

Xenicane-Type Diterpenes with Cytotoxicity from *Xenia florida*Yuan-Bin Cheng,[†] Jiun-Yang Jang,[†] Ashraf Taha Khalil,[†] Yao-Haur Kuo,[‡] and Ya-Ching Shen^{*,†}

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Chromatographic investigation of an acetone extract of the octocoral *Xenia florida* afforded three new xenicane diterpenes, namely, florxenilide A (**1**), florxenilide B (**2**), and florxenilide C (**3**), in addition to seven known xenicane diterpenes and two known cadinene sesquiterpenes. Structures were elucidated through spectroscopic analysis, especially 2D NMR, and chemical derivatization. The absolute configuration of **1** was determined by NOESY, CD, and Mosher's methods. Florxenilides A (**1**) and B (**2**) exhibited cytotoxicity against human colon cancer (WiDr) cells at 4.5 and 3.7 μ M, respectively.

Octocorals have been widely studied, as they produce a huge array of skeletal classes of terpenes with unique substitution patterns and functionalities.¹ *Xenia* (order Alcyonaceae, family Xenidiidae) commonly occurs in clear water of the tropics in the form of small yellowish cylindrical or clavate colonies.² They are a rich source of diterpenoids containing a nine-membered monocarbocyclic ring. The structures of *Xenia* diterpenoids have been divided into three groups: xenicins (containing a dihydropyran-cyclononane skeleton), xeniolides (possessing a δ -lactone-cyclononane skeleton), and xeniaphyllanes (with a bicyclo[7.2.0]undecane skeleton).^{3,4} In our survey of bioactive marine natural products, we have investigated the constituents of marine octocorals inhabiting Taiwan's coral reefs. Three new xenicane derivatives were isolated from *Xenia florida* together with seven known xenicane diterpenes, xeniafaraunol A,⁵ florlide C,⁶ florlide D,⁶ 9-deoxyxeniolide B,⁷ 9-deoxyxeniolide A,⁸ xeniafaraunol B,⁵ and florlide A.⁶ Two cadinene sesquiterpenes, xenitorin A⁹ and xenitorin B,⁹ were identified in the course of chromatographic fractionation. The new metabolites were named florxenilide A (**1**), florxenilide B (**2**), and florxenilide C (**3**). The structure elucidation was based on spectroscopic analysis, especially 2D NMR and chemical derivatization. The isolated compounds were evaluated against human KB, WiDr, and Hepa tumor cell lines for their cytotoxic activity.

The molecular formula $C_{33}H_{40}O_{10}$ was established for **1** by HRESIMS, which showed a quasi-molecular ion peak at m/z 619.2522 $[M + Na]^+$. The IR spectrum displayed absorption bands diagnostic of hydroxyl (3477 cm^{-1}), esters ($1744, 1728, 1711\text{ cm}^{-1}$), double bond (1653 cm^{-1}), and aromatic (1601 cm^{-1}) functionalities. The ^1H NMR spectroscopic data (Tables 1 and 2) indicated the presence of three acetate methyls (δ_{H} 2.10, 2.03, and 2.00) and a benzoyl ester (δ_{H} 8.01, 7.52, and 7.41). The oxymethine signal at δ_{H} 5.85, the olefinic at δ_{H} 6.40 (both br s), and three methyls at δ_{H} 1.73 (6H) and 1.89 (3H) suggested the presence of a xenicane skeleton.^{3,10,11} The proton at δ_{H} 5.85 (H-1) coupled with a proton at δ_{H} 2.46 (H-11a, COSY spectrum) and 3J -correlated with an olefinic CH at δ_{C} 137.2 (C-3) and an acetate carbonyl at δ_{C} 169.7 (HMBC spectrum). The proton at δ_{H} 6.40 (H-3, br s) coupled to a proton at δ_{H} 2.15 (H-4a) and 3J -correlated to a CH at δ_{C} 38.0 (C-4a), confirming the presence of a 1-acetoxy dihydropyran moiety. Furthermore, the COSY spectrum revealed vicinal coupling between H-4a/H-5/H-6, while the methyl proton at δ_{H} 1.89 (H-18) 3J -correlated with the methylene at δ_{C} 40.6 (C-6) and the olefinic CH at δ_{C} 122.3 (C-8), indicating C-7/C-8 unsaturation. The benzoyl carbonyl at δ_{C} 166.0 correlated with the proton at δ_{H} 5.74 (d, $J =$

Table 1. ^1H NMR Data (CDCl_3 , 300 MHz) of Compounds **1–3**^a

	1	2	3
1	5.85 br s	5.97 s	
3	6.40 br s	6.45 s	4.94 d (12.0)
4a	2.15 m	2.16 m	4.35 d (12.0)
5	2.01 m	2.14 m	2.81 m
	4.35 d (12.0)	1.70 m	1.86 m
6	2.30 m	2.35 m	1.85 m
	2.12 m	1.26 m	1.68 m
8	5.19 d (8.7)	3.11 d (9.3)	1.26 m
9	5.74 br d (8.7)	4.79 d (9.3)	3.42 br s
			2.05 m
10	4.37 br s	4.41 s	1.77 m
11a	2.46 br s	2.83 s	2.58 m
12	5.67 d (3.6)	5.66 d (3.6)	2.74 s
13	5.65 dd (9.2, 3.6)	5.62 dd (8.9, 3.6)	6.00 d (11.0)
14	5.28 d (9.2)	5.28 d (8.9)	6.18 dd (15.0, 11.0)
16	1.73 s	1.74 s	5.91 d (15.0)
17	1.73 s	1.77 s	1.34 s
18	1.89 s	1.55 s	1.34 s
19	5.04 s	5.18 s	1.06 s
	4.99 s	5.08 s	1.90 d (13.2)
1-OAc	2.10 s	2.12 s	1.50 d (13.2)
12-OAc	2.00 s	2.03 s	
13-OAc	2.03 s	2.11 s	
OBz			
3',7'	8.01 d (7.5)	8.07 d (7.5)	
4',6'	7.41 t (7.5)	7.47 t (7.5)	
5'	7.52 t (7.5)	7.60 t (7.5)	
OMe			3.23 s

^a J values (in Hz) are cited in parentheses.

8.7 Hz, H-9), which vicinally coupled with two protons at δ_{H} 5.19 (d, $J = 8.7$ Hz, H-8) and 4.37 (br s, H-10), thus indicating that a benzoyloxy group was attached to C-9 and a hydroxyl to C-10. HMBC correlations between H-9 and carbons at δ_{C} 137.5 (s, C-7) and 150.0 (s, C-11) as well as of exomethylene protons (δ_{H} 5.04, 4.99, H-19) with C-11a (CH, δ_{C} 41.9) and C-10 (CH, δ_{C} 81.5) constructed the cyclononane ring with exomethylene at C-11. On the other hand, H-3 was 3J -correlated with an oxymethine at δ_{C} 70.6, which was attached to a proton at δ_{H} 5.67 (d, $J = 3.6$ Hz, H-12), which in turn was coupled to δ_{H} 5.65 (dd, $J = 9.2, 3.6$ Hz, H-13). The chemical shifts of H-12 and H-13 as well as their HMBC correlation to two carbonyls at δ_{C} 169.6 and 170.2 located two acetoxy moieties at C-12 and C-13. Obviously, the olefinic proton at δ_{C} 5.28 (d, $J = 9.2$ Hz, H-14) was coupled to H-13 and attached to a carbon at δ_{C} 117.8 (d, C-14). The two equivalent methyl signals at δ_{H} 1.73 (6H, s) were 2J -correlated to the quaternary olefinic at δ_{C} 140.8 (C-15) and 3J -correlated to H-14 as well as to the methyl

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Table 2. ^{13}C NMR Data (CDCl_3 , 300 MHz) of Compounds 1–3^a

	1	2	3
1	91.8 (CH)	91.2 (CH)	171.4 (qC)
3	137.2 (CH)	138.1 (CH)	70.9 (CH ₂)
4	113.1 (qC)	112.8 (qC)	136.9 (qC)
4a	38.0 (CH)	38.4 (CH)	37.8 (CH)
5	28.7 (CH ₂)	26.0 (CH ₂)	34.0 (CH ₂)
6	40.6 (CH ₂)	40.5 (CH ₂)	37.9 (CH ₂)
7	137.5 (qC)	60.1 (qC)	36.9 (qC)
8	122.3 (CH)	61.6 (CH)	73.8 (CH)
9	78.0 (CH)	80.6 (CH)	27.8 (CH ₂)
10	81.5 (CH)	80.6 (CH)	21.2 (CH ₂)
11	150.0 (qC)	148.0 (qC)	75.7 (qC)
11a	41.9 (CH)	40.6 (CH)	53.9 (CH)
12	70.6 (CH)	70.5 (CH)	127.7 (CH)
13	71.5 (CH)	71.2 (CH)	120.5 (CH)
14	117.8 (CH)	118.0 (CH)	145.0 (CH)
15	140.8 (qC)	141.2 (qC)	70.8 (qC)
16	25.7 (CH ₃)	25.8 (CH ₃)	29.8 (CH ₃)
17	18.7 (CH ₃)	18.8 (CH ₃)	29.7 (CH ₃)
18	18.1 (CH ₃)	17.9 (CH ₃)	30.5 (CH ₃)
19	115.5 (CH ₂)	117.9 (CH ₂)	38.4 (CH ₂)
1-OAc	169.7 (qC)	169.7 (qC)	
	20.8 (CH ₃)	21.1 (CH ₃)	
12-OAc	169.6 (qC)	169.6 (qC)	
	20.8 (CH ₃)	21.0 (CH ₃)	
13-OAc	170.2 (qC)	170.2 (qC)	
	20.9 (CH ₃)	20.9 (CH ₃)	
benzoyl			
CO	166.0 (qC)	166.7 (qC)	
2'	129.9 (qC)	129.8 (qC)	
3',7'	129.4 (CH)	129.6 (CH)	
4',6'	128.3 (CH)	128.5 (CH)	
5'	133.1 (CH)	133.6 (CH)	
OMe			48.5 (CH ₃)

^a Assignments were guided by DEPT, HMQC, and HMBC spectral data.

Table 3. Cytotoxicity of Compounds 1–5 against Human Cancer Cells (ED_{50} , $\mu\text{g/mL}$)^a

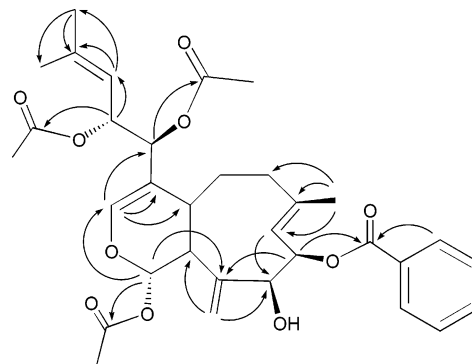
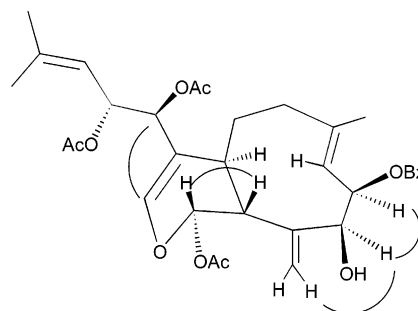
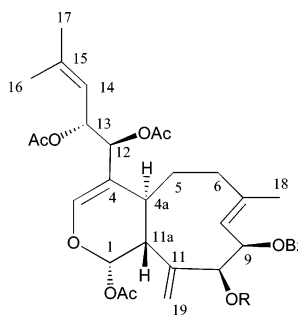
compound	KB ^b	WiDr ^c	Hepa ^d
1	>20	2.73	>20
2	11.4	1.88	8.03
3	>20	>20	>20
4	>20	>20	>20
5	>20	>20	>20
6	>20	>20	>20
mitomycin	0.56	0.35	0.04

^a The concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure according to the method described in the Experimental Section. ^b Oral epidermoid carcinoma. ^c Human colon adenocarcinoma. ^d Human liver carcinoma.

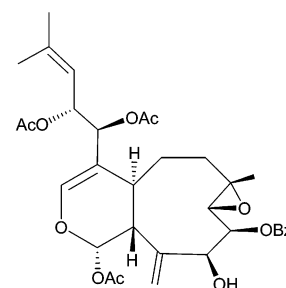
signals at δ_{C} 25.7 and 18.7, confirming the attachment of 2,3-diacetoxy-5-methylhex-4-ene group at C-4.¹²

The coupling constants $J_{12,13}$ and $J_{13,14}$ were identical to the reported values,¹² indicating the same configuration at C-12 and C-13. The NOE interaction between H-8/H-4a indicated the *E*-geometry of the C-7/C-8 double bond. The NOESY correlations between H-11a/H-1; H-18/H β -6, H-9; H-10/H-9, H-19; and H-4a/H-12 suggested the relative stereochemistry of 1 (Figure 2).

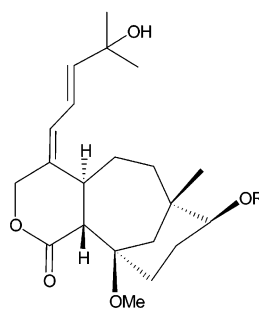
The absolute configuration of 1 was determined by application of Mosher's method.¹³ Compound 1 was converted to (R)- α -methoxy- α -(trifluoromethyl)phenylacetyl (MTPA) ester and (S)-MTPA ester. The ^1H NMR spectrum for each ester was measured, followed by calculation of chemical shift differences ($\Delta\delta = \delta_{\text{S}} - \delta_{\text{R}}$). The obtained differences were $\Delta\delta_{\text{H}} -0.18, +0.07, +0.12$, and $+0.32$ for H-1, H-8, H-9, and H-10, respectively, indicating the *R*-configuration at C-9 and the *S*-configuration at C-10. This was in accordance with the result of the circular dichroism (CD) exciton chirality of the dibenzoate ester (5) of 1, which exhibited a positive Cotton effect at 239 nm. On the basis of the dibenzoate rule, these

**Figure 1.** Selected HMBC correlations of 1.**Figure 2.** Selected NOESY correlations of 1.

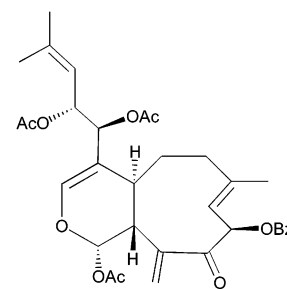
1 R = H
5 R = Bz



2



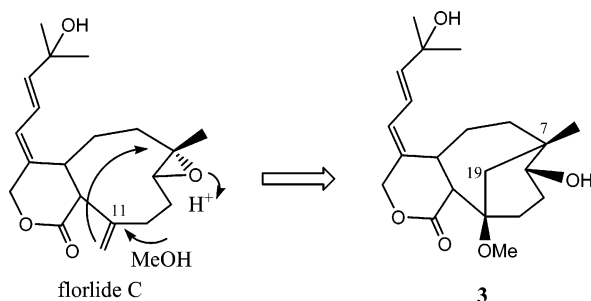
3 R = H
6 R = Ac



4

two benzoyl chromophores are right-handed and clockwise.¹⁴ It is noteworthy that this is the first report of oxy-substitution at both C-9 and C-10, although 9 α -oxy-substitution was previously reported in a few related xenicanes such as xenilactol.¹⁵ The structure of 1 was thus established as florxenilide A.

Compound 2 had the molecular formula $\text{C}_{33}\text{H}_{40}\text{O}_{11}$ as established by HRESIMS (m/z 635.2465, $[\text{M} + \text{Na}]^+$). The spectroscopic data of 2 were similar to those of 1 (Tables 1 and 2), implying the presence of the xenicanane skeleton with an exomethylene at C-11 and three acetoxy and one benzyloxy groups. A significant difference was found in the chemical shifts of C-7 and C-8 (δ_{C}

Scheme 1. Possible Biogenetic Pathway of **3**

60.1 and 61.6, respectively) that replaced the olefinic signals assignable to C-7 and C-8 in **1**. The oxymethine at δ_H 3.11 (d, $J = 9.3$ Hz, H-8), attached to a carbon at δ_C 61.6, revealed HMBC correlations to a quaternary carbon at δ_C 60.1 (C-7) and a methylene at δ_C 40.5 (C-6), whereas H-18 (δ_H 1.55, s) correlated with C-6, C-7, and C-8, indicating the presence of a 7,8-epoxy ring. NOESY correlations were detected between H-1/H-11a; H-8/H-4a; and H-18/H-9. These correlations favored the α -orientation of H-9 and H-10 as well as the β -orientation of H-1.⁸ Compound **2** was therefore identified as florxenilide B.

The HRESIMS of **3** revealed a quasi-molecular ion peak at m/z 387.2146 [$M + Na$]⁺, consistent with the molecular formula $C_{21}H_{32}O_5$ and six degrees of unsaturation. The IR spectrum suggested the presence of hydroxyl, carbonyl, and double bonds, whereas the UV spectrum suggested a conjugated diene system (λ_{max} 244 nm). The ¹H NMR displayed an *E*-diene system at δ_H 6.00 (d, $J = 11.0$ Hz, H-12), 6.18 (dd, $J = 15.0, 11.0$ Hz, H-13), and 5.91 (d, $J = 15.0$ Hz, H-14). The oxymethylene protons at δ_H 4.94 and 4.35 (each d, $J = 12.0$ Hz) were ³*J*-correlated to a carbonyl at δ_C 171.4 (C-1) and an olefinic CH at δ_C 127.7 (C-12). Three methyl singlets were observed at δ_H 1.34 (3H $\times$ 2) and 1.06 (3H) along with isolated AB methylene proton signals at δ_H 1.90 and 1.50 (each d, $J = 13.2$ Hz) with HMBC correlations to a quaternary carbon at δ_C 36.9 (C-7) and 75.7 (C-11) and an oxymethine at δ_C 73.8 (C-8). This suggested the presence of a bicyclic [4.3.1] ring system containing two hydroxyl groups at C-8 and C-11. The NMR data of **3** matched those of floride A except for the presence of a methoxy group at δ_H 3.23 (δ_C 48.5) whose protons correlated to an oxy-quaternary carbon at δ_C 75.7 (C-11). This proved that the hydroxyl group of floride A was substituted by an OCH₃ in **3**, and this was augmented by MS data at m/z 349 [$M - Me$]⁺ (see Experimental Section). Therefore, **3** was identified as floride A-11-*O*-methyl ether or florxenilide C.^{6,16} A possible biogenetic pathway of **3** might be explained by incorporation of a molecule of MeOH to floride C at C-11, as illustrated in Scheme 1.⁶

Chromium oxide oxidation of **1** was performed in anhydrous medium (Collin's reagent)¹⁷ to afford 10-dehydroflorxenilide A (**4**). The spectroscopic data are cited in the Experimental Section. The isolated natural xenicane diterpenes, including the new compounds **1**–**3** together with the new xenicane derivatives **4**–**6**, were tested for cytotoxic activities against three human tumor cell lines. The results revealed that florxenilides A (**1**) and B (**2**) exhibited significant cytotoxicity against human colon cancer cells with IC₅₀ values of 2.7 and 1.9 μ g/mL (4.5, 3.7 μ M), respectively, while florxenilide B (**1**) had a very weak activity against KB and Hepa tumor cells at 11.4 and 8.0 μ g/mL, respectively. The derivatives **4**–**6** were all inactive (>20 μ g/mL) against the three tumor cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. The CD spectrum was recorded on a Jasco J-715 CD polarimeter. The ¹H and ¹³C NMR, COSY, HMQC, HMBC, and NOESY spectra were

recorded on a Bruker FT-300 spectrometer or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ¹H and 125 MHz for ¹³C, respectively, using TMS as internal standard. The chemical shifts are given in δ values (ppm) and coupling constants in Hz. Low-resolution EIMS and FABMS were recorded on a VG Quattro 5022 mass spectrometer, and HREIMS were measured on a JEOL JMS-SX 102 spectrometer. Silica gel 60 (Merck) was used for column chromatography (CC), and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used. *S*-(+)- α -Methoxy- α -trifluoromethylphenyl acetyl chloride and *R*-(-)- α -methoxy- α -trifluoromethylphenyl acetyl chloride (MTPA esters) were obtained from ACROS Organics (NJ). Chromium oxide was obtained from Showa Chemicals Co. LTD (Japan).

Animal Material. The soft coral *Xenia florida* was collected from Green Island, off Taiwan, in December 2003, at a depth of 20 m, and immediately stored in a freezer. A voucher specimen (GSCII-2) is deposited at the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. The wet organism (1400 g) was extracted with acetone three times using a stirrer. The acetone extract was evaporated under vacuum and then partitioned between EtOAc–H₂O (1:1) to give a crude extract (29 g). The extract was chromatographed on a silica gel column using a gradient of *n*-hexane–EtOAc to give 18 fractions. Fraction F₃ (207 mg) was chromatographed on a silica gel column using a gradient of *n*-hexane–EtOAc to furnish xenitorin A (70 mg), xenitorin B (37 mg), and xenifloride A (8 mg). A similar separation of F₄ (280 mg) afforded an additional amount of xenifloride A (37 mg). Separation of F₅ (900 mg) on Sephadex LH-20, eluting with MeOH, followed by column chromatography on silica gel using *n*-hexane–EtOAc (10:1 to 1:2) yielded **1** (166 mg) and a mixture that gave **2** (2 mg) after NP-HPLC purification [*n*-hexane–EtOAc (2:1)]. Fraction F₇ (354 mg) was subjected to NP-HPLC using *n*-hexane–EtOAc (3:1) followed by RP-HPLC using MeOH–H₂O (4:1) to give floride C (8 mg) and floride D (9 mg). Fraction F₁₀ (179 mg) was chromatographed on NP-HPLC, eluting with *n*-hexane–EtOAc (2:1), to give 9-deoxyxenilide B (11 mg). Fraction F₁₁ (982 mg) was chromatographed on a silica gel column using a gradient of *n*-hexane–acetone to give eight subfractions (F₁₁-A to F₁₁-H). Subfraction F₁₁-C (191 mg) was separated using NP-HPLC, eluting with *n*-hexane–EtOAc (2:1), to yield 9-deoxyxenilide A (16 mg) and xeniafarunol B (18.3 mg). Fraction F₁₇ (371 mg) was separated on NP-HPLC, eluting with *n*-hexane–EtOAc (2:1), to give floride A (64 mg) and a mixture that was further purified using RP-HPLC, eluting with MeOH–H₂O–CH₃CN (10:10:1), to yield **3** (13 mg). Fraction F₁₈ (240 mg) was fractionated on a silica gel column using CH₂Cl₂–MeOH (20:1 to 10:1) for elution to furnish four subfractions (F₁₈-A to F₁₈-D). Subfraction F₁₈-B (143 mg) was further separated using RP-HPLC, eluting with MeOH–H₂O (1:1), to yield additional amounts of floride A (15 mg) and **3** (12 mg).

Florxenilide A (1): [α]_D²⁵ +8 (c 0.1, CH₂Cl₂); UV λ_{max} (log ϵ) 228 (3.16) nm; IR (CH₂Cl₂) ν_{max} 3477 (OH), 2936 (CH), 1744, 1728, 1711 (C=O esters), 1653 (double bond), 1601 and 1490 (Ar CH), 1260, 1231 (C–O), 1023, 947, 714 cm^{–1}; ¹H NMR (300 MHz, CDCl₃), see Table 1; ¹³C NMR (75 MHz, CDCl₃), see Table 2; ESIMS m/z 619 [$M + Na$]⁺; HRESIMS m/z 619.2522 (calcd for C₃₃H₄₀O₁₀Na, 619.2519).

Preparation of (R)- and (S)-MTPA Esters of Florxenilide A (1). *S*-(+)- or *R*-(-)-MTPA chloride (one drop) was added to a solution of **1** (5 mg in 0.5 mL of pyridine), and the solution was allowed to stand at room temperature for 7 h. After purification using preparative thin-layer chromatography, the ester (4.8 mg, 96% yield) was submitted to ¹H NMR analysis, and $\Delta\delta$ values ($\delta_S - \delta_R$) were calculated for H-1, H-8, H-9, and H-10.

Benzoylation of Florxenilide A (1). Thirty milligrams of **1** was treated with benzoyl chloride (0.1 mL) and pyridine (2 mL) at 50 °C for 15 h. Usual workup and extraction with EtOAc gave a residue. Purification of the product by LH-20 using MeOH as eluent gave 27 mg of florxenilide A-10-*O*-benzoate (**5**), which showed the following: [α]_D²⁵ +0.8 (c 2.5, CH₂Cl₂); UV (MeOH) λ_{max} (log ϵ) 231 (0.79), 215 (0.49) nm; CD λ_{max} (Mol. CD) 224 (–4.241), 239 (+4.438) nm; IR (CH₂Cl₂) ν_{max} 3061, 2931 (CH), 1737, 1730, 1715 (C=O esters), 1673, 1650 (C=C), 1601, 1484 (Ar CH), 1245 (C–O), 1025, 947, 711 cm^{–1}; ¹H NMR (CDCl₃, 300 MHz) δ 8.06 (2H, d, 3',7'-Bz), 7.98 (2H, d, 3'',7''-Bz), 7.57 (2H, m, 5', 5''-Bz), 7.46 (2H, t, 4',6'-Bz), 7.43 (2H, t, 4'',6''-Bz), 6.44 (s, H-3), 5.96 (d, $J = 8.6$ Hz, H-9), 5.78 (s, H-1), 5.74

(s, H-10), 5.69 (d, $J = 3.6$ Hz, H-12), 5.65 (dd, $J = 10.0, 3.6$ Hz, H-13), 5.41 (s, H-19a), 5.28 (d, $J = 10.0$ Hz, H-14), 5.26 (d, $J = 8.6$ Hz, H-8), 5.21 (s, H-19b), 2.49 (br s, H-11a), 2.38 (br d, $J = 12.2$ Hz, H₂-6), 2.10 (m, H₂-5), 2.10 (3H, s), 2.08 (m, H-4a), 2.04 (3H, s), 1.97 (s, H-18), 1.97 (3H, s), 1.79, 1.76 (s, H-16 and H-17); ^{13}C NMR (CDCl_3 , 75 MHz) δ 170.2 (C, CH_3CO), 169.8 (C, CH_3CO), 168.9 (C, CH_3CO), 165.2 (C, CO Bz), 164.6 (C, CO Bz), 146.3 (C, C-11), 141.1 (C, C-15), 137.9 (CH, C-3), 137.8 (C, C-7), 133.3 (CH, C-5 Bz), 133.1 (CH, C-5 Bz), 130.3 (C, C-2 Bz), 129.9 (CH, C-3 Bz), 129.6 (C, C-2 Bz), 128.4 (CH, C-4 Bz), 128.3 (CH, C-4 Bz), 122.8 (CH, C-8), 118.5 (CH₂, C-19), 117.8 (CH, C-14), 113.1 (C, C-4), 91.3 (CH, C-1), 82.0 (CH, C-10), 74.8 (CH, C-9), 71.5 (CH, C-13), 70.6 (CH, C-12), 43.6 (CH, C-11a), 38.4 (CH, C-4a), 29.0 (CH₂, C-5), 25.8 (CH₃, C-17), 20.9 (CH₃, CH_3CO), 20.8 (CH₃, CH_3CO), 20.6 (CH₃, CH_3CO), 18.6 (CH₃, C-16), 18.3 (CH₃, C-18).

Florfenilide B (2): $[\alpha]_D^{25} +114$ (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 228 (3.15) nm; IR (CH_2Cl_2) ν_{max} 3523 (OH), 2927 (CH), 1746, 1640, 1731 (C=O esters), 1228, 1025, 713 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz), see Table 1; ^{13}C NMR (CDCl_3 , 75 MHz), see Table 2; EIMS (30 eV) m/z 546, 490, 475, 398, 295, 277, 127, 105, 85, 77; ESIMS m/z 635 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 635.2465 (calcd for $\text{C}_{33}\text{H}_{40}\text{O}_{11}\text{Na}$, 635.2468).

Florfenilide C (3): $[\alpha]_D^{25} +204$ (c 0.02, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 244 (3.72) nm; IR (CH_2Cl_2) ν_{max} 3440 (OH), 3053 (C—H), 2930, 2931 (C—H), 1742 (C=O), 1655 (C=C), 1026, 735 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz), see Table 1; ^{13}C NMR (CDCl_3 , 75 MHz), see Table 2; EIMS m/z 364 $[\text{M}]^+$, 349 $[\text{M} - \text{Me}]^+$, 346 $[\text{M} - \text{H}_2\text{O}]^+$, 141, 123, 105, 91, 79; FABMS m/z 387 $[\text{M} + \text{Na}]^+$, 365 $[\text{M} + \text{H}]^+$; HRESIMS m/z 387.2146 (calcd for $\text{C}_{21}\text{H}_{32}\text{O}_5\text{Na}$, 387.2147).

Florfenilide C monoacetate (6): $[\alpha]_D^{25} +102$ (c 0.02, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3481 (OH), 2968, 2931 (C—H), 1746 (C=O), 1731 (C=O ester), 1653 (C=C), 1250 (C—O acetate), 1026, 733 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 6.17 (dd, $J = 15.0, 11.0$ Hz, H-13), 6.02 (d, $J = 11.0$ Hz, H-12), 5.91 (d, $J = 15.0$ Hz, H-14), 4.94 (d, $J = 12.0$ Hz, H-3a), 4.67 (br s, H-8), 4.37 (d, $J = 12.0$ Hz, H-3a), 3.24 (s, OMe), 2.83 (m, H-4a), 2.78 (br s, H-11a), 2.11 (s, OAc), 1.35 (s, H-16 and H-17), 0.99 (s, H-18); ESIMS m/z 429 $[\text{M} + \text{Na}]^+$.

Oxidation of 1 with Collin's Reagent $[(\text{C}_5\text{H}_5\text{N})_2\text{CrO}_3]$. Two drops of chromium trioxide (40 mg in 3 mL of pyridine) were added to a solution of **1** (20 mg in 2 mL of CH_2Cl_2), and the reaction mixture was stirred at RT for 30 min. After filtration to remove CrO_3 , the filtrate was concentrated and purified by NP-HPLC using n -hexane— CH_2Cl_2 —MeOH (20:15:1) for elution to provide 10-dehydroflorfenilide A (**4**, 5 mg): $[\alpha]_D^{25} +101.7$ (c 0.12, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 230 (3.11) nm; IR (CH_2Cl_2) ν_{max} 3060 (C=C—H), 2924 (CH), 1754, 1746, 1731, 1714 (C=O), 1681, 1601 (Ar C—H), 1271, 1226 (C—O), 1024, 948, 712 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz), δ 8.12 (2H, d, $J = 7.8$ Hz, 3',7'-Bz), 7.58 (t, $J = 7.8$ Hz, 5'-Bz), 7.46 (2H, t, $J = 7.8$ Hz, 4',6'-Bz), 6.40 (s, H-3), 6.24 (d, $J = 6.5$ Hz, H-9), 6.12 (br s, H-1), 5.57 (s, H-19), 5.52 (dd, $J = 9.4, 3.5$ Hz, H-13), 5.49 (d, $J = 3.5$ Hz, H-12), 5.44 (s, H-19), 5.42 (d, $J = 6.5$ Hz, H-8), 5.17 (d, $J = 9.4$ Hz, H-14), 2.25 (m, H-4a), 2.11 (s, OAc), 2.01 (s, OAc), 1.75 (3H, s, H-18), 1.67 (s, OAc), 1.26 (3H, s, H-16 & H-17); ^{13}C NMR (CDCl_3 , 75 MHz), δ 196.5 (C, C-10), 170.3, 169.8, 169.0 (each C, 3 $\times$ CH_3CO), 165.7 (C, C=O Bz), 147.4 (C, C-11), 141.4 (C, C-15), 138.2 (C, C-7), 138.1 (CH, C-3), 133.2 (CH, 5'-Bz), 131.7 (C, 2'-Bz), 129.9 (CH, 3',7'-Bz),

128.4 (CH, 4',6'-Bz), 125.5 (CH, C-8), 117.4 (CH₂, C-19), 116.1 (CH, C-14), 113.4 (C, C-4), 91.2 (CH, C-1), 77.2 (CH, C-9), 71.5 (CH, C-13), 71.1 (CH, C-12), 42.3 (CH, C-11a), 41.4 (CH₂, C-6), 37.7 (CH, C-4a), 28.1 (CH₂, C-5), 25.8 (CH₃, C-17), 21.1, 21.1, 21.0 (each CH₃, 3 $\times$ CH_3CO), 18.8 (CH₃, C-18), 18.2 (CH₃, C-16).

Cytotoxicity Assay. The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of three human tumor cell lines (KB, WiDr, and Hepa), respectively. Samples and control standard drugs were prepared at a concentration of 1, 10, 40, and 100 g/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 ED₅₀ value was defined, by comparison with the untreated cells, as the concentration of a test sample resulting in 50% reduction of absorbance. Mitomycin was used as a positive control.

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