Xenicane-Type Diterpenes with Cytotoxicity from *Xenia florida*

Yuan-Bin Cheng,[†] Jiun-Yang Jang,[†] Ashraf Taha Khalil,[†] Yao-Haur Kuo,[‡] and Ya-Ching Shen*[,†]

Institute of Marine Resources, National Sun Yat-sen University, 70 Lien-Hai Road, Kaohsiung, Taiwan 80424, Republic of China, and National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China

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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 μM, 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 Xeniidae) 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 A[9] 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 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 = 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

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)
			4.35 d (12.0)
4a	2.15 m	2.16 m	2.81 m
5	2.01 m	2.14 m	1.86 m
	4.35 d (12.0)	1.70 m	1.85 m
6	2.30 m	2.35 m	1.68 m
	2.12 m	1.26 m	1.26 m
8	5.19 d (8.7)	3.11 d (9.3)	3.42 br s
9	5.74 br d (8.7)	4.79 d (9.3)	2.05 m
			1.77 m
10	4.37 br s	4.41 s	2.58 m
11a	2.46 br s	2.83 s	2.74 s
12	5.67 d (3.6)	5.66 d (3.6)	6.00 d (11.0)
13	5.65 dd (9.2, 3.6)	5.62 dd (8.9, 3.6)	6.18 dd (15.0, 11.0)
14	5.28 d (9.2)	5.28 d (8.9)	5.91 d (15.0)
16	1.73 s	1.74 s	1.34 s
17	1.73 s	1.77 s	1.34 s
18	1.89 s	1.55 s	1.06 s
19	5.04 s	5.18 s	1.90 d (13.2)
	4.99 s	5.08 s	1.50 d (13.2)
1-OAc	2.10 s	2.12 s	
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.

* To whom correspondence should be addressed. Tel: 886-7-525-2000, ext. 5058. Fax: 886-7-525-5020. E-mail: ycshen@mail.nsysu.edu.tw.
[†] National Sun Yat-Sen University.
[‡] National Research Institute of Chinese Medicine.

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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}, μg/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 ($\Delta\delta = \delta_S - \delta_R$). The obtained differences were $\Delta\delta_H$ −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

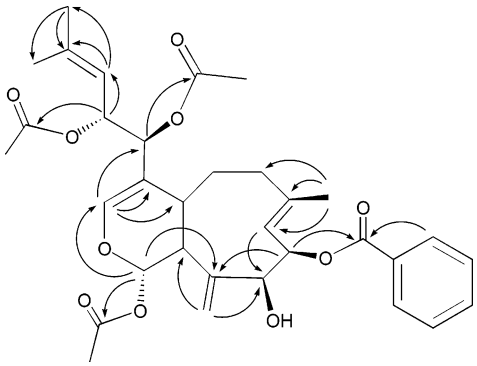


Figure 1. Selected HMBC correlations of **1**.

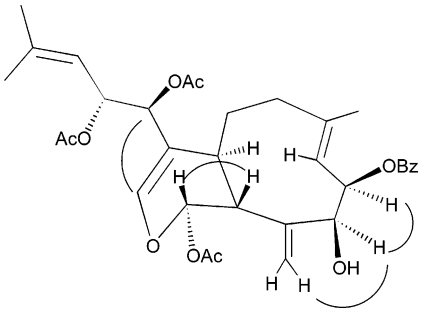
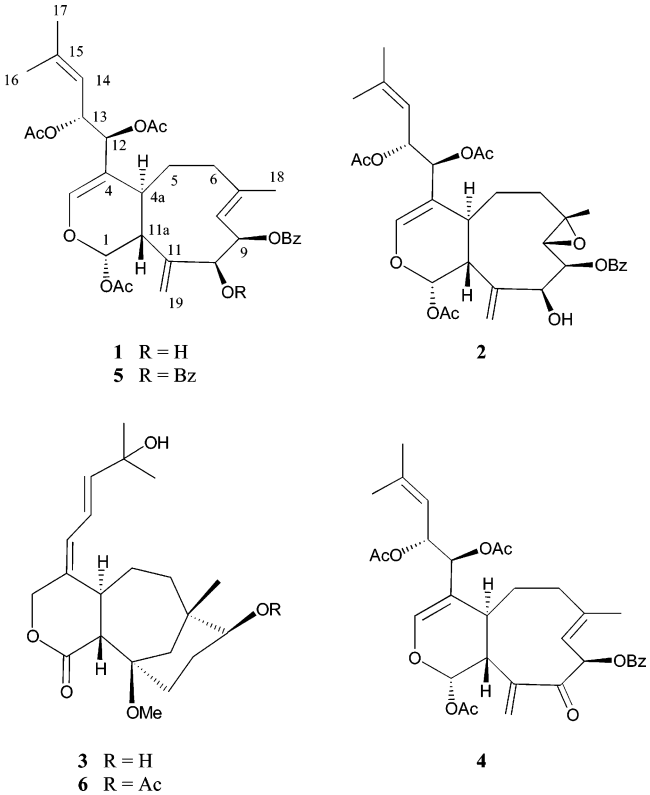


Figure 2. Selected NOESY correlations of **1**.



1 R = H
5 R = Bz

2

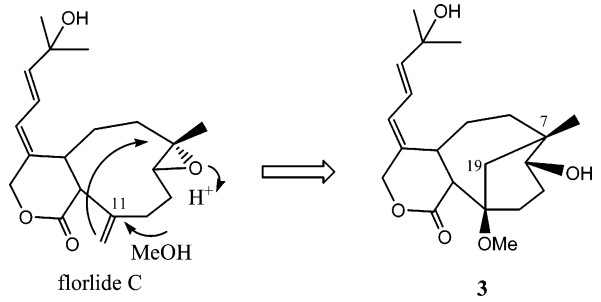
3 R = H
6 R = Ac

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 9α-oxy-substitution was previously reported in a few related xenicanes such as xenialactol.[15] The structure of **1** was thus established as florxenilide A.

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

Scheme 1. Pausible 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 ^{1}H 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 ^3J-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 × 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 florlide 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 florlide A was substituted by an OCH_3 in **3**, and this was augmented by MS data at m/z 349 $[M - Me]^+$ (see Experimental Section). Therefore, **3** was identified as florlide 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 IC_{50} values of 2.7 and 1.9 μg/mL (4.5, 3.7 μM), respectively, while florxenilide B (**1**) had a very weak activity against KB and Hepa tumor cells at 11.4 and 8.0 μg/mL, respectively. The derivatives **4**−**6** were all inactive (>20 μg/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 ^{1}H and ^{13}C 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 ^{1}H and 125 MHz for ^{13}C, 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−H_2O (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 F_3 (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 xeniflorlide A (8 mg). A similar separation of F_4 (280 mg) afforded an additional amount of xeniflorlide A (37 mg). Separation of F_5 (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 F_7 (354 mg) was subjected to NP-HPLC using *n*-hexane−EtOAc (3:1) followed by RP-HPLC using MeOH−H_2O (4:1) to give florlide C (8 mg) and florlide D (9 mg). Fraction F_{10} (179 mg) was chromatographed on NP-HPLC, eluting with *n*-hexane−EtOAc (2:1), to give 9-deoxyxeniolide B (11 mg). Fraction F_{11} (982 mg) was chromatographed on a silica gel column using a gradient of *n*-hexane−acetone to give eight subfractions (F_{11}-A to F_{11}-H). Subfraction F_{11}-C (191 mg) was separated using NP-HPLC, eluting with *n*-hexane−EtOAc (2:1), to yield 9-deoxyxeniolide A (16 mg) and xeniafarunol B (18.3 mg). Fraction F_{17} (371 mg) was separated on NP-HPLC, eluting with *n*-hexane−EtOAc (2:1), to give florlide A (64 mg) and a mixture that was further purified using RP-HPLC, eluting with MeOH−H_2O−CH_3-CN (10:10:1), to yield **3** (13 mg). Fraction F_{18} (240 mg) was fractionated on a silica gel column using CH_2Cl_2−MeOH (20:1 to 10:1) for elution to furnish four subfractions (F_{18}-A to F_{18}-D). Subfraction F_{18}-B (143 mg) was further separated using RP-HPLC, eluting with MeOH−H_2O (1:1), to yield additional amounts of florlide A (15 mg) and **3** (12 mg).

Florxenilide A (1): $[\alpha]^{25}_D$ +8 (*c* 0.1, CH_2Cl_2); UV λ_{max} (log ϵ) 228 (3.16) nm; IR (CH_2Cl_2) 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}; ^{1}H NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 2; ESIMS m/z 619 $[M + Na]^+$; HRESIMS m/z 619.2522 (calcd for $C_{33}H_{40}O_{10}Na$, 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 ^{1}H 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: $[\alpha]^{25}_D$ +0.8 (*c* 2.5, CH_2Cl_2); 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 (CH_2Cl_2) 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}; ^{1}H NMR ($CDCl_3$, 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, H_2-6), 2.10 (m, H_2-5), 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 (CH_2, 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 (CH_2, C-5), 25.8 (CH_3, C-17), 20.9 (CH_3, CH_3-CO), 20.8 (CH_3, CH_3CO), 20.6 (CH_3, CH_3CO), 18.6 (CH_3, C-16), 18.3 (CH_3, C-18).

Florxenilide B (2): $[\alpha]^{25}_D$ +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}; ^{1}H 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 $[M + Na]^+$; HRESIMS m/z 635.2465 (calcd for $C_{33}H_{40}O_{11}Na$, 635.2468).

Florxenilide C (3): $[\alpha]^{25}_D$ +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=C−H), 2930, 2931 (C−H), 1742 (C=O), 1655 (C=C), 1026, 735 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; EIMS m/z 364 $[M]^+$, 349 $[M - Me]^+$, 346 $[M - H_2O]^+$, 141, 123, 105, 91, 79; FABMS m/z 387 $[M + Na]^+$, 365 $[M + H]^+$; HRESIMS m/z 387.2146 (calcd for $C_{21}H_{32}O_5Na$, 387.2147).

Florxenilide C monoacetate (6): $[\alpha]^{25}_D$ +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}; ^{1}H 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 $[M + Na]^+$.

Oxidation of 1 with Collin's Reagent [$(C_5H_5N)_2CrO_3$]. 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-dehydroflorxenilide A (**4**, 5 mg): $[\alpha]^{25}_D$ +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}; ^{1}H 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 × 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 (CH_2, 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 (CH_2, C-6), 37.7 (CH, C-4a), 28.1 (CH_2, C-5), 25.8 (CH_3, C-17), 21.1, 21.1, 21.0 (each CH_3, 3x CH_3-CO), 18.8 (CH_3, C-18), 18.2 (CH_3, 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 ED_{50} 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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References and Notes

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2005**, *22*, 15−61, and references therein.
(2) Fabricius, K.; Alderslade, P. *Soft Corals and Sea Fans*; Australian Institute of Marine Science: Townsville, MC, 2001; p 138.
(3) Kashman, Y.; Groweiss, A. *J. Org. Chem.* **1980**, *45*, 3814−3824.
(4) Iwagawa, T.; Amano, Y.; Nakatani, M.; Hase, T. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1309−1312.
(5) Kashman, Y.; Saltoun, M.; Rudi, A.; Benayahu, Y. *Tetrahedron Lett.* **1994**, *35*, 8855−8858.
(6) Iwagawa, T.; Kawasaki, J.; Hase, T. *J. Nat. Prod.* **1998**, *61*, 1513−1515.
(7) Vervoort, H. C.; Fenical, W. *Nat. Prod. Lett.* **1995**, *6*, 49−55.
(8) Rho, J. R.; Oh, M. S.; Jang, K. H.; Cho, K. W.; Shin, J. *J. Nat. Prod.* **2001**, *64*, 540−543.
(9) Duh, C. Y.; Chien, S. C.; Song, P. Y.; Wang, S. K.; El-Gamal, A. A.; Dai, C. F. *J. Nat. Prod.* **2002**, *65*, 1853−1856.
(10) Almourabit, A.; Gillet, B.; Ahond, A.; Beloeil, J.-C.; Poupat, C.; Potier, P. *J. Nat. Prod.* **1989**, *52*, 1080−1087.
(11) Hooper, G. J.; Davies-Coleman, M. T. *Tetrahedron* **1995**, *51*, 9973−9984.
(12) Almourabit, A.; Ahond, A.; Chiaroni, A.; Poupat, C.; Riche, C.; Potier, P. *J. Nat. Prod.* **1988**, *51*, 282−292.
(13) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(14) Harada, N.; Nakanishi, K. Circular Dichroic Spectroscopy. *Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983; pp 15−17 and 79−80.
(15) Hiyaoka, H.; Mitome, H.; Nakano, M.; Yamada, Y. *Tetrahedron* **2000**, *56*, 7737−7740.
(16) Iwagawa, T.; Nakamura, K.; Hirose, T.; Okamura, H.; Nakatani, M. *J. Nat. Prod.* **2000**, *63*, 468−472.
(17) Carey, F. A. *Organic Chemistry*; McGraw-Hill: New York, 1992; p 613.

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