Sorbifolivaltrates A−D, Diene Valepotriates from *Valeriana sorbifolia*[1]

Ya-ming Xu, Steven P. McLaughlin, and A. A. Leslie Gunatilaka*

SW Center for Natural Products Research and Commercialization, Office of Arid Lands Studies, College of Agriculture and Life Sciences, The University of Arizona, 250 E. Valencia Road, Tucson, Arizona 85706-6800

Four new diene valepotriates, sorbifolivaltrates A−D (**1–4**), and the known compounds isovaltrate (**5**), valtrate (**6**), seneciovaltrate (**7**), valtrate hydrine B3 (**8**), and valtrate hydrine B7 (**9**), have been isolated by bioassay-guided fractionation of the cytotoxic hexanes and methyl ethyl ketone crude extracts of the aerial parts of *Valeriana sorbifolia* occurring in the Sonoran desert. The structures of **1–4** were determined on the basis of their high-resolution mass spectrometric and NMR spectroscopic data. All compounds exhibited weak to moderate cytotoxicity against the human metastatic prostate cancer cell line, PC-3M.

The genus *Valeriana* (Valerianaceae) is a moderately large, but poorly understood genus consisting of about 200 species.[2] In Arizona, this genus is represented by five species, namely, *V. arizonica*, *V. capiata* ssp. *acutiloba*, *V. edulis*, *V. occidentalis*, and *V. sorbifolia*.[3] Of these, *V. arizonica*, *V. capiata* ssp. *acutiloba*, *V. edulis*, and *V. occidentalis* are closely related to *V. officinalis*.[4] *V. edulis* and *V. sorbifolia* are more closely related to each other than either is to *V. officinalis*.[4] Root preparations of *V. officinalis*, popularly known as valerian, find applications in phytomedicine as a treatment for insomnia and anxiety.[5] *V. sorbifolia* is a perennial species with a habitat ranging from southeastern Arizona throughout Central America to northern South America.[2] Despite a number of analytical and pharmacological studies[6] on the closely related species *V. edulis* and *V. edulis* ssp. *procera* (commonly known as *Valeriana mexicana*), there are no reports on the investigation of *V. sorbifolia* for its constituents. In continuing our search for novel natural product-based potential anticancer agents from arid land plants and their associated microorganisms,[7] hexanes and methyl ethyl ketone extracts of *V. sorbifolia* HBK were selected for further investigation on the basis of their cytotoxic activity. Herein we report the isolation, structure elucidation, and cytotoxic activity of four new diene valepotriates, named sorbifolivaltrates A−D (**1–4**), and five known diene valepotriates (**5–9**). Valepotriates represent a small group of iridoid esters encountered in several species of *Valeriana*, of which some are known to be responsible for the sedative activity of the crude drug valerian.[5]

Cytotoxicity-guided fractionation of the hexanes extract of the aerial parts of *V. sorbifolia* afforded compounds **1**, **2**, **5–7**, and **9**, whereas similar fractionation of the methyl ethyl ketone extract furnished **3**, **4**, **8**, and **9**. The known compounds were identified as isovaltrate (**5**),[8] valtrate (**6**),[9] seneciovaltrate (**7**),[10] valtrate hydrine B3 (**8**),[11] and valtrate hydrine B7 (**9**)[10] by comparison of their spectroscopic data with those reported in the literature. Sorbifolivaltrates A−D (**1–4**) were isolated as colorless, amorphous solids. The molecular formula of sorbifolivaltrate A (**1**) was determined to be $C_{25}H_{34}O_8$ by a combination of HRESIMS and ^{13}C NMR data and indicated nine degrees of unsaturation. Its ^1H NMR spectrum (Table 1) exhibited signals due to two olefinic protons at δ 6.72 (1H, s) and 5.86 (1H, dd, $J = 2.5$ Hz) and two oxirane proton signals at δ 3.02 (1H, d, $J = 5$ Hz) and 2.93 (1H, d, $J = 5$ Hz), typical of diene valepotriates with an oxirane moiety.[12] The signal at δ 0.96 (12H, d, $J = 6.5$ Hz) in the ^1H NMR spectrum due to four methyl groups suggested the presence two *iso*-valeryl groups and those at δ 1.96 (3H, s) and 2.23 (3H, s) together with an olefinic proton signal at δ 5.67 (1H, brs) indicated the presence of a

3-methylcrotonyl group in **1**. The foregoing suggested that **1** is an iridoid triester containing one 3-methylcrotonyl and two *iso*-valeryl groups. Assignments of ^1H and ^{13}C NMR data (Tables 1 and 2, respectively) of **1** were made by comparison with those reported[12] for similar valtrates and by the interpretation of its HMQC and HMBC spectra. However, in the absence of HMBC correlations between the acyl groups and the iridoid moiety in **1**, chemical shift data had to be used to locate the acyl groups. It has been reported that the chemical shifts of the acyl carbonyl groups connected to different positions of the iridoid ring of valepotriates exhibit differences of 0.2–0.3 ppm in their ^{13}C NMR spectra due to the so-called γ-effect.[10,13,14] The carbonyl signal of the 3-methylcrotonyl (Cr) moiety of **1** appeared at δ 163.3, which was almost identical to that of seneciovaltrate (**7**), suggesting that the 3-methylcrotonyl group should be placed at C-1 and the two *iso*-valeryl (Iv) groups at C-7 and C-11. Thus, the structure of sorbifolivaltrate A was identified as 1-(3-methylcrotonyl)-7,11-di-*iso*-valeryl valtrate (**1**).

Sorbifolivaltrate B (**2**) analyzed for $C_{23}H_{30}O_8$ by a combination of HRESIMS and ^{13}C NMR spectroscopy and indicated nine degrees of unsaturation. The ^1H and ^{13}C NMR data of **2** (Tables 1 and 2, respectively) closely resembled those of seneciovaltrate (**7**)[10] except that one *iso*-valeryl group appeared to have been replaced with a 3-methylvaleryl moiety [δ_H 0.87 (3H, t, $J = 7.2$ Hz), 0.90 (3H, d, $J = 7.2$ Hz); δ_C 172.7 (s), 41.5 (t), 32.0 (d), 29.3 (t), 19.3 (q), and 11.3 (q)]. The positions of the ester carbonyl signals of the acetyl (δ 170.8) and 3-methylcrotonyl (δ_C 163.2) groups of **2** were similar to those of **7** (see Table 2), suggesting that the acetyl (Ac) and the 3-methylcrotonyl (Cr) groups should be located at C-11 and C-1, respectively. The remaining 3-methylvaleryl (Mv) moiety in **2** was therefore placed at C-7. On the basis of the above arguments the structure of sorbiforlivaltrate B was identified as 1-(3-methylcrotonyl)-7-(3-methylvaleryl)-11-acetylvaltrate (**2**). The molecular formula of sorbifolivaltrate C (**3**) was determined to be $C_{30}H_{46}O_{11}$ by a combination of HRESIMS and ^{13}C NMR data and indicated eight degrees of unsaturation. Its ^1H NMR spectrum (Table 1) exhibited signals due to two olefinic protons at δ 6.66 (1H, s) and 5.75 (1H, dd. $J = 2.4$ Hz) and two oxymethylene proton signals at δ 4.44 (1H, d, $J = 11.4$ Hz) and 4.28 (1H, d, $J = 11.4$ Hz), typical of diene valepotriate hydrines.[12] The methyl signals at δ 0.94 and 0.93 (each 3H, d, $J = 6.6$ Hz) and δ 0.92 and 0.91 (each 6H, d, $J = 6.6$ Hz) in the ^1H NMR spectrum suggested the presence of three *iso*-valeryl groups and those at δ 1.34 and 1.33 (3H each, s) indicated the occurrence of a 3-hydroxylvaleryl group in **3**. Thus, these data suggested that **3** is an iridoid tetra-ester containing one 3-hydroxylvaleryl and three *iso*-valeryl groups. Assignment of the ^1H and ^{13}C NMR data of **3** (Tables 1 and 2, respectively) were made by comparison with those reported for related valtrates[13] and by the interpretation of its HMQC and HMBC spectra. The location

* To whom correspondence should be addressed. Tel: (520) 741-1691. Fax: (520) 741-1468. E-mail: leslieg@ag.arizona.edu.

Table 1. ^{1}H NMR Data (600 MHz) for Sorbifolivaltrates A−D (**1–4**) in CDCl$_3$

	position	**1**[a,b] δ	**2**[a] δ	**3**[a] δ	**4**[a] δ
	1	6.02 (1H, d, 10.5)	6.00 (1H, d, 10.2)	6.21 (1H, d, 10.2)	6.26 (1H, d, 10)
	3	6.72 (1H, s)	6.69 (1H, s)	6.66 (1H, s)	6.67 (1H, s)
	6	5.86 (1H, dd, 2.5)	5.84 (1H, br s)	5.75 (1H, dd, 2.4)	5.76 (1H, dd, 2.5)
	7	5.38 (1H, d, 2.5)	5.35 (1H, br s)	5.47 (1H, d, 2.4)	5.46 (1H, d, 2.5)
	9	3.45 (1H, dd, 2.5, 10.5)	3.43 (1H, d, 10.2)	2.91 (1H, dd, 2.4, 9.6)	2.96 (1H, dd, 2.4, 9.6)
	10	3.02 (1H, d, 5)	2.99 (1H, d, 4.8)	4.44 (1H, d, 11.4)	4.39 (1H, d, 11.5)
		2.93 (1H, d, 5)	2.90 (1H, d, 4.8)	4.28 (1H, d, 11.4)	4.27 (1H, d, 11.5)
	11	4.78 (1H, d, 12.5)	4.72 (1H, d, 12.6)	4.71 (1H, d, 12.6)	4.66 (1H, d, 12.6)
		4.68 (1H, d, 12.5)	4.65 (1H, d, 12.6)	4.63 (1H, d, 12.6)	4.63 (1H, d, 12.6)
R$_1$	2	5.67 (1H br s)	5.84 (1H, br s)	2.63 (1H, d, 15)	5.73 (1H, br s)
				2.58 (1H, d, 15)	
	4	2.23 (3H, s)	2.19 (3H, s)	1.34 (3H, s)	2.00 (3H, s)
	5	1.96 (3H, s)	1.93 (1H, s)	1.33 (3H, s)	2.00 (3H, s)
R$_7$	2	2.20 (2H, d, 7.0)[c]	2.1−2.3 (2H, m)	2.1−2.3 (2H, m)	2.1−2.3 (2H, m)
	3	2.10 (1H, m)	2.0−2.1 (1H, m)	2.0−2.1 (1H, m)	2.1−2.3 (1H, m)
	4	0.96 (3H, d, 6.5)	1.22 (1H, m)	0.94 (3H, d, 6.6)[c]	1.19 (1H, m)
			1.32 (1H, m)		1.31 (1H, m)
	5	0.96 (3H, d, 6.5)	0.87 (3H, t, 7.2)	0.93 (3H, d, 6.6)[c]	0.85 (3H, t, 7.8)
	6		0.90 (3H, d, 7.2)		0.88 (3H, d, 6.6)
R$_{10}$	2			2.1−2.3 (2H, m)	2.1−2.3 (2H, m)
	3			2.0−2.1 (1H, m)	2.0−2.1 (1H, m)
	4			0.92 (3H, d, 6.6)[c]	0.93 (3H, d, 6.6)
	5			0.92 (3H, d, 6.6)[c]	0.93 (3H, d, 6.6)
R$_{11}$	2	2.19 (2H, d, 7.0)[c]	2.04 (3H, s)	2.1−2.3 (2H, m)	2.02 (3H, s)
	3	2.10 (1H, m)		2.0−2.1 (1H, m)	
	4	0.96 (3H, d, 6.5)		0.91 (3H, d, 6.6)[c]	
	5	0.96 (3H, d, 6.5)		0.91 (3H, d, 6.6)[c]	

[a] Coupling constants (*J* values) are in Hz. [b] 500 MHz NMR instrument was used for these determinations. [c] Assignments may be interchanged in each column.

**Table 2.** ^{13}C NMR Data (125 MHz) for Sorbifolivaltrates A−D (**1–4**), **7**, and **9** in CDCl$_3$[a]

position	**7**	**1**	**2**	**9**	**3**	**4**
1	92.3, CH	92.3, CH	92.3, CH	92.4, CH	92.8, CH	92.4, CH
3	148.6, CH	148.5, CH	148.6, CH	148.1, CH	147.7, CH	148.1, CH
4	108.2, C	108.4, C	108.2, C	108.6, C	109.1, C	108.6, C
5	141.1, C	141.1, C	141.1, C	139.2, C	138.7, C	139.2, C
6	118.4, CH	118.4, CH	118.4, CH	117.5, CH	117.9, CH	117.4, CH
7	83.1, CH	83.1, CH	83.1, CH	83.2, CH	83.2, CH	83.2, CH
8	64.3, C	64.3, C	64.3, C	80.2, C	80.2, C	80.2, C
9	43.0, CH	43.1, CH	43.0, CH	48.7, CH	48.5, CH	48.7, CH
10	47.9, CH$_2$	48.0, CH$_2$	47.9, CH$_2$	65.6, CH$_2$	65.4, CH$_2$	65.6, CH$_2$
11	60.9, CH$_2$	60.5, CH$_2$	60.9, CH$_2$	61.0, CH$_2$	60.5, CH$_2$	61.0, CH$_2$
R$_1$-1	163.2, C	163.3, C	163.2, C	163.9, C	170.3, C	163.9, C
2	114.2, CH	114.2, CH	114.2, CH	114.4, CH	46.7, CH$_2$	114.4, CH
3	161.7, C	161.6, C	161.6, C	162.1, C	69.5, C	162.0, C
4	27.7, CH$_3$	27.7, CH$_3$	27.7, CH$_3$	27.8, CH$_3$	28.9, CH$_3$	27.7, CH$_3$
5	20.9, CH$_3$	20.7, CH$_3$	20.7, CH$_3$	20.8, CH$_3$	29.8, CH$_3$	20.8, CH$_3$
R$_7$-1	172.4, C	172.9, C	172.7, C	171.8, C	171.8, C	172.3, C
2	43.4, CH$_2$	43.4, CH$_2$	41.5, CH$_2$	43.4, CH$_2$	43.35, CH$_2$	41.4, CH$_2$
3	25.8, CH	25.8, CH	32.0, CH	25.7, CH	25.7, CH	31.9, CH
4	22.3, CH$_3$	22.4, CH$_3$	29.3, CH$_2$	22.4, CH$_3$	22.4, CH$_3$	29.2, CH$_2$
5	22.3, CH$_3$	22.4, CH$_3$	11.3, CH$_3$	22.4, CH$_3$	22.4, CH$_3$	11.3, CH$_3$
6			19.3, CH$_3$			19.2, CH$_3$
R$_{10}$-1				173.0, C	173.4, C	173.0, C
2				43.1, CH$_2$	43.1, CH$_2$	43.1, CH$_2$
3				25.6, CH	25.7, CH	25.6, CH
4				22.4, CH$_3$	22.4, CH$_3$	22.4, CH$_3$
5				22.3, CH$_3$	22.4, CH$_3$	22.4, CH$_3$
R$_{11}$-1	170.9, C	172.9, C	170.8, C	170.8, C	172.9, C	170.8, C
2	21.0, CH$_3$	43.5, CH$_2$	21.0, CH$_3$	21.0, CH$_3$	43.4, CH$_2$	21.0, CH$_3$
		25.8, CH			25.7, CH	
		22.4, CH$_3$			22.3, CH$_3$	
		22.4, CH$_3$			22.3, CH$_3$	

[a] Multiplicities deduced from DEPT and HSQC spectra.

of each acyl group was determined as for **1** and **2** above, by the application of ^{13}C NMR chemical shift data for the acyl carbonyl carbon. The carbonyl chemical shifts of the three *iso*-valeryl carbonyls (δ_C 171.8, 173.4, and 172.9) of **3** were almost identical with those of seneciovaltrate (**7**) and valtrate hydrine B7 (**9**),[10] locating the 3-hydroxyvaleryl group at C-1. Thus, the structure of sorbifolivaltrate C was identified as 1-(3-hydroxylvaleryl)-7,10,11-tri-*iso*-valerylvaltrate hydrine (**3**).

Sorbifolivaltrate D (**4**) analyzed for C$_{28}$H$_{40}$O$_{10}$ by a combination of HRESIMS and ^{13}C NMR data and indicated nine degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **4** (Tables 1 and 2, respectively) closely resembled those of valtrate hydrine B7 (**9**)[10]
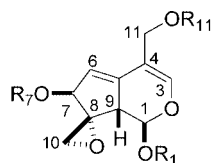
	R$_1$	R$_7$	R$_{11}$
1	Cr	Iv	Iv
2	Cr	Mv	Ac
5	Iv	Ac	Iv
6	Iv	Iv	Ac
7	Cr	Iv	Ac
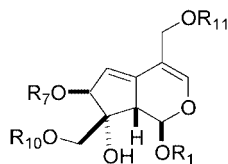
	R$_1$	R$_7$	R$_{10}$	R$_{11}$
3	Hiv	Iv	Iv	Iv
4	Cr	Mv	Iv	Ac
8	Hiv	Iv	Iv	Ac
9	Cr	Iv	Iv	Ac

3-Methylvaleryl (Mv)   3-Methylcrotonyl (Cr)

3-Hydroxylisovaleryl (Hiv)   Isovaleryl (Iv)

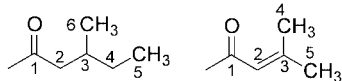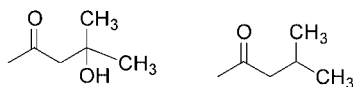except that one *iso*-valeryl group is replaced by a 3-methylvaleryl group [δ_H 0.88 (3H, d, *J* = 6.6 Hz) and 0.85 (3H, t, *J* = 7.8 Hz); δ_C 172.3 (s), 41.4 (t), 31.9 (d), 29.2 (t), 19.2 (q), and 11.3 (q)]. The positions of the ester carbonyl signals of acetyl (δ_C 170.8), *iso*-valeryl (δ_C 173.0), and 3-methylcrotonyl (δ_C 163.9) groups of **4** were similar to those of **9** (see Table 2), suggesting that acetyl (Ac), *iso*-valeryl (Iv), and the 3-methylcrotonyl (Cr) groups are located at C-11, C-10, and C-1, respectively. The remaining 3-methylvaleryl (Mv) moiety in **4** was therefore placed at C-7. On the basis of the above arguments, the structure of sorbifolivaltrate D was identified as 1-(3-methylcrotonyl)-7-(3-methylvaleryl)-10-*iso*-valeryl-11-acetylvaltrate hydrine (**4**).

A number of valepotriates have been reported to be cytotoxic for cancer cells.[15] It has been noted that the presence of double bond(s) in these compounds is essential for cytotoxic activity, whereas the oxirane ring, a potential alkylating moiety, is not required for this activity.[16] When tested in our cancer cell proliferation inhibition (cytotoxicity) assay,[17] compounds **1**, **5**, and **6** showed stronger activity (IC$_{50}$ 2.6, 3.1, and 1.7 μM, respectively) against the human metastatic prostate cancer cell line, PC-3M, compared with compounds **2**, **3**, **4**, **7**, **8**, and **9** (IC$_{50}$ 12.2, 8.9, 7.3, 20.0, 11.1, and 8.9 μM, respectively), suggesting the importance of the oxirane ring for cytotoxicity of these compounds. Although an obvious structure–activity relationship was not discernible, these data suggest that cytotoxic activity of valepotriates may be partly dependent on the location and relative size of the acyl groups and on the overall conformation of these compounds besides the functional groups present.

Experimental Section

General Experimental Procedures. UV data were recorded with a Shimadzu UV-1601 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker DRX-500 and DRX-600 NMR spectrometers. Mass spectra were recorded on an IonSpec FT mass spectrometer. Lichroprep diol (40–63 μm) used for column chromatography was purchased from EM Industries, Inc. Bakerbond C$_{18}$ (40 μm) is a product of J.T. Baker, Inc. The Kromasil C$_{18}$ reversed-phase column (250 × 10 mm, 5 μm) for HPLC was obtained from Phenomenex Inc. Sephadex LH-20 was obtained from Amersham Biosciences.

Plant Material. The aerial parts of *V. sorbifolia* were collected in Huachuca Mountain, AZ, in September 1998, and this species was identified by Dr. S. P. McLaughlin. A voucher specimen is deposited at the University of Arizona Herbarium (ARIZ) under the accession number 376843.

Extraction and Isolation. Dried and powdered *V. sorbifolia* (50 g) was extracted (× 3) sequentially with hexanes, methyl ethyl ketone, and MeOH at room temperature. The hexanes and methyl ethyl ketone extracts (0.602 and 1.785 g, respectively) showed cytotoxic activity against the PC-3M cell line at 25 μg/mL. The hexanes extract (600 mg) was subjected to gel-permeation chromatography over Sephadex LH-20 (50 g) and eluted successively with CH$_2$Cl$_2$−hexanes (4:1), CH$_2$Cl$_2$−acetone (3:2), CH$_2$Cl$_2$−acetone (1:4), and MeOH. The cyto-toxic fraction (596 mg) obtained with CH$_2$Cl$_2$−hexanes (4:1) was subjected to column chromatography over Lichroprep diol (30 g) and eluted with various mixtures of hexanes−CHCl$_3$−MeOH. The fraction eluted with hexanes−CHCl$_3$ (1:1) afforded an active fraction (360.7 mg), which was further fractionated by reversed-phase column chromatography (RP-18; 20 g), eluted with a gradient of water in MeOH. The 85% aqueous MeOH fraction (42 mg) also showed cytotoxic activity and was subjected to column chromatography over Lichroprep diol (30 g), using CHCl$_3$−MeOH (9:1) as the eluent, to afford fractions A (7.5 mg), B (7.6 mg), and C (12.7 mg). Each of these fractions was further separated by preparative HPLC [250 × 10 mm Kromasil C$_{18}$ (5 μm) column; eluent: 75% MeOH; UV detection at 254 nm] to afford compounds **1** (1.9 mg), **5** (0.9 mg), and **6** (1.2 mg) from fraction A, compounds **2** (0.7 mg) and **7** (2.5 mg) from fraction B and compound **9** (3.2 mg) from fraction C. The methyl ethyl ketone extract (1.5 g) was subjected to solvent−solvent partitioning with hexanes−80% aqueous MeOH and CHCl$_3$−50% aqueous MeOH to yield hexanes, CHCl$_3$, and 50% aqueous MeOH fractions.[7] The CHCl$_3$ fraction (569.8 mg), which showed cytotoxic activity at 12.5 μg /mL, was subjected to gel-permeation chromatography over Sephadex LH-20 (50 g) and eluted successively with CH$_2$Cl$_2$−hexanes (4:1), CH$_2$Cl$_2$−acetone (3:2), CH$_2$Cl$_2$−acetone (1:4), and MeOH. The cytotoxic fraction (231.3 mg), eluted with CH$_2$Cl$_2$−hexanes (4:1), was further fractionated by column chromatography over RP-18 (20 g), eluted with MeOH−H$_2$O (1:1), MeOH−H$_2$O (7:3), MeOH−H$_2$O (9:1), MeOH, and finally acetone. The cytotoxic fraction (116.2 mg) obtained with MeOH−H$_2$O (9:1) was fractionated by column chromatography over Lichroprep diol (30 g) by elution with hexanes, hexanes−CHCl$_3$ (1:1), CHCl$_3$, and MeOH. The cytotoxic fraction (92.7 mg) eluted with hexanes−CHCl$_3$ (1:1) was further separated by preparative HPLC [250 × 10 mm Kromasil C$_{18}$ (5 μm) column; eluent: 80% aqueous MeOH; UV detection at 254 nm] to afford compounds **3** (1.9 mg), **4** (1.0 mg), **8** (15.6 mg), and **9** (6.8 mg).

Sorbifolivaltrate A (1): amorphous powder; UV (MeOH) λ_{max} (log ε) 219.5 (3.88), 256 (3.69) nm; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; positive HRESIMS *m/z* 485.2142 [M + Na]$^+$ (calcd for C$_{25}$H$_{34}$O$_8$·Na, 485.2151).

Sorbifolivaltrate B (2): amorphous powder; UV (MeOH) λ_{max} (log ε) 220.5 (3.13), 254 (3.01) nm; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; positive HRESIMS *m/z* 457.1820 [M + Na]$^+$ (calcd for C$_{23}$H$_{30}$O$_8$·Na, 457.1838).

Sorbifolivaltrate C (3): amorphous powder; UV (MeOH) λ_{max} (log ε) 256 (2.87) nm; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; positive HRESIMS *m/z* 605.2955 [M + Na]$^+$ (calcd for C$_{30}$H$_{46}$O$_{11}$·Na, 605.2938).

Sorbifolivaltrate D (4): amorphous powder; UV (MeOH) λ_{max} (log ε) 223 (3.63), 255 (3.67) nm; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; positive HRESIMS *m/z* 559.2523 [M + Na]$^+$ (calcd for C$_{28}$H$_{40}$O$_{10}$·Na, 559.2519).

Cytotoxicity Assay.[17] PC-3M human metastatic prostate cancer cells were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM glutamax, 100 U/mL penicillin, and 100 μg/mL streptomycin and were harvested at or above 80% confluence. Cells were plated onto a 96-well clear, flat-bottom plate at a cell density of 2000 per well and were allowed to recover overnight. A serial dilution of drugs was prepared

in medium, the positive control doxorubicin and the negative control DMSO were also prepared in the same medium, and all samples were added onto the plate in quadruplicate. Each plate was incubated for approximately 40 h at 37 °C in a 5% CO_2 atmosphere, and after incubation, 0.4 mg/mL MTT was added per well and the plate incubated for 4 h. After incubation, the medium and MTT were removed and DMSO was added. The plate was read on a Molecular Devices ThermoMax microplate reader at 650 nm, and percent inhibition calculated and plotted to determine IC_{50} (inhibition concentration of 50%) values. An IC_{50} of 1.26 μM was obtained for the positive control (doxorubicin).

References and Notes

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NP0704553