Cytotoxic Diterpenoids from *Euphorbia helioscopia*

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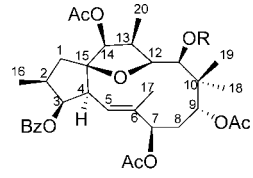
Four new jatrophane-type diterpenoids (**1**−**4**), together with 16 known related compounds, were isolated from the Chinese medicinal plant *Euphorbia helioscopia*. The structures of **1**−**4** were determined on the basis of spectroscopic and chemical methods. Cytotoxicity of the isolated compounds against HeLa and MDA-MB-231 cells was evaluated, with only helioscopinolide A (**5**) and euphornin (**3a**) being active.

The genus *Euphorbia* (Euphorbiaceae) has been reported to be a rich source of skin-irritating, cytotoxic, and tumor-promoting diterpenoids.[1,2] *Euphorbia helioscopia* L. is widely distributed in mainland China[3] and has been used as a traditional folk medicine for the treatment of malaria, bacillary dysentery, and osteomyelitis.[4] Up to now, more than 30 diterpenoids have been isolated and structurally characterized from *E. helioscopia*.[5–9] As a part of our ongoing program on phytochemical investigations on medicinally important *Euphorbia* species,[10] four new jatrophane-type diterpenoids (**1**−**4**), together with 16 known compounds, were isolated from the whole plants of *E. helioscopia*. Details of the isolation and structural elucidation of compounds **1**−**4** are presented herein. The cytotoxicity against HeLa and MDA-MB-231 cancer cells was evaluated for all the compounds obtained.



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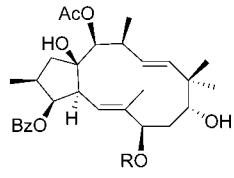

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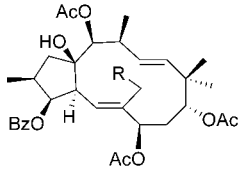

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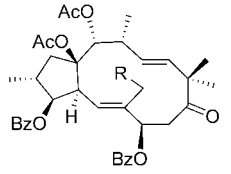

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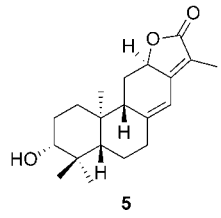

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The NMR data of compounds **1**−**4** showed that they are all jatrophane-type diterpenoids.[7–9]

Compound **1**, a colorless oil, was assigned a molecular formula of $C_{33}H_{44}O_{10}$, as established by its HRESIMS ($[M + Na]^+$ at *m*/*z* 623.2851, calcd 623.2832) and NMR data. The IR spectrum displayed absorption bands at 3421 and 1718 cm^{-1}, indicating the presence of hydroxy and ester functionalities. The NMR data (Tables 1 and 2) showed the presence of three acetoxy groups [δ_H 1.32, 1.99, 2.17 (each 3H, s); δ_C 170.5, 169.4, 169.3 (CO); 21.1, 20.7, 20.0 (CH_3)] and one benzoyloxy group [δ_H 8.05 (2H, d, J = 8.0 Hz), 7.41 (2H, t, J = 8.0 Hz), 7.50 (1H, t, J = 8.0 Hz); δ_C 165.7, 132.6, 130.4, 129.8, and 128.3]. Further signals were observed for the presence of a trisubstituted double bond [δ_H 5.68 (1H, d, J = 11.2 Hz); δ_C 123.0 and 132.1], six oxymethines [δ_H 5.39 (t, J = 6.8 Hz), 4.96 (d, J = 4.8 Hz), 6.20 (d, J = 5.2 Hz), 2.91 (dd, J = 6.4, 11.2 Hz), 3.60 (t, J = 8.8 Hz), and 5.15 (d, J = 4.8 Hz); δ_C 77.0, 73.9, 70.7, 77.4, 84.4, and 80.3], two tertiary methyls [δ_H 0.85, 0.91; δ_C 17.6 and 17.8], one vinyl methyl [δ_H 1.63 (d, J = 1.2 Hz), δ_C 15.5], and two secondary methyls [δ_H 1.05, 1.12 (each, d, J = 6.8 Hz); δ_C 13.9 and 12.8]. The aforementioned data of **1** clearly indicated that it was a jatrophane tetraester bearing a benzoyloxy and three acetoxy groups.[5–9] The carbon skeleton of **1** was assigned by careful analysis of the $^1H-^1H$ COSY, HSQC, and HMBC data (Figure 1). Chemical shift considerations and HMBC correlations indicated that ester moieties were located at hydroxy groups connected to the C-3, C-7, C-9, and C-14 positions, respectively (Figure 1). The molecular formula indicated the presence of 12 units of unsaturation. Therefore, the skeleton of **1** must be tricyclic since only one double bond, one benzene ring group, and four carbonyl groups were found to be present. Acetylation of **1** with acetic anhydride in pyridine afforded only a monoacetate (**1a**) and caused a downfield shift of H-11 ($\Delta\delta$ +1.57 ppm). These observations implied that an ether functionality (deduced from the molecular formula and substituents discussed above) must be present between C-12 and C-15. The relative stereochemistry of **1** was assessed by analyzing the NOESY spectrum (Figure 2), taking into account the data reported for a large number of jatrophane diterpenoids already isolated. The stereochemistry at C-4 and C-15 was proposed from the observation that all jatrophane diterpenoids subjected to X-ray crystallographic analysis exhibit a *trans* ring junction.[11] Since the angular proton H-4 was assumed to be α-oriented on a biogenetic basis,[7–9] the diagnostic NOE correlations of H-4/H-2, H-4/H-3, H-4/H-13, H-4/H-14, H-4/Me-17, H-13/H-14, and H-13/H-17 showed that H-2, H-3, H-13, and H-14 are all in an α-orientation. Meanwhile, the strong NOE effects of H-7/Me-17, H-9/H-5, H-9/Me-19, H-11/H-13, and H-11/Me-18 indicated that H-7, H-11, and Me-18 all have an α-orientation, whereas H-9 and Me-19 are β-oriented. The relative configuration elucidated here is in accordance with those of diterpenoids isolated earlier from *E. helioscopia*.[7–9] Therefore, the structure of **1** was elucidated as 7β,9α,14β-triacetoxy-3β-benzoyloxy-12β,15β-epoxy-11β-hydroxyjatropha-5*E*-ene.

Compound **2**, a colorless oil, was assigned a molecular formula of $C_{29}H_{40}O_7$ from the pseudomolecular ion peak at *m*/*z* 523.2684 (calcd for $C_{29}H_{40}O_7Na$, 523.2672) in the HRESIMS. The NMR data

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**−**4**[a]

position	1	2	3	4 [b]
1α	2.05 m	2.07 m	2.12 m	2.98 dd (4.4, 15.6)
1β	2.05 m	1.77 m	1.79 m	1.48 dd (8.0, 15.6)
2	2.09 m	2.17 m	2.18 m	2.11 m
3	5.39 t (6.8)	5.39 t (4.0)	5.47 s	5.22 dd (2.4, 6.4)
4	3.10 dd (6.8, 11.2)	2.90 dd (5.2, 11.2)	3.11 dd (9.6, 6.0)	3.52 dd (6.8, 9.6)
5	5.68 d (11.2)	5.62 d (11.2)	5.89 d (9.6)	5.97 d (9.6)
6				
7	4.96 d (4.8)	4.20 d (6.0)	5.26 br s	5.67 dd (7.2, 11.2)
8α	1.85 dt (5.2, 16.0)	1.76 m	1.98 m	3.34 dd (8.4, 16.0)
8β	1.68 d (16.0)	1.92 m	1.98 m	2.91 dd (4.4, 16.0)
9	6.20 d (5.2)	3.33 t (3.2)	4.83 br s	
10				
11	2.91 dd (6.4, 11.2)	5.08 d (15.6)	4.99 d (16.0)	5.35 d (16.0)
12	3.60 t (8.8)	5.56 dd (8.8, 15.6)	5.56 dd (9.6, 16.0)	5.13 dd (8.8, 16.0)
13	2.26 m	2.53 m	2.54 m	2.44 m
14	5.15 d (4.8)	4.91 d (2.8)	4.94 br s	5.93 br s
15				
16	1.05 d (6.8)	0.98 d (7.2)	1.00 d (6.4)	1.07 d (6.8)
17	1.63 d (1.2)	1.66 d (1.2)	4.41 d (12.0)	4.33 s
			3.92 d (12.0)	4.35 s
18	0.85 s	0.86 s	0.87 s	1.13 s
19	0.91 s	1.08 s	0.95 s	1.33 s
20	1.12 d (6.8)	0.96 d (7.2)	0.95 d (6.8)	0.93 d (6.8)
OBz-3	8.05 d (8.0)	8.04 d (8.0)	8.04 d (8.0)	7.64 d (8.0)
	7.41 t (8.0)	7.44 t (8.0)	7.44 t (8.0)	7.12 t (8.0)
	7.50 t (8.0)	7.55 t (8.0)	7.54 t (8.0)	7.36 t (8.0)
OAc-7	1.32 s		1.14 s	
OAc-9	1.99 s		1.96 s	
OAc-14	2.17 s	2.22 s	2.22 s	2.20 s
OAc-15				2.19 s

[a] Recorded at 400 MHz in $CDCl_3$. δ_H in ppm, J in Hz. [b] Other signals for **4**: δ OBz-7 7.59 d (8.0), 7.16 t (8.0), 7.32 (8.0). (Assignments of OBz-3 and OBz-7 are interchangeable.)

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **1**−**4**[a]

position	1	2	3	4 [b]
1	38.6 (CH_2)	45.4 (CH_2)	46.4 (CH_2)	43.7 (CH_2)
2	35.0 (CH)	36.7 (CH)	36.4 (CH)	37.9 (CH)
3	77.0 (CH)	81.2 (CH)	81.8 (CH)	86.2 (CH)
4	50.6 (CH)	48.0 (CH)	47.1 (CH)	43.9 (CH)
5	123.0 (CH)	117.9 (CH)	124.2 (CH)	127.4 (CH)
6	132.1 (qC)	139.3 (qC)	138.2 (qC)	139.9 (qC)
7	73.9 (CH)	72.3 (CH)	70.8 (CH)	72.6 (CH)
8	32.2 (CH_2)	36.3 (CH_2)	33.7 (CH_2)	43.5 (CH_2)
9	70.7 (CH)	72.5 (CH)	73.4 (CH)	207.7 (qC)
10	43.1 (qC)	39.7 (qC)	39.8 (qC)	49.1 (qC)
11	77.4 (CH)	140.5 (CH)	139.0 (CH)	133.0 (CH)
12	84.4 (CH)	127.6 (CH)	128.5 (CH)	134.2 (CH)
13	43.4 (CH)	39.7 (CH)	39.5 (CH)	37.7 (CH)
14	80.3 (CH)	80.7 (CH)	80.5 (CH)	75.3 (CH)
15	92.9 (qC)	83.9 (qC)	83.5 (qC)	92.2 (qC)
16	13.9 (CH_3)	13.5 (CH_3)	13.4 (CH_3)	19.2 (CH_3)
17	15.5 (CH_3)	16.2 (CH_3)	60.3 (CH_2)	63.0 (CH_2)
18	17.6 (CH_3)	18.7 (CH_3)	20.5 (CH_3)	25.5 (CH_3)
19	17.8 (CH_3)	22.7 (CH_3)	22.6 (CH_3)	24.7 (CH_3)
20	12.8 (CH_3)	18.9 (CH_3)	19.2 (CH_3)	23.2 (CH_3)
OBz-3	165.7 (qC)	168.1 (qC)	166.3 (qC)	165.2 (qC)
	130.4 (qC)	130.0 (qC)	129.8 (qC)	130.0 (qC)
	129.8 (CH)	129.4 (CH)	129.8 (CH)	129.2 (CH)
	128.3 (CH)	128.4 (CH)	128.5 (CH)	128.0 (CH)
	132.6 (CH)	132.9 (CH)	133.1 (CH)	132.6 (CH)
OAc-7	169.4 (qC)		169.2 (qC)	
	20.0 (CH_3)		19.8 (CH_3)	
OAc-9	169.3 (qC)		169.8 (qC)	
	21.1 (CH_3)		21.0 (CH_3)	
OAc-14	170.5 (qC)	171.0 (qC)	171.0 (qC)	170.1 (qC)
	20.7 (CH_3)	20.8 (CH_3)	20.9 (CH_3)	22.1 (CH_3)
OAc-15				169.9 (qC)
				21.0 (CH_3)

[a] Recorded at 100 MHz in $CDCl_3$. [b] Other signals for **4**: δ OBz-7 166.5 (qC), 129.6 (qC), 129.3 (CH), 128.1 (CH), 132.56 (CH). (Assignments of OBz-3 and OBz-7 are interchangeable.)

of **2** showed a close similarity to those of euphornin B (**2a**), with the only difference being due to the absence of a C-7 acetoxy group

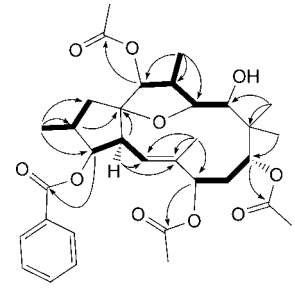

Figure 1. Selected COSY (bold lines) and HMBC (H→C) correlations of **1**.

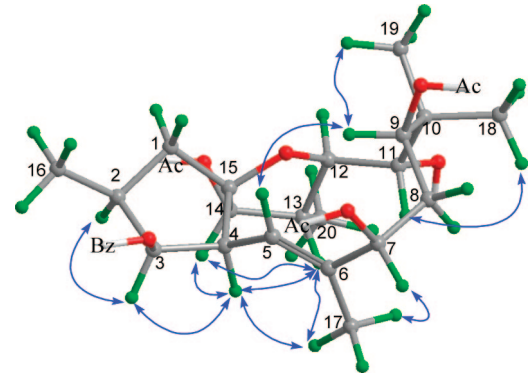

Figure 2. Key NOESY (H↔H) correlations of **1**.

in **2**, which was confirmed by the fact that the chemical shift of H-7 [δ_H 4.20 (1H, d, J = 6.0 Hz)] was shifted upfield by about 0.86 ppm. The NOESY correlations of H-4/H-2, H-4/H-3, H-4/H-13, H-4/H-14, H-4/H-17, H-7/H-17, H-9/H-5, H-9/Me-19, H-11/H-18, and H-12/H-19, as well as the overall NMR data, similar to euphornin B (**2a**), suggested that **2** shared the same relative configuration as **2a**. Therefore, the structure of compound **2** was

assigned as 14β-acetoxy-3β-benzoyloxy-7β,9α,15β-trihydroxyjatropha-5*E*,11*E*-diene.

Compound **3**, a colorless oil, gave a molecular formula of $C_{33}H_{44}O_{10}$, as established on the basis of HRESIMS ([M + Na]$^+$ at *m*/*z* 623.2838). The NMR data of **3** were closely comparable to analogous data of euphornin (**3a**), a jatrophane-type diterpenoid isolated previously from this plant.[7] Spectroscopic analysis revealed that the only structural difference between these two compounds was the presence of a C-17 hydroxy group in **3**. The NMR data of **3** also showed the presence of a hydroxymethyl group [δ_H 4.41 and 3.92 (each 1H, d, J = 12.0 Hz); δ_C 60.3]. The HMBC correlations from H_2-17 to C-5, C-6, and C-7 confirmed the hydroxyl group to be attached to C-17. The relative configuration of **3** was assigned as the same as that of euphornin (**3a**) by comparing their NMR data and the ROESY correlations of H-4/H-2, H-4/H-3, H-4/H-13, H-4/H-14, H-4/H-17, H-7/H-17, H-9/H-5, and H-9/Me-19. Therefore, the structure of **3** was assigned as 7β,9α,14β-triacetoxy-3β-benzoyloxy-15β,17-dihydroxyjatropha-5*E*,11*E*-diene.

Compound **4** was assigned a molecular formula of $C_{38}H_{44}O_{10}$, as established on the basis of HRESIMS at *m*/*z* 683.2850 [M + Na]$^+$ (calcd for $C_{38}H_{44}O_{10}Na$, 683.2832), 16 mass units greater than that of euphoscopin C (**4a**). Detailed analysis of the NMR spectra of **4** and **4a** made it clear that these two compounds are very similar except for the presence of a hydroxy group at C-17 in **4**. The assignments of the proton and carbon NMR signals were also confirmed by the HSQC and HMBC spectra. The NOESY correlations of H-4/H-3, H-4/Me-16, H-4/H_2-17, H-7/H_2-17, H-13/H-11, and H-13/H-14, as well as the overall NMR data's similarity to euphoscopin C (**4a**), suggested that **4** shared the same relative configuration as **4a**. Thus, the structure of **4** was established as 14α,15β-diacetoxy-3β,7β-dibenzoyloxy-17-hydroxy-9-oxo-2βH,13βHjatropha-5*E*,11*E*-diene.

In addition to the new compounds, 16 known compounds were also obtained, including 12 jatrophane-type diterpenoids: euphornin (**3a**),[7] euphornin A,[7] euphornin B (**2a**),[7] euphornin G,[7] euphoheliosnoid A,[8] euphoheliosnoid B,[8] euphoheliosnoid C,[8] euphoscopin A,[7] euphoscopin B,[7] euphoscopin C (**4a**),[7] euphoscopin E,[7] euphoscopin J,[7] one jolkinolide-type diterpenoid, helioscopinolide A (**5**),[12] and three other compounds, dehydrovomifoliol,[13] ficusic acid,[14] and loliolide.[15]

All isolated compounds were evaluated for cytotoxicity against HeLa human cervical carcinoma cells and MDA-MB-231 breast tumor cells. Only two of these compounds, helioscopinolide A (**5**) (IC_{50} 0.11 and 2.1 μM, respectively) and euphornin (**3a**) (IC_{50} 3.1 and 13.4 μM, respectively), were found to be cytotoxic for the HeLa and MDA-MB-231 cells. All other compounds were inactive (IC_{50} > 10 μM) for both cell lines. No clear correlations between structure and cytotoxicity could be found.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectrometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer. EIMS (70 eV) was carried out on a Finnigan-MAT 95 mass spectrometer, and ESIMS was carried out on a Finnigan LC Q^{DECA} instrument. Semipreparative HPLC was performed on a Agilent 1100 with a Agilent DAD spectrophotometer and a Phenomenex ODS column (10 × 250 mm, 5 μm). All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd.), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were also used for column chromatography.

Plant Material. The whole plants of *E. helioscopia* were collected from Xuzhou, Jiangsu Provence, People's Republic of China, in May 2006, and the plant was identified by Prof. Hu-Biao Chen, School of Pharmaceutical Sciences, Peking University, People's Republic of China. A voucher specimen (SC0052006) was deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and powdered whole plants of *E. helioscopia* (4.0 kg) were extracted with 95% EtOH at room temperature (×3, each for 4 days) to give 580 g of crude extract, which was suspended in water (1.0 L) and then partitioned with ethyl acetate to give an ethyl acetate-soluble fraction (130 g). This organic-soluble fraction was chromatographed on a silica gel column eluted successively with a petroleum ether/acetone gradient (100:0 to 0:100) to obtain six fractions. Fraction 2 (39 g) was subjected to a silica gel column eluting with petroleum ether−EtOAc (10:1 to 3:1) to afford four major fractions, F2a−F2d. F2c was extensively subjected to column chromatography over silica gel ($CHCl_3$−EtOAc, 20:1), MCI gel (MeOH−H_2O, 9:1), and Sephadex LH-20 (MeOH−$CHCl_3$, 1:1), followed by semipreparative HPLC (MeOH−H_2O, 85:15), to yield euphoscopin A (4.8 mg), euphoscopin J (10 mg), and euphornin G (5.0 mg). F2d was subjected to column chromatography over MCI gel (MeOH−H_2O, 9:1) and silica gel ($CHCl_3$−EtOAc, 20:1) to obtain four major fractions, which were further purified by semipreparative HPLC (MeOH−H_2O, 80:20) to afford euphoscopins B (3.5 mg), C (2.0 mg) (**4a**), and E (2.8 mg) and **4** (1.8 mg). Fraction 3 (18 g) was subjected to column chromatography over MCI gel, silica gel, and Sephadex LH-20 (ether−$CHCl_3$−MeOH, 2:1:1) to obtain three major fractions, F3a−F3c. F3a was further purified by semipreparative HPLC (MeCN−H_2O, 70:30) to afford **1** (3.0 mg), **2** (2.4 mg), and **3** (2.7 mg). F3b was purified by semipreparative HPLC (MeOH−H_2O, 75:25) to afford euphornin (4.5 mg) (**3a**), euphornin A (12.0 mg), and euphornin B (4.7 mg) (**2a**). F3b was purified by semipreparative HPLC (MeOH−H_2O, 80:20) to afford helioscopinolide A (12.5 mg) (**5**). In the same manner, fraction 4 (15 g) was separated on a silica gel column eluting with petroleum ether−EtOAc (10:1 to 3:1) to afford three major fractions, F4a−F4c. F4b was separated on a Sephadex LH-20 column (eluting with ether−$CHCl_3$−MeOH, 2:1:1) to afford three major farction, F4b1−F4b3. F4b1 was further purified by semipreparative HPLC (MeOH−H_2O, 65:35) to afford euphoheliosnoids A (14 mg), B (5 mg), and C (8.0 mg). F4b2 was further purified by emipreparative HPLC (MeOH−H_2O, 65:35) to afford floliolide (18 mg) and ficusic acid (25 mg). F4b3 was subjected to HPLC (MeOH−H_2O, 70:30) to give dehydrovomifoliol (3.5 mg).

7β,9α,14β-Triacetoxy-3β-benzoyloxy-12β,15β-epoxy-11β-hydroxyjatropha-5*E*-ene (1): colorless oil; $[\alpha]^{25}_D$ +81.0 (*c* 0.10, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 229 (3.95) nm; IR (KBr) ν_{max} 3421, 2966, 1718, 1280 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m*/*z* 623.3 [M + Na]$^+$ (100); HRESIMS *m*/*z* 623.2851 [M + Na]$^+$ (calcd for $C_{33}H_{44}O_{10}Na$, 623.2832).

14β-Acetoxy-3β-benzoyloxy-7β,9α,15β-trihydroxyjatropha-5*E*,11*E*-diene (2): colorless oil; $[\alpha]^{25}_D$ −25.0 (*c* 0.09, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 229 (4.13) nm; IR (KBr) ν_{max} 3469, 2968, 1736, 1452, 1371, 1240 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m*/*z* 523.3 [M + Na]$^+$; HRESIMS *m*/*z* 523.2684 [M + Na]$^+$ (calcd for $C_{29}H_{40}O_7Na$, 523.2672).

7β,9α,14β-Triacetoxy-3β-benzoyloxy-15β,17-dihydroxyjatropha-5*E*,11*E*-diene (3): colorless oil; $[\alpha]^{25}_D$ −16.0 (*c* 0.08, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 229 (4.05) nm; IR (KBr) ν_{max} 3450, 2950, 1723, 1456, 1241 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m*/*z* 623.2 [M + Na]$^+$; HRESIMS *m*/*z* 623.2838 [M + Na]$^+$ (calcd for $C_{33}H_{44}O_{10}Na$, 623.2832).

14α,15β-Diacetoxy-3β,7β-dibenzoyloxy-17-hydroxy-9-oxo-2βH,13βHjatropha-5*E*,11*E*-diene (4): colorless oil; $[\alpha]^{25}_D$ +34.0 (*c* 0.05, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 229 (4.32) nm; IR (KBr) ν_{max} 3462, 2968, 1745, 1703, 1452, 1218 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m*/*z* 623.2 [M + Na]$^+$; HRESIMS *m*/*z* 683.2850 [M + Na]$^+$ (calcd for $C_{38}H_{44}O_{10}Na$, 683.2832).

Acetylation of 7β,9α,14β-Triacetoxy-3β-benzoyloxy-12β,15β-epoxy-11β-hydroxyjatropha-5*E*-ene (1). A solution of **1** (1.2 mg) in pyridine (0.3 mL) was added to acetic anhydride (0.3 mL), and the mixture was stirred at room temperature for 24 h. After evaporation of excess reagent under vacuum, the residue was separated by column chromatography of silica gel eluted with petroleum ether−EtOAc (2:1) to give compound **1a** (0.6 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 6.11 (1H, d, J = 4.0 Hz) (H-9), 5.65 (1H, d, J = 12.0 Hz) (H-8), 5.39 (1H, t, J = 8.0 Hz) (H-3), 5.16 (1H, d, J = 4.0 Hz) (H-14), 5.00 (1H, d, J = 4.4 Hz) (H-7), 4.48 (1H, d, J = 9.6 Hz) (H-11), 3.80 (1H, t, J = 5.2 Hz) (H-12), 3.12 (1H, dd, J = 7.2, 12.0 Hz) (H-4), 2.17 (3H, s),

2.05 (3H, s), 1.99 (3H, s), 1.75 (3H, d, $J = 0.8$ Hz) (Me-17), 1.34 (3H, s), 1.06 (3H, d, $J = 6.4$ Hz), 0.98 (3H, s), 0.93 (3H, d, $J = 6.4$ Hz), 0.70 (3H, s).

Cytotoxicity Assay. Cytotoxity against the HeLa and MDA-MB-231 cells was evaluated by using the MTT method according to the protocols described in previous literature[16] and with adriamycin as a positive control ($IC_{50} = 0.41$ μM against HeLa cells and 0.34 μM against MDA-MB-231 cells).

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Supporting Information Available: 1D and 2D NMR spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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