data of **1**-**11** are included in the experimental section.

The cytotoxic activities of compounds **1**-**11** were tested against human cervical epitheloid carcinoma (Hela), human oral epidermoid (KB), human medulloblastoma (Daoy), and human colon adenocarcinoma (WiDr) tumor cell lines. Table 1 displays the assay results of the *in vitro* cytotoxic activities, expressed as IC_{50} of **1**-**11**. All tested compounds showed significant activity against Hela and KB cell lines. Compounds **1** and **5** exhibited the strongest activity against the WiDr cell line with IC_{50} 0.16 and 0.35, respectively, while compounds **3** and **11** showed moderate activity against the same cell line. Compounds **5** and **11**

Table 1. Cytotoxic activity of compounds **1-11**[a]

Compound	IC_{50} (μg/mL)			
	Hela	KB	Daoy	WiDr
1	3.56	6.06	(-)[b]	0.16
2	(-)	(-)	(-)	(-)
3	3.13	5.45	(-)	8.00
4	2.36	2.19	(-)	10.50
5	3.05	4.10	9.93	0.35
6	2.70	2.48	(-)	15.93
7	2.49	2.09	(-)	(-)
8	4.79	3.86	(-)	(-)
9	3.02	2.77	(-)	(-)
10	3.10	2.96	(-)	(-)
11	2.63	1.92	5.42	6.30
Mitomycin C	0.09	0.08	0.07	0.06

[a] Cell lines: Hela, human cervical epitheloid carcinoma; KB, human oral epidermoid; Daoy, human medulloblastoma; WiDr, human colon adenocarcinoma.
[b] (-): IC_{50} > 20 μg/mL.

showed exclusive moderate cytotoxicity against the Daoy cell line, while all other compounds were inactive. The benzoyl ester **2** was inactive against all tested cell lines but it seems that substitution at the *p*-position increased the cytotoxic activity, especially against Hela and KB cells. All tested compounds showed significant cytotoxicity against Hela and KB cell lines with the exception of benzoyl ester **2**. The activity of almost all esters against Hela and KB cells was superior compared to the parent compound **1**, especially in the case of **4**, **7**, and **11**. This may be attributed to greater penetration of the cytotoxic ester into the cancer cells.

EXPERIMENTAL SECTION

General Methods

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on a Hitachi T-2001 and a Hitachi U-3210 spectrophotometer, respectively. Mass spectra were recorded on a VG Quattro 5022 mass spectrometer. NMR spectra were run on a Bruker FT-300 NMR instrument. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Multiplicities and assignments were monitored by DEPT and HMBC techniques. Sephadex LH-20 was obtained from Amersham Pharmacia Biotech AB, Uppsala, Sweden. Other chemicals were obtained from E. Merck (Germany) unless otherwise specified.

Animal Material

The soft coral *Capnella* sp. was collected on Green Island, Taiwan, in June 2005. A voucher specimen (GSC-25) was deposited at the School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation

The soft coral *Capnella* sp. (4 kg, wet weight) was homogenized with CH_2Cl_2/MeOH (1:1) twice (2 × 6 L). After filtration and concentration under vacuum, the crude extract (20 g) was partitioned between EtOAc/H_2O to produce EtOAc extract. The EtOAc extract (5 g) was re-extracted with *n*-hexane/MeOH/H_2O (4:3:1) to afford *n*-hexane extract and a hydro-alcoholic mixture. Concentration of the latter under vacuum led to isolation of **1** (2.8 g).

Capnellene-8β,10α-diol (1)

Colorless amorphous solid; $[\alpha]_D^{25}$ +40.8 (*c* 0.05, CH_2Cl_2); UV λ_{max} 207 nm; IR (CH_2Cl_2) ν_{max} 3419 (OH), 1042 (OH) cm^{-1}; EIMS *m/z* 218 $[M-H_2O]^+$; HRESIMS *m/z* 259.1676 $[M+Na]^+$ (calc for $C_{15}H_{24}O_2$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.71 (2H, m, H-3), 1.97 (m, H_a-5), 1.30 (m, H_b-5), 2.49 (m, H-6), 2.33 (m, H_a-7), 1.50 (m, H_b-7), 4.81 (m, H-8), 1.87 (s, H-11), 5.34 (2H, br s, H-12), 1.08 (3H, s, H-13), 1.15 (3H, s, H-14), 1.26 (3H, s, H-15); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.0 (C-1), 43.2 (C-2), 42.0 (C-3), 49.9 (C-4), 46.2 (C-5), 49.6 (C-6), 38.1 (C-7), 73.6 (C-8), 162.4 (C-9), 90.2 (C-10), 65.7 (C-11), 109.8 (C-12), 32.8 (C-13), 31.4 (C-14), 24.1 (C-15).

General Procedure for the Preparation of 2-11

0.1 mL of an appropriate acid chloride (benzoyl chloride, toluoylchloride, 4-chlorobenzoyl chloride, 2-furoylcarbonyl chloride, 2-thienoylcarbonyl chloride, 4-fluorobenzoyl chloride, 4-propylbenzoyl chloride, cinnamoyl chloride, 4-nitrobenzoyl chloride, and anisoyl chloride) was added to a solution of **1** (50 mg, 0.212 mmole) in anhydrous pyridine (2 mL) at 50° C and stirred for 21 h. After completion of the reaction (as monitored by TLC) the reaction mixture was poured into ice-water and stirred for 45 min and then extracted with CH_2Cl_2. The organic layer was washed with $NaHCO_3$ solution, then with sufficient water, dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The crude product was first purified through a Sephadex LH-20 column using CH_2Cl_2-MeOH (1:1) to afford the pure products. The respective products were **2** (23 mg, yield 32%), **3** (15.5 mg, yield 21%), **4** (35.7 mg, yield 45%), **5** (44 mg, yield 63%), **6** (46 mg, yield

63%), **7** (24 mg, yield 33%), **8** (17.8 mg, yield 22%), **9** (34 mg, yield 44%), 10 (32.4 mg, yield 40%), **11** (27 mg, yield 34%).[11]

10α-Hydroxy-8β-*O*-benzoylcapnellene (2)

$[\alpha]_D^{25}$ +108.7 (*c* 0.05, CH_2Cl_2); UV λ_{max} 255, 273 nm; IR (CH_2Cl_2) ν_{max} 3503 (OH), 1716 (C=O ester) cm^{-1}; EIMS *m/z* 340; HRESIMS *m/z* 363.1938 $[M+Na]^+$ (calc for $C_{22}H_{28}O_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.51 (2H, m, H-2), 1.71 (2H, m, H-3), 1.94 (m, H_a-5), 1.43 (m, H_b-5), 2.67 (m, H-6), 2.49 (m, H_a-7), 1.53 (m, H_b-7), 5.48 (dd, *J* = 4.8, 1.5 Hz, H-8), 1.92 (s, H-11), 5.48 (2H, br s, H-12), 1.13 (3H, s, H-13), 1.20 (3H, s, H-14), 1.27 (3H, s, H-15), 8.04 (2H, d, *J* = 7.3 Hz, H-3′,7′), 7.45 (2H, d, *J* = 7.3 Hz, H-4′,6′), 7.57 (t, *J* = 7.3 Hz, H-5′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.1 (C-1), 43.4 (C-2), 42.4 (C-3), 49.8 (C-4), 45.5 (C-5), 49.3 (C-6), 35.1 (C-7), 76.0 (C-8), 158.8 (C-9), 90.3 (C-10), 66.1 (C-11), 113.8 (C-12), 32.5 (C-13), 31.3 (C-14), 23.8 (C-15), 166.3 (C-1′), 130.5 (C-2′), 129.6 (C-3′,7′), 128.4 (C-4′,6′), 132.9 (C-5′).

10α-Hydroxy-8β-*O*-*p*-toluoylcapnellene (3)

$[\alpha]_D^{25}$ +114.2 (*c* 0.05, CH_2Cl_2); UV λ_{max} 243, 251 nm; IR (CH_2Cl_2) ν_{max} 3497 (OH), 1713 (C=O ester), 1611, 1455 cm^{-1}; EIMS *m/z* 354; HRESIMS *m/z* 377.2092 $[M+Na]^+$ (calc for $C_{23}H_{30}O_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (2H, m, H-2), 1.70 (2H, m, H-3), 1.97 (m, H_a-5), 1.30 (m, H_b-5), 2.64 (m, H-6), 2.48 (m, H_a-7), 1.50 (m, H_b-7), 6.04 (d, *J* = 7.8 Hz, H-8), 1.91 (s, H-11), 5.47 (s, H_a-12), 5.46 (s, H_b-12), 1.12 (3H, s, H-13), 1.20 (3H, s, H-14), 1.27 (3H, s, H-15), 7.92 (2H, d, *J* = 7.8 Hz, H-3′,7′), 7.24 (2H, d, *J* = 7.8 Hz, H-4′,6′), 2.41 (s, H-8′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.1 (C-1), 43.4 (C-2), 42.4 (C-3), 49.8 (C-4), 45.5 (C-5), 49.3 (C-6), 35.1 (C-7), 75.7 (C-8), 158.9 (C-9), 90.3 (C-10), 66.1 (C-11), 113.8 (C-12), 32.5 (C-13), 31.3 (C-14), 23.8 (C-15), 166.4 (C-1′), 127.7 (C-2′), 129.6 (C-3′,7′), 129.1 (C-4′,6′), 143.6 (C-5′), 21.6 (C-8′).

10α-Hydroxy-8β-*O*-4-chlorobenzoylcapnellene (4)

$[\alpha]_D^{25}$ +60.0 (*c* 0.05, CH_2Cl_2); UV λ_{max} 237, 252 nm; IR (CH_2Cl_2) ν_{max} 3494 (OH), 1715 (C=O ester), 1593, 1488 cm^{-1}; EIMS *m/z* 374; HRESIMS *m/z* 397.1548 $[M+Na]^+$ (calc for $C_{22}H_{27}ClO_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.70 (2H, m, H-3), 1.93 (m, H_a-5), 1.38 (m, H_b-5), 2.65 (m, H-6), 2.46 (m, H_a-7), 1.46 (m, H_b-7), 6.03 (d, *J* = 7.8 Hz, H-8), 1.90 (s, H-11), 5.46 (2H, br s, H-12), 1.11 (3H, s, H-13), 1.19 (3H, s, H-14), 1.26 (3H, s, H-15), 7.95 (2H, d, *J* = 7.1 Hz, H-3′,7′), 7.40 (2H, d, *J* = 7.1 Hz, H-4′,6′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.1 (C-1), 43.4 (C-2), 42.3 (C-3), 49.7 (C-4), 45.5 (C-5), 49.3 (C-6), 35.1 (C-7), 76.2 (C-8), 158.6 (C-9), 90.2 (C-10), 66.1 (C-11), 113.9 (C-12), 32.5 (C-13), 31.3 (C-14), 23.7 (C-15), 165.4 (C-1′), 128.7 (C-2′), 130.9 (C-3′,7′), 128.9 (C-4′,6′), 139.4 (C-5′).

10α-Hydroxy-8β-*O*-2-furoylcapnellene (5)

$[\alpha]_D^{25}$ +68.0 (*c* 0.05, CH_2Cl_2); UV λ_{max} 248, 260 nm; IR (CH_2Cl_2) ν_{max} 3497 (OH), 1713 (C=O ester) cm^{-1}; EIMS *m/z* 330; HRESIMS *m/z* 353.1728 $[M+Na]^+$ (calc for $C_{20}H_{26}O_4$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.70 (2H, m, H-3), 1.91 (m, H_a-5), 1.41 (m, H_b-5), 2.66 (m, H-6), 2.44 (m, H_a-7), 1.59 (m, H_b-7), 6.02 (br d, *J* = 6.9 Hz, H-8), 1.89 (s, H-11), 5.47 (s, H_a-12), 5.46 (d, *J* = 1.1 Hz, H_b-12), 1.10 (3H, s, H-13), 1.18 (3H, s, H-14), 1.25 (3H, s, H-15), 7.59 (br s, H-4′), 6.50 (t, *J* = 3.1 Hz, H-5′), 7.12 (d, *J* = 3.1 Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.1 (C-1), 43.4 (C-2), 42.4 (C-3), 49.8 (C-4), 45.5 (C-5), 49.3 (C-6), 35.0 (C-7), 76.0 (C-8), 158.6 (C-9), 90.3 (C-10), 66.3 (C-11), 114.2 (C-12), 32.5 (C-13), 31.3 (C-14), 23.8 (C-15), 158.6 (C-1′), 144.9 (C-2′), 146.4 (C-4′), 111.7 (C-5′), 117.7 (C-6′).

10α-Hydroxy-8β-*O*-2-thiophenoylcapnellene (6)

$[\alpha]_D^{25}$ +78.4 (*c* 0.05, CH_2Cl_2); UV λ_{max} 253, 278 nm; IR (CH_2Cl_2) ν_{max} 3503 (OH), 1701 (C=O ester), 1593, 1488 cm^{-1}; EIMS *m/z* 346; HRESIMS *m/z* 369.1502 $[M+Na]^+$ (calc for $C_{20}H_{26}O_3S$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.71 (2H, m, H-3), 1.90 (m, H_a-5), 1.40 (m, H_b-5), 2.64 (m, H-6), 2.44 (m, H_a-7), 1.48 (m, H_b-7), 6.00 (d, *J* = 7.8 Hz, H-8), 1.90 (s, H-11), 5.49 (s, H_a-12), 5.47 (s, H_b-12), 1.12 (3H, s, H-13), 1.19 (3H, s, H-14), 1.25 (3H, s, H-15), 7.55 (d, *J* = 4.8 Hz, H-4′), 7.09 (t, *J* = 4.0 Hz, H-5′), 7.79 (d, *J* = 3.6 Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.1 (C-1), 43.4 (C-2), 42.5 (C-3), 49.9 (C-4), 45.4 (C-5), 49.3 (C-6), 35.0 (C-7), 76.2 (C-8), 158.7 (C-9), 90.3 (C-10), 66.3 (C-11), 114.5 (C-12), 32.5 (C-13), 31.3 (C-14), 23.7 (C-15), 161.9 (C-1′), 127.7 (C-2′), 134.1 (C-4′), 132.4 (C-5′), 133.3 (C-6′).

10α-Hydroxy-8β-*O*-4-fluorobenzoylcapnellene (7)

$[\alpha]_D^{25}$ +124.0 (*c* 0.05, CH_2Cl_2); UV λ_{max} 241 nm; IR (CH_2Cl_2) ν_{max} 3502 (OH), 1715 (C=O ester), 1603, 1508 (Ar.) cm^{-1}; EIMS *m/z* 358; HRESIMS *m/z* 381.1847 $[M+Na]^+$ (calc for $C_{22}H_{27}FO_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.75 (2H, m, H-3), 1.93 (m, H_a-5), 1.39 (m, H_b-5), 2.66 (m, H-6), 2.48 (m, H_a-7), 1.47 (m, H_b-7), 6.04 (br d, *J* = 6.5 Hz, H-8), 1.91 (s, H-11), 5.47 (2H, br s, H-12), 1.12 (3H, s, H-13), 1.20 (3H, s, H-14), 1.27 (3H, s, H-15), 8.04 (2H, d, *J* = 6.6 Hz, H-3′,7′), 7.12 (2H, d, *J* = 6.6 Hz, H-4′,6′); ^{13}C NMR (75 MHz, $CDCl_3$), δ

44.2 (C-1), 43.4 (C-2), 42.4 (C-3), 49.8 (C-4), 45.5 (C-5), 49.3 (C-6), 35.1 (C-7), 76.1 (C-8), 158.7 (C-9), 90.2 (C-10), 66.1 (C-11), 113.9 (C-12), 32.5 (C-13), 31.3 (C-14), 23.8 (C-15), 165.3 (C-1′), 126.7 (C-2′), 132.1 (C-3′,7′), 115.5 (C-4′,6′), 164.0 (C-5′).

10α-Hydroxy-8β-*O*-4-propylbenzoylcapnellene (8)

$[\alpha]_D^{25}$ +74.2 (*c* 0.05, CH_2Cl_2); UV λ_{max} 234, 250 nm; IR (CH_2Cl_2) ν_{max} 3481 (OH), 1714 (C=O ester), 1610, 1457 (Ar.) cm^{-1}; EIMS *m/z* 382; HRESIMS *m/z* 405.2408 $[M+Na]^+$ (calc for $C_{25}H_{34}O_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.69 (2H, m, H-3), 1.92 (2H, m, H_a-5), 1.46 (m, H_b-5), 2.65 (m, H-6), 2.45 (m, H_a-7), 1.49 (m, H_b-7), 6.04 (d, *J* = 7.7 Hz, H-8), 1.92 (s, H-11), 5.47 (s, H_a-12), 5.46 (s, H_b-12), 1.13 (3H, s, H-13), 1.20 (3H, s, H-14), 1.27 (3H, s, H-15), 7.95 (2H, d, *J* = 8.0 Hz, H-3′,7′), 7.25 (2H, d, *J* = 8.0 Hz, H-4′,6′), 2.64 (2H, t, *J* = 7.4 Hz, H-8′), 1.65 (m, H-9′), 0.94 (t, *J* = 7.3 Hz, H-10′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.1 (C-1), 43.4 (C-2), 42.4 (C-3), 49.8 (C-4), 45.4 (C-5), 49.3 (C-6), 35.1 (C-7), 75.7 (C-8), 158.9 (C-9), 90.3 (C-10), 66.1 (C-11), 113.8 (C-12), 32.5 (C-13), 31.3 (C-14), 23.8 (C-15), 166.4 (C-1′), 127.9 (C-2′), 129.6 (C-3′,7′), 128.5 (C-4′,6′), 148.3 (C-5′), 38.0 (C-8′), 24.2 (C-9′), 13.7 (C-10).

10α-Hydroxy-8β-*O*-cinnamoylcapnellene (9)

$[\alpha]_D^{25}$ +105.6 (*c* 0.05, CH_2Cl_2); UV λ_{max} 280, 305 nm; IR (CH_2Cl_2) ν_{max} 3481 (OH), 1705 (C=O ester), 1636 (double bond), 1457 cm^{-1}; EIMS *m/z* 366; HREIMS 389.2092 *m/z* $[M+Na]^+$ (calc for $C_{24}H_{30}O_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (2H, m, H-2), 1.69 (2H, m, H-3), 1.91 (m, H_a-5), 1.37 (m, H_b-5), 2.63 (m, H-6), 2.44 (m, H_a-7), 1.51 (m, H_b-7), 5.94 (d, *J* = 6.8 Hz, H-8), 1.90 (s, H-11), 5.43 (2H, br s, H_2-12), 1.12 (3H, s, H-13), 1.19 (3H, s, H-14), 1.27 (3H, s, H-15), 6.46 (d, *J* = 16.0 Hz, H-2′), 7.69 (d, *J* = 16.0 Hz, H-3′), 7.53 (2H, m, H-5′,9′), 7.39 (3H, m, H-6′,7′,8′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.1 (C-1), 43.3 (C-2), 42.3 (C-3), 49.8 (C-4), 45.4 (C-5), 49.4 (C-6), 35.0 (C-7), 75.4 (C-8), 158.7 (C-9), 90.2 (C-10), 66.0 (C-11), 113.2 (C-12), 32.5 (C-13), 31.4 (C-14), 23.8 (C-15), 166.7 (C-1′), 113.2 (C-2′), 144.8 (C-3′), 134.3 (C-4′), 128.1 (C-5′,9′), 128.8 (C-6′,8′), 118.4 (C-5′).

10α-Hydroxy-8β-*O*-4-nitrobenzoylcapnellene (10)

$[\alpha]_D^{25}$ +91.2 (*c* 0.05, CH_2Cl_2); UV λ_{max} 255, 276 nm; IR (CH_2Cl_2) ν_{max} 3525 (OH), 1722 (C=O ester), 1607, 1529, 1347 (NO_2) cm^{-1}; EIMS *m/z* 385; HRESIMS *m/z* 408.1784 $[M+Na]^+$ (calc for $C_{22}H_{27}NO_5$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (2H, m, H-2), 1.72 (2H, m, H-3), 1.95 (m, H_a-5), 1.38 (m, H_b-5), 2.66 (m, H-6), 2.50 (m, H_a-7), 1.56 (m, H_b-7), 6.04 (d, *J* = 7.8 Hz, H-8), 1.91 (s, H-11), 5.50 (s, H_a-12), 5.48 (s, H_b-12), 1.11 (3H, s, H-13), 1.19 (3H, s, H-14), 1.26 (3H, s, H-15), 8.18 (2H, d, *J* = 8.5 Hz, H-3′,7′), 8.29 (2H, d, *J* = 8.5 Hz, H-4′,6′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.2 (C-1), 43.2 (C-2), 42.3 (C-3), 49.7 (C-4), 45.5 (C-5), 49.3 (C-6), 35.1 (C-7), 77.1 (C-8), 158.4 (C-9), 90.2 (C-10), 66.1 (C-11), 114.3 (C-12), 32.6 (C-13), 31.3 (C-14), 23.7 (C-15), 164.4 (C-1′), 135.8 (C-2′), 130.6 (C-3′,7′), 123.5 (C-4′,6′), 150.5 (C-5′).

10α-Hydroxy-8β-*O*-4-anisoylcapnellene (11)

$[\alpha]_D^{25}$ +100.3 (*c* 0.05, CH_2Cl_2); UV λ_{max} 255, 273 nm; IR (CH_2Cl_2) ν_{max} 3488 (OH), 1711 (C=O ester), 1606, 1511 (Ar.) cm^{-1}; EIMS *m/z* 370; HRESIMS *m/z* 393.2040 $[M+Na]^+$ (calc for $C_{23}H_{30}O_4$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (2H, m, H-2), 1.71 (2H, m, H-3), 1.92 (m, H_a-5), 1.46 (m, H_b-5), 2.54 (m, H-6), 2.46 (m, H_a-7), 1.54 (m, H_b-7), 6.02 (d, *J* = 7.1 Hz, H-8), 1.91 (s, H-11), 5.47 (s, H_a-12), 5.46 (s, H_b-12), 1.12 (3H, s, H-13), 1.19 (3H, s, H-14), 1.27 (3H, s, H-15), 7.99 (2H, d, *J* = 8.7 Hz, H-3′,7′), 6.92 (2H, d, *J* = 8.7 Hz, H-4′,6′), 3.86 (3H, s, H-8′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 44.1 (C-1), 43.4 (C-2), 42.4 (C-3), 50.0 (C-4), 45.5 (C-5), 49.3 (C-6), 35.1 (C-7), 75.6 (C-8), 163.3 (C-9), 90.2 (C-10), 66.1 (C-11), 113.6 (C-12), 32.5 (C-13), 31.3 (C-14), 23.8 (C-15), 166.0 (C-1′), 122.9 (C-2′), 131.6 (C-3′,7′), 113.7 (C-4′,6′), 159.0 (C-5′), 55.4 (OCH_3).

Cytotoxicity Assay

The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of four human tumor cell lines (Hela, KB, Daoy, and WiDr), respectively. Samples and control standard drugs were prepared at a concentration of **1**, 10, 40, and 100 μg/mL. After seeding 2880 cells/well in a 96-well microtiter plate for 3 h, 20 L of sample or standard agent was placed in each well and incubated at 37 °C for 3 days. The absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at 650 nm. The IC_{50} value was defined, by comparison with the untreated cells, as the concentration of a test sample resulting in 50% reduction of absorbance. Mitomycin C was used as a positive control.

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