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Full Papers

Cytotoxic Diterpenoids from the Hybrid Soft Coral *Sinularia maxima* × *Sinularia polydactyla*

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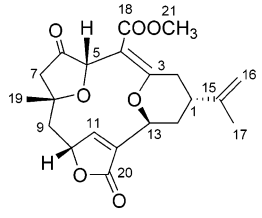
Chemical investigation of the hybrid soft coral *Sinularia maxima* × *Sinularia polydactyla* yielded four new cembranolide diterpenes (**1**−**4**), the norcembranoid 5-episinuleptolide (**5**), and two known sesquiterpenes. The structures and configurations of the new compounds were determined by spectroscopic techniques and comparison of NMR data with those of related metabolites. Compound **3** shows strong cytotoxicity on the breast cancer SK-BR3 cell line and cervix cancer HeLa and HeLa-Apl cell lines with GI_{50} values of 0.039, 0.48, and 0.56 μM, respectively.

Soft corals, particularly those of the genus *Sinularia*, have been recognized as rich sources of terpenoids possessing a wide range of biological activities.[1−4] Of the diterpenes from the genus *Sinularia*, most are derived from the 14-membered cembrane nucleus.[2] Pukalide, the first cembranoid from the marine environment, was reported from *S. abrupta*.[5] Several bioactive compounds with related carbon skeletons were obtained from other *Sinularia* species.[2]

Hybridization is defined as "successful mating in nature between individuals from two populations, or groups of populations, that are distinguishable on the basis of one or more heritable characters".[6] It is widespread in many natural plant populations. Although hybridization is known to occur in the ocean, little attention has been paid to this phenomenon in the marine system.[7] DNA sequence data were used to show that the coral *Acropora prolifera* in the Caribbean is in fact an F_1 hybrid of *A. cervicornis* and *A. palmata*.[8] Hybridization represents a new source of molecular and structural diversity, and it was shown that hybridization results in progeny that differ quantitatively and qualitatively from the parents in the expression of secondary metabolites.[9] In a continuation of our investigation of the hybrid soft coral *Sinularia maxima* × *Sinularia polydactyla*,[10] we now report the structure and cytotoxicity of four new cembranoid diterpenes (**1**−**4**), as well as the presence of the known norcembranoid 5-episinuleptolide (**5**)[11] and two known sesquiterpenes.



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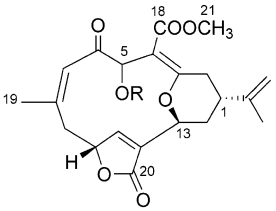
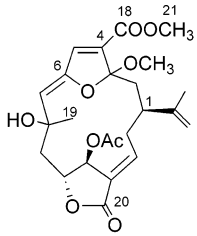


R= 23CH3 — CHCH2CH3 (22, 24, 25)

2- 5*R* or *S*
3- 5*R* or *S*



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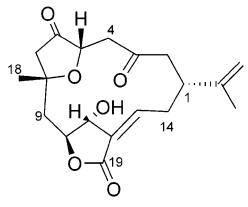

5 5-episinuleptolide

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **5**

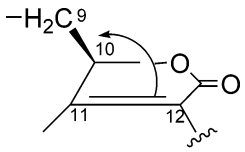
	5-episinuleptolide,[a] **5**		compound **1**[b]			
position	δ_C	δ_H (mult., *J*)[c]	δ_C	δ_H (mult., *J*)	HMBC	COSY
1	39.2	2.52 (m)	36.3	3.43 (m)		$H14_b$, $H2_b$
2	44.1	2.47 (m)	32.9	H_a: 3.69 (m)	1, 15	$H2_b$
		2.63 (dd 15, 2.4)		H_b: 2.35 (m)	14, 15	
3	206.5		171.6			
4	45.4	2.44 (d 15)	111.7			
		2.71 (m)				
5	75.4	4.15 (dd 10.2, 2.4)	75.7	4.82 (s)	3, 6, 8, 18	
6	216.3		211.9			
7	51.5	2.41 (d 18)	48.5	H_a: 2.63 (bd, 22.0)	8, 19	$H7_b$
		2.51 (m)		H_b: 2.30 (d, 22.0)	8, 19	$H7_a$
8	79.4		79.9			
9	42.6	2.33 (dd 15, 7.2)	41.7	H_a: 2.73 (dd, 19, 3)	10, 11	
		2.07 (dd 15, 2.4)		H_b: 2.27 (dd, 19, 4.5)		
10	83.8	4.40 (d 7.2)	79.2	5.16 (brs)	8, 12, 20	H11
11	74.9	4.43 (d 4)	152.3	7.55 (s)	10, 12, 13, 20	H10
12	133.6		134.2			
13	142.7	6.25 (dd 11, 3)	74.7	5.29 (dd, 10, 4)	1, 3, 11, 12, 14	$H14_a$, $H14_b$
14	27.5	2.02 (brd 15.6)	28.8	H_a: 2.38 (m)	1	H13
		3.55 (ddd 15.6, 11, 6)		H_b: 2.12 (m)		H13
15	148.0		147.2			
16	110.8	4.80 (brs)	110.6	H_a: 4.75 (s)	1, 15, 17	H17
		4.82 (brs)		H_b: 4.82 (s)		
17	22.5	1.75 (brs)	21.5	1.77 (s)	1, 15	
18	26.6	1.28 (s)	167.7			
19	169.3		28.4	1.32 (s)	8	
20			171.6			
21			51.1	3.68 (s)	18	

[a] Spectra recorded at 600 MHz in DMSO. [b]Spectra recorded at 400 MHz in $CDCl_3$. [c] Chemical shifts are in parts per million relative to TMS.

Results and Discussion

The organism *S. maxima* × *S. polydactyla* was exhaustively extracted with 1:1 MeOH−CH_2Cl_2. The concentrated crude extract was subjected to vacuum liquid chromatography using a hexanes−EtOAc−MeOH gradient followed by further chromatographic purifications to afford the known 5-episinuleptolide (**5**) as the major constituent (0.6%) of the extract. New cembranes (**1**−**4**) and two known furanosesquiterpenes were obtained after routine application of Si gel chromatography and reversed-phase HPLC chromatography.

Compound **1** was obtained as a colorless oil. Its HREIMS showed an $[M + H]^+$ ion at *m/z* 389.1613. When considered in conjunction with ^{1}H and ^{13}C NMR data, this indicated a molecular formula of $C_{21}H_{24}O_7$ with 10 sites of unsaturation. The IR spectrum indicated the presence of α,*β*-unsaturated *γ*-lactone (1756 cm^{-1}) and ketone (1703 cm^{-1}) functionalities, while the UV absorption at 285 nm confirmed the presence of an enone system. The ^{13}C NMR spectrum in $CDCl_3$ showed resonances for 21 carbons differentiated by DEPT into three methyl, five methylene, five methine, and eight quaternary carbons. The spectrum revealed the presence of three oxymethine functionalities at *δ* 75.7, 74.7, and 79.2, one oxygenated quaternary carbon at *δ* 79.9, and a ketone carbonyl at *δ* 211.9. The carbon resonances at *δ* 152.3, 134.2, and 171.6 were assigned to the α,*β*-unsaturated *γ*-lactone functionality, which was further supported by the deshielded olefinic proton at *δ* 7.55 (s). The methyl resonance at *δ* 1.77 and the olefinic methylene protons at *δ* 4.75 (s) and 4.82 (s) were ascribed to the isopropenyl group residing at C-1 according to HMBC data. Comparison of the above data with those of similar metabolites, including 5-episinuleptolide (**5**), strongly indicated a cembranoid molecular framework possessing the rare 3,13-epoxy-bridged carbocycle, which was confirmed via HMBC correlation of H-13 and C-3 (*δ* 171.6). The position of the methyl ester group at C-4 was established through ^{1}H/^{13}C three-bond correlation between H-5 (*δ* 4.82) and the C-18 carbonyl carbon. The different structural moieties were connected using extensive HMBC and COSY correlations (Table 1). Known cembranoids possessing a 3,13-bridged skeleton are restricted to the Caribbean sea feather, *Pseudopterogorgia bipinnata*.[12]



M-helicity

Figure 1. Correlation of the n → *π** and *π* → *π** Cotton effects with absolute configuration in compounds **1**, **2**, and **3**.

Definition of the absolute configuration of compound **1** at C-10 was done via the circular dichroic method developed for the determination of the absolute configuration of 5-substituted 2(*5H*)-furanones.[13,14] The CD spectrum is completely dominated by Cotton effects due to the electronic transitions of the 2(*5H*)-furanone moiety (i.e., high-amplitude positive and negative Cotton effects for the *π* → *π** and n → *π** transitions at 228.4 and 253.4 nm, respectively). These Cotton effects $\{[\theta]_{253.4} +1.48 \times 10^4, [\theta]_{228.4} -4.8 \times 10^4\}$ indicate a left-handed (*M*) helicity of the five-membered α,*β*-unsaturated lactone moiety (Figure 1) and hence *R* absolute configuration at C-10. Owing to the presence of the three oxygen heterocyclic moieties, the macrocycle in compound **1** is sufficiently rigid in conformational terms to permit firm configurational conclusions based on observed NOE associations. An NOE correlation between H-10 and H-9*β* and the C-19 methyl protons indicates an 8*R* absolute configuration. An NOE correlation between the C-19 methyl protons and H-5 indicates their *cis*-cofacial relationship and hence a 5*S* absolute configuration. An NOE correlation between H-13 and both H-14α and H-14*β* and between H-14α and H-2α (1,3 diaxial) imply a chair conformation of the six-membered pyran ring in which both H-13 and the C-1 isopropenyl group are equatorially positioned. On the basis of the 1*R* absolute configuration of 5-episinuleptolide **5**, this indicates a 1*R*,13*S* absolute configuration for compound **1**.

Compounds **2** and **3** were first isolated as a mixture of two closely related compounds, analyzing for $C_{25}H_{33}O_7$ {*m/z* 445.22208 $[M + H]^+$} by high-resolution ESIMS. The 500 MHz ^{1}H NMR spectrum displayed duplication of four signals in both $CDCl_3$ and

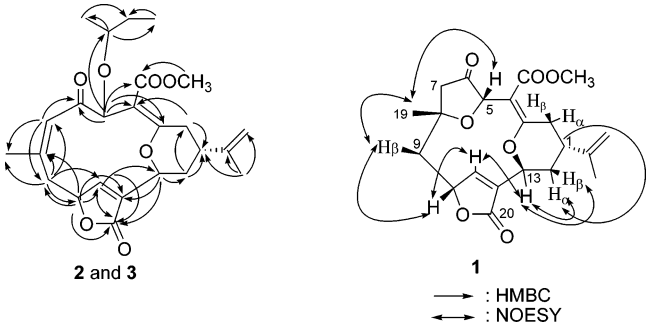


Figure 2. NOESY correlations of compound **1** and HMBC correlations of compounds **2** and **3**.

C_6D_6, which may be attributed to the existence of either two stereoisomers or two rotamers. Additional chromatographic purification resulted in the separation of the mixture into two diastereomeric components (**2** and **3**). The ^{13}C NMR spectrum of **2**, supported by DEPT data, indicated the presence of 25 carbon atoms. These resonances comprised five methyl, five methylene, seven methine, and eight quaternary carbons. As in **1**, the ^{13}C NMR spectrum of **2** revealed the presence of a lactone and an ester carbonyl at δ 170.0 and 169.1, respectively, four oxygen-bearing carbon functionalities at δ 72.9, 77.1, 79.2, and 81.5, and eight olefinic carbons resonating between δ 108.7 and 168.5. Conspicuous features of the ^1H NMR spectrum of **2** compared to those of compound **1** were the presence of two methyl moieties at δ 0.90 (t) and 1.33 (d), two methylene protons at δ 1.64 (m) and 1.48 (m), and a methine proton at δ 3.54 (m). These resonances were ascribed to an *O*-butyl group, which was placed at C-5 on the basis of the HMBC correlation observed between H-5 (δ 5.15) and the methine carbon (δ 77.1) of the *O*-butyl group (Figure 2). Additionally, the ^1H NMR spectrum exhibited an extra olefinic proton at δ 6.21 (s). When combined with the considerable shift in the C-6 carbonyl resonance, this suggested unsaturation at C-7−C-8, which was further supported by the $^1H/^{13}C$ correlation observed in the HMBC spectrum between the C-19 methyl protons and C-7 (δ 123.9), C-8 (δ 149.0), and C-9 (δ 36.4) and between H-7 and the C6 carbonyl carbon (δ 197.8). Information gleaned from COSY, HMQC, HMBC, and NOESY and the structural similarity with compound **1** led to the formulation of structure **2**. The *Z*-geometry of the C-7−C-8 double bond was elucidated via the NOESY correlations between H-7 and C-19 methyl protons. Comparison of the NMR data of **2** and its isomer **3** (Table 2) revealed significant differences in the chemical shifts of protons 22, 23, 24, and 25 and the methyl ester protons, which implied that compounds **2** and **3** are epimeric at either C-5 or C-22. In view of the anticipated lability of the α-proton at C-5, we favor diastereoisomerism at this stereocenter. The 10*R* absolute configuration was again established by the CD spectrum, which showed Cotton effects commensurate with *M*-helicity (i.e., positive and negative Cotton effects for the $\{[\theta]_{257.1} +4.4 \times 10^4, [\theta]_{220.4} -2.7 \times 10^4\}$ for the n → π^* and $\pi \rightarrow \pi^*$ transitions at 257.1 and 220.4 nm, respectively). Similar NOE effects to those observed at the C-1 and C-13 stereocenters of compound **1** and based on the 1*R* absolute configuration of 5-episinuleptolide **5** presumably indicated the same 1*S*,13*S* absolute configuration as in compound **1**. However, we were unable to define the configurations at C-5 and C-22 in both compounds **2** and **3**.

Compound **4** was obtained as a pale yellow oil that analyzed as $C_{24}H_{30}O_9$ (485.1975 *m*/*z* $[M + Na]^+$) by HRESIMS. The IR bands at 3490, 1728, 1721, and 1607 cm^{-1} indicated the presence of hydroxy, carbonyl, and ester functionalities. The ^{13}C NMR spectrum of compound **4** was typical of a furanocembranoid[15,16] and indicated the presence of eight olefinic carbons between δ 113 and 150, two ester carbonyls at δ 162.3 and 170.2, a hemiacetal carbon at δ 117.3, and a lactone carbonyl at δ 167.5. The ^1H NMR spectrum of **4** indicated the presence of two trisubstituted α,β-unsaturated double-bond protons at δ 6.7 (1H, s) and 6.4 (1H, t, J = 8 Hz), a trisubstituted double-bond proton at δ 4.8 (1H, s), an isopropenyl group [δ 4.75 (1H, brs), 4.9 (1H, brs), and 1.65 (3H, brs)], a methyl ester at δ 3.37 (3H, s), an *O*-methyl resonance at δ 2.90 (3H, s), and a tertiary methyl connected to an oxygen-bearing carbon at δ 1.3 (3H, s). The NMR data (Table 3) and a literature survey revealed that compound **4** is related to the furanocembranoids isolated from *S. maxima*[17] except for the presence of a γ-lactone moiety bridging C-10 and C-12, indicated by ^{13}C NMR resonances at δ 79.3, 76.5, 128.4, and 167.5. The exocyclic H-13 of the α,β-unsaturated γ-lactone system in **4** resonates at δ 6.47. The corresponding endocyclic vinylic H-11's in **1** and **2** resonate at δ 7.55 and 7.20, respectively. Extensive HMQC, HMBC, and COSY correlations confirmed the new structure of **4** as shown. The relative configuration of the stereocenters of the γ-lactone in **4** was established by NOESY experiments and analogy with model compounds. Further, the magnitude of the H-10−H-11 coupling constant ($J_{10,11}$ <1 Hz) suggested a disposition of these protons with a dihedral angle approaching 90°. This is possible only if the protons are *trans*-oriented. The NOE experiments, however, did not permit configurational assignment at C-8 or C-3.

The structure and absolute configuration of 5-episinuleptolide (**5**) were determined via comparison of its NMR and specific rotation data with those reported.[11] Two furanosesquiterpenes were also isolated as a mixture from the less polar fraction of the extract and identified as furanosesquiterpene geometrical isomers. Subsequent separation on silica gel impregnated with silver nitrate led to the identification of methyl 5*Z*-5-(2,6-dimethylocta-5,7-dienyl)-furano-3-carboxylate and methyl 5*E*-5-(2,6-dimethylocta-5,7-dienyl)furano-3-carboxylate by comparison of their spectroscopic data (^1H, ^{13}C NMR, COSY, HMQC, and HMBC) with those of reported compounds.[18]

Table 2. ^1H and ^{13}C NMR Data of Compounds **2** and **3**

	compound **2**[a]		compound **3**[a]	
position	δ_C	δ_H (mult., J)[b]	δ_C	δ_H (mult., J)[b]
1	34.9	3.00 (brt, 12.5)	34.8	2.99 (brt, 12.6)
2	32.3	3.73 (brd, 15.5)	32.3	3.73 (brd, 15.5)
		2.17 (dd, 15.5, 12.5)		2.12 (dd, 15.5, 12.6)
3	168.5		168.5	
4	108.7		108.9	
5	81.5	5.15 (s)	81.1	5.14 (s)
6	197.8		197.9	
7	123.9	6.21 (s)	124.0	6.21 (s)
8	149.0		148.9	
9	36.4	2.09 (dd, 12.0, 7.2)	36.4	2.08 (dd, 12.2, 7.0)
		3.71 (brd, 12.0)		3.71 (bd, 12.2)
10	79.2	4.43 (brd, 7.2)	79.2	4.42 (brd, 7.5)
11	159.4	7.20 (brs)	159.3	7.17 (d, 1)
12	129.0		129.0	
13	72.9	4.82 (d, 7.8)	72.8	4.78 (d, 10)
14	29.2	2.25 (brdt, 12.5, 1.5)	29.2	2.24 (brd, 13.0)
		1.48 (m)		1.61 (m)
15	147.8		147.8	
16	110.2	4.78 (s)	110.2	4.77 (s)
		4.83 (s)		4.82 (s)
17	20.8	1.69 (s)	20.7	1.68 (s)
18	169.1		169.0	
19	29.5	1.56 (brd, 1.3)	29.5	1.56 (brd, 1)
20	170.0		170.0	
21	51.5	3.30 (s)	51.5	3.46 (s)
22	77.1	3.54 (m)	76.9	3.58 (m)
23	20.1	1.33 (d, 6.1)	19.9	1.10 (d, 6)
24	29.7	1.64 (m)	30.4	1.61 (m)
		1.48 (m)		1.42 (ddd, 13.5, 12.5, 7.5)
25	10.1	0.90 (t, 7.4)	10.4	1.10 (t, 7.5)

[a] Spectra recorded at 500 MHz in C_6D_6. [b]Chemical shifts are in parts per million relative to TMS.

Table 3. ^{1}H and ^{13}C NMR Data of Compound **4**[a]

position	δ_C	δ_H (mult., *J*)[b], HMBC (H to C)
1	41.3	2.50 (m)
2	38.9	2.65 (dd 10 ,8.7), 1, 3, 15
		1.85 (brd 14), 3, 4, 15
3	117.3	
4	131.6	
5	139.7	6.76 (s), 4, 6, 18
6	150.6	
7	117.6	4.86 (s), 5, 6, 8, 9
8	71.4	
9	41.1	1.65 (m), 7, 8, 19
		2.85 (m)
10	79.3	4.65 (dd 11.7, 5.5), 11, 12, 20
11	76.5	5.10 (s), 9, 10, 12, 20
12	128.4	
13	148.4	6.47 (t, 8), 1, 11, 20
14	32.8	1.74 (m), 12, 13
		2.82 (m), 1, 13
15	148.1	
16	113.1	4.75 (brs), 1
		4.93 (brs), 1, 15, 16
17	18.7	1.65 (brs), 1, 15, 17
18	162.3	
19	30.8	1.30 (s), 7, 8, 9
20	167.5	
21	51.3	3.37 (s), 4, 18
22	170.2	
23	20.5	1.53 (s), 22
24	50.0	2.93 (s), 3

[a] Spectra recorded at 400 MHz in C_6D_6. [b]Chemical shifts are in parts per million relative to TMS.

Assessment of the cytotoxicity of compounds **1**−**3** against a number of cancer cell lines showed that cembranoid **3** strongly inhibited the growth of ovary cancer IGROV, breast cancer SK-BR3, melanoma SK-MEL-28, pancreas PANC1, colon LOVO and LOVO-DOx, and cervix HeLa and HeLa-Apl cell lines with GI_{50} values of 0.52, 0.039, 0.54, 0.58, 0.51, 0.75, 0.48, and 0.56 μM, respectively. Compounds **1** and **2** showed moderate cytotoxicity (Supporting Information).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. CD spectra were recorded on a JASCO J-715 spectropolarimeter. UV spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. IR spectra were recorded on an ATI Mattson Genesis series FTIR spectrometer. NMR spectra were measured on Bruker Advance DRX-400 and DRX-500 spectrometers. ^{1}H and ^{13}C NMR spectra were measured and reported in ppm by using the chloroform-d_6, benzene-d_6, and DMSO-d_6 solvent peaks (δ_H 7.24 and δ_C 77.2, δ_H 7.16 and δ_C 128.3, δ_H 2.50 and δ_C 39.5, respectively) as an internal standard. ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. HPLC was carried out on a Waters 510 model system (column 1: Phenomenex Ultracarbon, 5 μm, ODS 30, 250 × 21.5 mm, flow rate 20 mL/min; column 2: Phenomenex Ultracarbon, C18, 5 μm, 250 × 10 mm; flow rate, 2 mL/min; detector wavelength, 254 nm).

Animal Material. The hybrid soft coral *S. maxima* × *S. polydactyla* was collected at Piti Bomb holes, a shallow reef on the leeward side of Guam (13°25′ N, 144°55′ E). Hybrids were verified by classical taxonomic procedures (i.e., spicule morphology).

Extraction and Isolation. The frozen soft coral was exhaustively extracted with 1:1 MeOH−CH_2Cl_2, and the combined solvent extracts were concentrated under reduced pressure. The extract (28 g) was subjected to Si gel vacuum liquid chromatography (VLC) eluted with hexanes, hexanes−EtOAc, EtOAc−MeOH, to MeOH to yield 11 fractions, which were concentrated under reduced pressure. Fraction 5 eluting with 2:8 EtOAc−hexanes (1.2 g) was further chromatographed on a Si gel column (gradient elution with $CHCl_3$−acetone). Further Si gel column flash chromatography gradiently eluting with hexanes−EtOAc was necessary before selected fractions could be subjected to normal-phase HPLC (isocratic elution with 3.5:6.5 EtOAc−hexanes) and RP HPLC (isocratic elution with 1:1 MeOH−H_2O) to yield compounds **1** (28 min, 4 mg) and **2** (21 min, 3 mg). Fraction 6 (4.9 g), eluted with 4:6 EtOAc−hexanes, was subjected to successive Si gel liquid chromatography, yielding 24 fractions. Fractions 6:8 and 6:9 were subjected to VLC followed by NP HPLC (isocratic elution with 7:3 EtOAc−hexanes) to yield compound **3** (16 min, 19 mg), while fraction 6:13 was subjected to LC followed by an RP HPLC (gradient elution with MeOH−H_2O) to yield compound **4**. An earlier fraction eluted with 5% EtOAc−hexanes was subjected to successive liquid chromatography to yield a mixture of furanosesquiterpenes, which were further separated on preparative TLC impregnated with $AgNO_3$.

Cytotoxicity Assay. Human-derived established cell lines used in this study were purchased from ATCC (American Type Culture Collection) unless otherwise specified. A panel of 14 human tumor cell lines was used to evaluate the cytotoxic potential of the tested compounds: A549 lung carcinoma NSCL, SK-MEL-28 malignant melanoma, HT-29 colon carcinoma, LoVo lymph node metathesis cells and the corresponding LoVo-Dox cells resistant to Doxorubicin, DU-145 and LNCaP prostate carcinoma tumor cells, SK-BR-3 breast adenocarcinoma, ovarian cells sensitive (IGROV) or resistant (IGROV-ET) to ET-743, cervix epitheloid carcinoma (HeLa) or resistant (HeLa-Apl) to Aplidine, K-562 chronic myelogenous leukaemia, and PANC1 pancreatic epitheloid carcinoma. All cell lines are maintained in DMEM (Dulbecco's modified Eagle's medium) culture medium supplemented with 10% FBS (fetal bovine serum), 2 mM L-glutamine and 100 units/mL penicillin and streptomycin at 37 °C and 5% CO_2. Triplicate cultures were incubated for 72 h in the presence or absence of test compounds (at 10 concentrations typically ranging from 10 to 0.0026 mg/mL). A colorimetric assay using sulforhodamine B (SRB) was adapted for a quantitative measurement of cell growth and viability, following a previously described method.[19] Cells were plated in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. One plate from each different cell line was fixed and stained and used for T_z reference (see next paragraph). After that, cells were treated with vehicle alone (control) or compounds at the concentrations indicated. Treated cells were further incubated for 72 h, and cytotoxic evaluation was performed by colorimetric analysis. In brief, cells were washed twice with phosphate-buffered saline (PBS), fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. Cells were then rinsed several times in 1% HOAc solution and air-dried. SRB was then extracted in 10 mM trizma base solution and the absorbance measured at 490 nm. Cell survival is expressed as percentage of control cell growth. Dose−response curves are performed by using the NCI algorithm[20] where T_z is the number of control cells at time t_0, C is the number of control cells at time t, and T is the number of treated cells at time t. If $T_z < T < C$ (growth inhibition), then the following equation is used: $100 \times ([T - T_z]/[C - T_z])$. If $T < T_z$ (net cell killing), then the following equation is used: $100 \times ([T - T_z]/T_z)$. After dose−curve generation, the following parameters are calculated by interpolation: GI_{50}, concentration that causes 50% growth inhibition; TGI, concentration that causes total growth inhibition; LC_{50}, concentration that causes 50% net cell killing.

Compound 1: colorless oil; $[\alpha]^{25}_D$ +9.4 (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.16) nm, 258 (sh, 3.79) nm; CD (MeOH) $[\theta]_{253.4}$ $+1.48 \times 10^4$, $[\theta]_{228.4}$ -4.8×10^5; IR (NaCl pellet) 3081, 3018, 2928, 1756, 1703, 1607, 1435, 1288, 1252, 1187, 1064, 893, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ESI (+)MS *m*/*z* 389 $[M + H]^+$; HRESIMS *m*/*z* 389.1613 $[M + H]^+$ (calcd for $C_{21}H_{25}O_7$, 389.1600), 406.1902 $[M + NH_4]^+$ (calcd for $C_{21}H_{28}O_7N$, 406.1865), 411.1351 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_7Na$, 411.1419).

Compound 2: colorless oil; $[\alpha]^{25}_D$ +32.5 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log *c*) 242 (3.33) nm; CD (MeOH) $[\theta]_{257.1}$ $+4.4 \times 10^4$, $[\theta]_{220.4}$ -2.7×10^4; IR (NaCl pellet) 2958, 2927, 1757, 1698, 1621, 1436, 1374, 1229, 1075, 888, 668 cm^{-1}; ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz), see Table 2; ESI (+)-FTMS *m*/*z* 445 $[M + H]^+$; HRESI-FTMS *m*/*z* 445.22208 $[M + H]^+$ (calcd for $C_{25}H_{33}O_7$, 445.2148).

Compound 3: colorless oil; $[\alpha]^{25}_D$ +10 (*c* 0.05, $CHCl_3$); UV λ_{max} (same as **1**); CD (MeOH) $[\theta]_{257.4}$ $+3.8 \times 10^4$, $[\theta]_{228.4}$ -1.24×10^5; IR (NaCl pellet) 2966, 2934, 1757, 1698, 1621, 1435, 1373, 1229, 1075,

1046, 960, 888, 754; ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz), see Table 2; HRESIMS (same as **2**).

Compound 4: pale yellow oil; $[\alpha]^{25}_D$ +7.4 (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.08) nm; (log ϵ) 306 (3.68) nm, IR (NaCl pellet) 3490, 2930, 1758, 1721, 1607, 1437, 1368, 1231, 969, 736 cm^{-1}; ^{1}H NMR (C_6D_6, 400 MHz); ^{13}C NMR (C_6D_6, 100 MHz), see Table 3; HRESIMS *m*/*z* 485.1975 $[M + Na]^+$ (calcd for $C_{24}O_9H_{29}Na$, 485.1889).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and cytotoxic activities of compounds **1**−**3**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Anjaneyulu, A. S. R.; Venkateswarlu, R. *J. Sci. Ind. Res*. **1995**, *54*, 637−649.
(2) Kamel, H. N.; Slattery, M. *Pharm. Biol*. **2005**, *43*, 253−269.
(3) Zhang, C.; Yan, S.; Zhang, G.; Lu, W.; Su, J.; Zeng, L.; Gu, L.; Yang, X.; Lian, Y. *J. Nat. Prod*. **2005**, *68*, 1087−1089.
(4) Su, J.; Ahmed, A. F.; Sung, P.; Chao, C.; Kuo, Y.; Sheu, J. *J. Nat. Prod*. **2006**, *69*, 1134−1139.
(5) Missakian, M. G.; Burreson, B. J.; Sheuer, P. J. *Tetrahedron* **1975**, *31*, 2513−2515.
(6) Arnold, M. L.; Burke, J. M. *Natural Hybridization. Evolutionary Genetics*: *Concepts and Case Studies*; Oxford University Press: Oxford, 2004.
(7) Gardner, J. P. A. Hybridization in the Sea. In *Advances in Marine Biology*; Academic Press Inc.: San Diego, CA, 1997; Vol. 31, pp 1−78.
(8) Vollmer, S. V.; Palumbi, S. R. *Science* **2002**, *296*, 2023−2025.
(9) Orians, C. M. *Am. J. Bot.* **2000**, *87*, 1749−1756.
(10) Kamel, H. N.; Fronczek, F. R.; Fischer, N. H.; Slattery, M. *Tetrahedron Lett.* **2004**, *49*, 1997−1998.
(11) Tseng, Y.-J.; Ahmed, A. F.; Dai, C.-F.; Chiang, M. Y.; Sheu, J.-H. *Org. Lett*. **2005**, *7*, 3813−3816.
(12) Rodriguez, A. D.; Shi, J.; Huang, S. D. *J. Nat. Prod*. **1999**, *62*, 1228−1237.
(13) Gawtonski, J. K.; Van, Oeveren, A.; Van, der Deen, H.; Leung, C. W.; Feringa, B. L. *J. Org. Chem.* **1996**, *61*, 1513−1515.
(14) Gutierrez, M.; Capson, T. L.; Guzman, H. M.; Gonzalez, J.; Ortega-Barria, E.; Quinoa, E.; Riguera, R. *J. Nat. Prod*. **2005**, *68*, 614−616.
(15) Bowden, B. F.; Coll, J. C.; Wright, A. D. *Aust. J. Chem*. **1989**, *42*, 757−763.
(16) Ksebati, M. B.; Ciereszko, L. S.; Schmitz, F. J. *J. Nat. Prod*. **1984**, *47*, 1009−1012.
(17) Venkateswarlu, Y.; Sridevi, K. V.; Rao, M. R. *J. Nat. Prod.* **1999**, *62*, 756−768.
(18) Bowden, B. F.; Coll, J. C.; De Silva, E. D.; De Costa, M. S. L.; Djura, P. J.; Mahendran, M.; Tapiolas, D. M. *Aust. J. Chem*. **1983**, *36*, 371−376.
(19) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Waren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(20) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−104.

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