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Cytotoxic Triterpenoids from the Rhizomes of *Astilbe chinensis*

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Six new triterpenoids (**1**−**6**) with a carboxylic acid functionality at C-27 were isolated from the rhizomes of a Korean native perennial herb, *Astilbe chinensis,* along with nine known triterpenoids. The structures of **1**−**6** were elucidated on the basis of spectroscopic data interpretation. All compounds isolated were evaluated for cytotoxic effects against a small panel of human cancer lines.

Astilbe chinensis (Maxim.) Franch. et Sav. (Saxifragaceae) is a perennial herb found in Korea, mainland China, Japan, and eastern Russia. As an herbal remedy, the rhizomes of *A. chinensis* have been used to treat arthralgia, chronic bronchitis, headache, and stomachalgia.[1,2] In previous investigations, the extracts of *A. chinensis* rhizomes were reported to have potential anti-inflammatory[3] and antitumor activity.[4,5] Triterpenoids with a C-27 carboxylic acid functionality found in some members of the genus of Saxifragaceae are reported as major active compounds and possess anti-inflammatory or cytotoxic effects.[6−10] We describe herein the structure elucidation of six new triterpenoids along with nine known compounds and the evaluation for their cytotoxicity in a small panel of cancer cell lines.

Results and Discussion

Repeated column chromatography of the hexane-soluble fraction of the rhizomes of *A. chinensis* was performed on normal- and reversed-phase silica gel, whereby 14 compounds were isolated. The structures of the new compounds **1**−**6** were elucidated on the basis of 2D NMR spectroscopy and high-resolution ESIMS. The known compounds were in good agreement with previously reported NMR data and were consequently identified as 3β-hydroxyolean-12-en-27-oic acid (**7**),[11] 3α-acetoxyolean-12-en-27-oic acid (**8**),[12] 3β,6β,24-trihydroxyolean-12-en-27-oic acid (**9**),[13] 3β-hydroxyurs-12-en-27-oic acid (**10**),[13] 3β-acetoxy-6β-hydroxyurs-12-en-27-oic acid (**11**),[13] and 3α-acetoxyurs-12-en-27-oic acid (**12**).[14]

Compound **1** was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{30}H_{48}O_4$ on the basis of the $[M - H]^-$ peak at *m/z* 471.3458 (calcd 471.3474) in the HRESIMS. The ^{1}H NMR spectrum showed signals of an olefinic proton at δ 5.78 (1H, t, $J = 2.5$ Hz, H-12), an oxygenated methine proton at δ 3.48 (1H, dd, $J = 11.6$, 4.4 Hz, H-3), an oxygenated methylene at δ 3.71, 4.51 (1H, each, d, $J = 10.9$ Hz, H-24), and six tertiary methyl signals at δ 0.74, 0.90, 1.02, 1.03, 1.10, 1.40 (3H, each, s, H_3-29, 30, 25, 28, 26, 23). The ^{13}C NMR and DEPT spectra revealed 30 carbon signals for **1**, comprised of six methyls, 11 methylenes, five methines, and eight quaternary carbons. The downfield resonances at δ 125.8 and 139.1 were indicative of olefinic carbons, whereas the methine carbon at δ 80.4 and a methylene carbon at δ 65.1 were oxygenated. From the above information, compound **1** was assigned as an olean-12-ene type of triterpene, and its ^{13}C NMR spectroscopic data were similar to those of the known compound **9**. HMBC interactions were observed between protons at δ_H 3.71, 4.51 (H-24), δ_H 1.40 (H-23) and carbons at δ_C 80.4 (C-3), 43.6 (C-4), 56.8 (C-5), a methine at δ_H 3.48 (H-3) and carbons at δ_C 29.0 (C-2), 43.6 (C-4), and a methyl at δ_H 1.03 (H-28) and carbons at δ_C 28.9 (C-16), 33.8 (C-17), 50.3 (C-18), 37.5 (C-22) (Figure 1). The presence of a carbonyl group at C-14 was confirmed by the HMBC interaction between a methylene at δ_H 1.88, 2.43 (H-15) and a carbon at δ_C 178.9 (C-27). The configuration of the C-3 OH group was assigned by the H-3 coupling constant in the ^{1}H NMR spectrum, which appeared as a doublet of doublets at δ_H 3.48 ($J = 11.6$, 4.4 Hz). This implied that H-3 is in an α-orientation. In the NOESY spectrum, correlations between the hydroxymethyl protons at δ_H 3.71 and 4.51 (H-24) and a proton at δ_H 1.02 (H-25) indicated that the configuration of C-24 is β. Thus, the structure of **1** was assigned as 3β,24-dihydroxyolean-12-en-27-oic acid.

Compound **2** was purified as a white, amorphous powder. Its molecular formula was determined to be $C_{32}H_{50}O_5$ on the basis of the $[M - H]^-$ peak at *m/z* 513.3567 (calcd 513.3580) in the HRESIMS. The NMR data of compound **2** were quite similar to those of compound **1** (Tables 1 and 2), but differences were found in the resonances of the attached functional groups. HMBC interactions between δ_H 1.28 (H-24)/δ_H 0.90 (H-23) and δ_C 81.4 (C-3)/37.9 (C-4)/51.4 (C-5), δ_H 4.53 (H-3) and δ_C 29.2 (C-2)/37.9 (C-4)/172.7 (C-31), δ_H 4.32 (H-6) and δ_C 51.4 (C-5), and δ_H 2.00 (H-32) and δ_C 172.7(C-31) indicated that a hydroxy group and an acetate are attached to C-6 and C-3, respectively. The configuration of the acetyl group was determined by the coupling constant of H-3 in the ^{1}H NMR spectrum, in which a very narrow triplet at δ_H 4.53 ($J = 2.4$ Hz) indicated the proton of H-3 to be equatorial with the acetate in an α-orientation. The configuration of the C-6 OH group was assigned as β on the basis of the magnitude of the coupling constant of H-6, as reported previously.[16] In contrast, the configuration of OH-6α in missourin appeared as a doublet of triplets with J values of 11.4 and 7.2 Hz.[17] Thus, the structure of compound **2** was determined as 3β-acetoxy-6β-hydroxyolean-12-en-27-oic acid.

Compound **3** was purified as a white, amorphous powder. Its molecular formula was assigned as $C_{29}H_{44}O_4$, on the basis of the $[M - H]^-$ peak at *m/z* 455.3143 (calcd 455.3161) in the HRESIMS. The NMR data of compound **3** were similar to those of compound **1** (Tables 1 and 2), but with differences evident in the region of C-4. HMBC interactions were found between a methylene at δ_H 5.96, 6.06 (H-23) and carbons at δ_C 73.4 (C-3), 153.7 (C-4), 53.1 (C-5), a methine at δ_H 4.20 (H-3) and carbons at δ_C 34.0 (C-2), 153.7 (C-4), and a methine at δ_H 4.72 (H-6) and carbons at δ_C 53.1 (C-5), 40.5 (C-8), 39.4 (C-10). Both the C-3 and C-6 hydroxy groups were assigned as having a β-orientation on the basis of the magnitude of their coupling constants in the ^{1}H NMR spectrum.[16,17] Thus, the structure of compound **3** was determined as 3β,6β-dihydroxy-24-norolean-12,4(23)-dien-27-oic acid.

Compound **4** was purified as a white, amorphous powder. Its molecular formula was determined as $C_{30}H_{46}O_3$ on the basis of the $[M - H]^-$ peak at *m/z* 453.3373 (calcd 453.3369) in the HRESIMS. The NMR data of compound **4** were similar to those of compound **1** (Tables 1 and 2), with the main difference found in the resonances of C-5 to C-7. HMBC interactions appeared between δ_H 5.09

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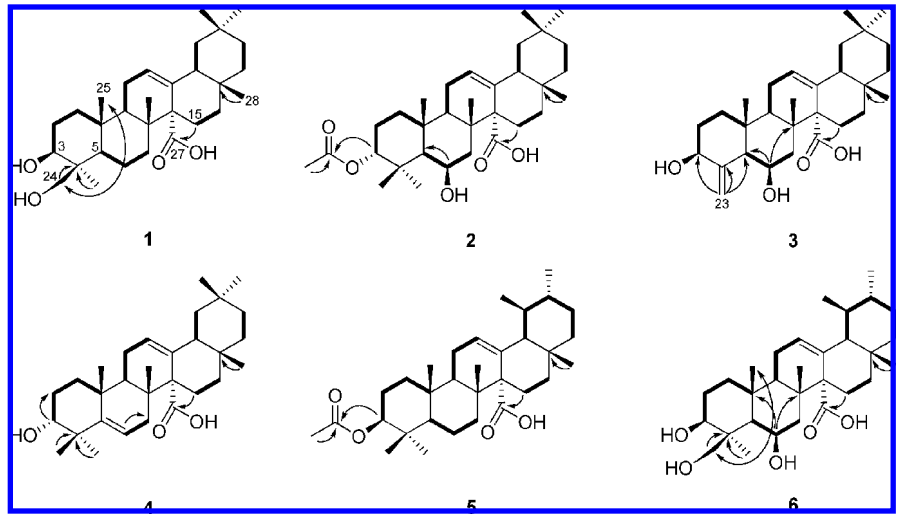

Figure 1. Key HMBC (→) and NOESY (↔) correlations of compounds **1**−**6**.

Table 1. ^{13}C NMR Data (δ) of **1**−**6** (100 MHz, pyridine-d_5)[a]

position	1	2	3	4	5	6
1	39.5	37.5	41.8	35.6	39.0	42.3
2	29.0	29.2	34.0	19.6	24.4	28.9
3	80.4	81.4	73.4	77.1	81.1	79.5
4	43.6	37.9	153.7	41.6	38.3	45.8
5	56.8	51.4	53.1	144.4	56.0	57.9
6	19.7	68.5	69.7	125.6	19.0	66.9
7	38.2	45.0	44.1	25.4	37.7	43.7
8	40.4	40.4	40.5	38.7	40.4	40.2
9	48.0	48.6	46.0	46.3	47.6	48.6
10	37.7	38.5	39.4	36.8	37.6	37.7
11	24.1	23.9	24.9	24.7	23.7	24.1
12	125.8	127.0	126.5	121.3	128.4	128.9
13	139.1	138.7	139.1	132.4	135.2	134.9
14	56.9	57.9	57.7	30.9	57.1	57.7
15	23.5	23.6	23.5	29.4	23.7	23.8
16	28.9	24.0	28.9	30.4	30.2	30.3
17	33.8	34.2	34.0	37.4	34.6	34.6
18	50.3	50.9	50.6	52.0	61.2	61.3
19	44.9	45.5	45.0	42.0	40.3	40.3
20	31.7	32.1	31.7	33.2	38.5	38.5
21	35.2	35.8	35.3	42.7	31.3	31.4
22	37.5	38.0	37.6	34.1	41.8	41.9
23	23.9	28.6	105.8	30.2	28.5	23.5
24	65.1	24.4		26.2	17.5	64.1
25	17.5	17.8	17.6	18.1	17.1	18.6
26	18.9	20.4	21.6	16.1	19.0	21.0
27	178.9	180.5	179.2	172.9	178.3	178.7
28	29.1	29.1	29.2	21.8	29.9	30.0
29	33.9	34.0	34.0	26.0	19.0	19.1
30	24.3	24.3	24.3	17.9	21.9	22.0
OAc		172.7			170.9	
OAc		21.3			21.5	

[a] Assignments are based on the ^{13}C, DEPT, HMQC, and HMBC spectra.

(H-6) and δ_C 25.4 (C-7), δ_H 5.58 (H-12) and δ_C 46.3 (C-9)/24.7 (C-11)/30.9 (C-14), and δ_H 1.54, 1.91 (H-15) and δ_C 172.9 (C-27). The configuration of the hydroxy group at C-3 was ascertained by examining the ^{1}H NMR coupling constants, in which a very narrow triplet for H-3 at δ_H 3.45 (1H, t, $W/2$ = 7.0 Hz) was consistent with an equatorial proton, and hence the C-3 hydroxy group could be assigned with an α-orientation. Thus, the structure of compound **4** was determined to be 3α-hydroxyolean-5,12-dien-27-oic acid.

Compound **5** was obtained as a white, amorphous powder. The molecular formula was determined as $C_{32}H_{50}O_4$, on the basis of the $[M - H]^-$ peak at *m/z* 497.3639 (calcd 497.3631) in the HRESIMS. The ^{1}H NMR spectrum showed an olefinic proton at δ 5.69 (1H, t, J = 2.8 Hz, H-12), an oxygenated methine proton at δ 4.61 (1H, dd, J = 9.9, 6.4 Hz, H-3), six tertiary methyl signals at δ 0.75, 0.90, 0.96, 0.99, 1.11 (3H, each, s, H_3-23, 24, 25, 28, 26), and two secondary methyl signals at δ 0.81 (3H, d, J = 6.3 Hz, H-30) and δ 1.13 (3H, d, J = 6.1 Hz, H-29). The ^{13}C NMR and DEPT spectra revealed 32 carbons, including eight methyls, nine methylenes, seven methines, and seven quaternary carbons. The downfield resonances at δ 128.4 and 135.2 were assigned to the C-12 and C-13 olefinic carbons, respectively. One methine carbon at δ 81.1 was assigned to C-3 (Table 1). From the above information, compound **5** was assumed to be an urs-12-ene-type triterpenoid, similar to the known compound 3β,24-dihydroxyurs-12-en-27-oic acid.[15] HMBC interactions were found between methyl groups at δ_H 0.90 (H-24)/δ_H 0.75 (H-23) and carbons at δ_C 81.1 (C-3), 38.3 (C-4), 56.0 (C-5), a methyl at δ_H 0.99 (H-28) and carbons at δ_C 30.2 (C-16), 34.6 (C-17), 61.2 (C-18), 41.8 (C-22), a methine at δ_H 4.61 (H-3) and carbons at δ_C 24.4 (C-2), 38.3 (C-4), 170.9 (C-31), and a methyl at δ_H 2.04 (H-32) and a carbon at δ_C 170.9 (C-31). The presence of a carbonyl group at C-14 was confirmed by HMBC interactions between δ_H 1.95, 2.40 (H-15) and δ_C 177.3 (C-27) (Figure 1). The configuration of the C-3 acetate group was assigned from the H-3 coupling constant in the ^{1}H NMR spectrum, in which a doublet of doublets appeared at δ_H 4.61 (J = 9.9, 6.4 Hz). This indicated that H-3 is axial, so the C-3 OH group is β-equatorial. Thus, the structure of compound **5** was determined to be 3β-acetoxyurs-12-en-27-oic acid.

Compound **6** was purified as a white, amorphous powder. The molecular formula was found to be $C_{30}H_{48}O_5$ on the basis of the $[M - H]^-$ peak at *m/z* 487.3375 (calcd 487.3423) in the HRESIMS. The ^{1}H NMR spectrum showed an olefinic proton at δ 5.80 (1H, t, J = 3.5 Hz, H-12), an oxygenated methine proton at δ 3.50 (1H, dd, J = 11.9, 4.2 Hz, H-3), an oxygenated methylene at δ 4.45, 4.68 (1H, each d, J = 11.3 Hz, H-24), four tertiary methyl signals at δ 1.02, 1.48, 1.71, 1.77 (3H, each, s, H_3-28, 23, 26, 25), and two secondary methyl signals at δ 0.81 (3H, d, J = 6.3 Hz, H-30) and δ 1.17 (3H, d, J = 6.1 Hz, H-29). The ^{13}C NMR and DEPT spectra revealed 30 carbons, including six methyls, nine methylenes, eight methines, and seven quaternary carbons. The downfield resonances at δ 128.9 and 134.9 were assigned to the C-12 and C-13 olefinic carbons, respectively. Two methine carbons at δ 79.5 and 66.9 were assigned, in turn, to C-3 and C-6, and an oxygenated methylene carbon at δ 64.1 was assigned to C-24 (Table 1). From the above information, compound **6** was determined to be an ursan-12-ene-type triterpene, and its ^{13}C NMR spectroscopic data were seen to be quite similar to those of compound **1**. HMBC interactions were observed between protons at δ_H 4.45, 4.68 (H-24), 1.48 (H-23) and carbons at δ_C 79.5 (C-3), 45.8 (C-4), 57.9 (C-5), a methine at δ_H 3.50 (H-3) and carbons at δ_C 28.9 (C-2), 45.8 (C-4), a methyl

Table 2. ^{1}H NMR (δ) Data for **1**−**6** (400 MHz, pyridine-d_5)[a]

position	1	2	3	4	5	6
1	1.16, 1.68 m	1.29, 1.42 m	1.40, 1.86 m	1.54 m	1.06, 1.58 m	1.31, 1.78 m
2	0.95, 2.07 m	0.83, 2.12 m	1.93, 2.16 m	1.43 m	1.67 m	1.85, 2.10 m
3	3.48 dd, 11.6, 4.4	4.53 t, 2.4	4.20 dd, 11.2, 5.5	3.45 t, *W*/2 = 7.0	4.61 dd, 9.9, 6.4	3.50 dd, 11.9, 4.2
5	1.08 m	1.30 m	1.97 m		0.99 m	1.26 m
6	1.44, 1.72 m	4.32 br s, *W*/2 = 7.2	4.72 br s, *W*/2 = 6.4	5.09 t, 5.6	1.34, 1.47 m	4.84 br s, *W*/2 = 6.4
7	1.73, 1.9 m	1.44, 1.84 m	2.04, 2.35 m	1.95 m	1.68, 1.88 m	2.05, 2.51 m
9	2.75 dd, 11.4, 5.2	2.38 dd, 10.5, 6.2	3.09 dd, 11.1, 5.3	1.46 m	2.75 dd, 11.4, 5.2	3.00 t, 8.3
11	1.96, 2.15 m	1.55, 2.07 m	2.29 m	1.83, 1.95 m	1.93, 2.07 m	2.29 m
12	5.78 t, 2.5	5.61 dd, 4.1, 2.9	5.87 t, 2.1	5.58, br d	5.69 d, 2.8	5.80 t, 3.5
15	1.88, 2.43 m	1.79, 2.02 m	2.02, 2.49 m	1.54, 1.91 m	1.95, 2.40 m	2.06, 2.51 m
16	1.85, 2.48 m	2.01 m	0.94, 2.50 m	1.35 m	1.04, 2.47 m	2.51 m
18	2.18 dd, 13.6, 3.6	2.02 m	2.22 m	2.12 m	1.48 d, 10.6	1.53 d, 11.2
19	1.35, 1.8 m	1.03, 1.43 m	1.38, 1.81 m	1.77 m	0.91 m	0.92 m
20					1.34 m	1.36 m
21	1.06, 1.37 m	1.07, 1.38 m	1.04, 1.37 m	1.33, 1.92 m	1.31 m	1.32 m
22	1.25, 1.49 m	1.22, 1.41 m	1.22, 1.47 m	0.97, 1.66 m	1.31, 1.49 m	1.31, 1.46 m
23	1.40 s	0.90 s	5.96, 6.06 br s	1.03 s	0.75 s	1.48 s
24	3.71 d, 10.9, Hb 4.51 d, 10.9, Ha	1.28 s		1.67 s	0.90 s	4.45 d, 11.3, Hb 4.68 d, 11.3, Ha
25	1.02 s	1.38 s	1.52 s	0.95 s	0.96 s	1.77 s
26	1.1 s	1.28 s	1.74 s	1.03 s	1.11 s	1.71 s
28	1.02 s	0.88 s	1.01 s	1.14 s	0.99 s	1.02 s
29	0.74 s	0.84 s	0.75 s	1.13 s	1.13 d, 6.1	1.17 d, 6.1
30	0.90 s	0.86 s	0.88 s	1.59 s	0.81 d, 6.3	0.81 d, 6.3
OAc		2.00, s			2.04 s	

[a] Assignments are based on the ^{1}H, ^{1}H−^{1}H COSY, HMQC, and HMBC spectra.

Table 3. Cytotoxicity of Compounds from *A. chinensis*[a]

	IC$_{50}$ (μM)[b]	
compound	HL-60	SNU-1
2	5.1	>10
5	9.4	>10
7	8.9	9.3
8	9.3	>10
9	3.5	>10
10	8.2	>10
11	9.6	>10
12	3.9	>10
camptothecin	0.8	
adriamycin[c]		4.0

[a] Compounds **1**, **3**, **4**, and **6** were inactive against all cell lines (IC$_{50}$ >10 μM). [b] All compounds were inactive against the K562, A549, and HepG2 cell lines (IC$_{50}$ >10 μM). [c] Adriamycin was used as a positive control and exhibited (IC$_{50}$ μM): K562 (2.5), A549 (2.2), and HepG2 (1.2).

at δ_H 1.02 (H-28) and carbons at δ_C 30.3 (C-16), 34.6 (C-17), 61.3 (C-18), 41.9 (C-22), and a methine at δ_H 4.84 (H-6) and carbons at δ_C 40.2 (C-8), 37.7 (C-10). The presence of a carbonyl group at C-14 was confirmed by the HMBC interaction between a methylene at δ_H 2.06, 2.51 (H-15) and a carbon at δ_C 178.7 (C-27) (Figure 1). The configuration of the C-3 hydroxy group was assigned as β from the ^{1}H NMR spectrum. As described above for compounds **1** and **5**, the signal at δ_H 3.50 (H-3) appeared as a doublet of doublets (J = 11.9, 4.2 Hz), implying that H-3 is in the axial orientation and the hydroxy group is β. In the NOESY spectrum, the hydroxymethyl protons at δ_H 4.45, 4.68 (H-24) correlated with carbons at δ_C 1.77 (H-25), indicating that the configuration of C-24 is β. Thus, the structure of compound **6** was determined to be 3β,6β,24-trihydroxyurs-12-en-27-oic acid.

All compounds isolated were evaluated against a small panel of human cancer cell lines, and the results are summarized in Table 3. Although all of the compounds tested were cytotoxic in at least one cell line, none of those compounds were very potent, with the HL-60 human promyelocytic leukemia cell line being the most susceptible.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler microhot stage (uncorrected). Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-1601 UV−visible spectrophotometer. NMR spectra were recorded on Varian Unity 400 FT-NMR spectrometer with tetramethylsilane as an internal standard. Chemical shifts are presented in ppm. HRESIMS were measured on a Waters Q-TOF Premier mass spectrometer.

Plant Material. The rhizomes of *A. chinensis* were collected at Jeju, Korea, in July 2005 and dried at room temperature. The plant material was identified by Dr. Joongku Lee (KRIBB), and a voucher specimen (00250) has been deposited at the Plant Extract Bank in KRIBB, Daejeon, Korea.

Extraction and Isolation. The rhizomes of *A. chinensis* (15 kg) were extracted with MeOH at room temperature (3 × 20 L) to obtain 3 kg of a solid extract. The MeOH extract was suspended in H_2O and extracted with hexane (3 × 3 L) to give a hexane-soluble fraction (160 g). A portion of this fraction (155 g) was chromatographed on a silica gel column eluted with a stepwise gradient of hexane and EtOAc, to yield four fractions (fr. A−D: 20, 10, 3, and 120 g). Fr. A was chromatographed on a silica gel column eluted with a hexane−EtOAc stepwise gradient (8:1, 4:1, 2:1), to yield six subfractions (fr. A1−A6: 14, 2, 2, 0.2, 0.1, and 0.1 g). Fr. A1 (14 g) was chromatographed on a silica gel column eluted with $CHCl_3$−MeOH (40:1) to give compound **7** (1.24 g). Compound **8** (77 mg) was obtained from fr. A4 (0.2 g) using a reversed-phase (RP) C_{18} column eluted with CH_3CN−H_2O (10:1). Fr. C (3 g) was chromatographed on a RP C_{18} column eluted with CH_3CN to yield two subfractions (fr. C1−C2: 1.5 and 1 g). Compound **1** (100 mg) was isolated from fr. C1 (1.5 g) using a RP C_{18} column eluted with CH_3CN. Fr. B (10 g) was chromatographed on a RP C_{18} column (CH_3CN−H_2O, 2:1) to yield four subfractions (fr. B1−B4: 0.1, 0.2, 5, and 4 g). Astilbic acid (2.1 g) and 3β,24-dihydroxyurs-12-en-27-oic acid (0.6 g) were obtained from fr. B3 (5 g) using a RP C_{18} column eluted with CH_3CN−H_2O (2:1). Fr. A6 (0.1 g) was chromatographed on a RP C-18 column (CH_3CN−H_2O, 3:1) to give compounds **2** (43 mg), **4** (10 mg), and **11** (17 mg). Compound **9** (253 mg) was isolated from fr. C2 (1 g) using a RP C_{18} column eluted with CH_3CN−H_2O (3:2). Fr. B2 (0.2 g) was chromatographed on a RP C_{18} column (CH_3CN−H_2O, 3:1) to afford compound **3** (12 mg). Compound **5** (12 mg) was obtained from fr. A5 (0.1 g) using a C_{18} column eluted with CH_3CN−H_2O (6:1). Fr. A2 (2 g) was chromatographed on a silica gel column eluted with hexane−EtOAc (4:1) to give compound **10** (1300 mg). Fr. B4 (4 g) was chromatographed on a RP C_{18} column (CH_3CN−H_2O, 3:1) to yield 3β,6β-dihydroxyurs-12-en-27-oic acid (566 mg) and **6** (700 mg). Compound **12** (10 mg) was obtained from fr. A1 (14 g) using a RP C_{18} column eluted with CH_3CN.

3β,24-Dihydroxyolean-12-en-27-oic acid (1): white, amorphous powder; mp 235−236 °C; $[\alpha]^{25}_D$ +110.3 (*c* 0.1, MeOH); UV (MeOH)

λ_{max} (log ε) 203 (4.00) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 471.3458 [M − H]$^-$ (calcd for $C_{30}H_{47}O_4$, 471.3474).

3β-Acetoxy-6β-hydroxyolean-12-en-27-oic acid (2): white, amorphous powder; mp 171−172 °C; $[\alpha]^{25}_D$ +45.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.00) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 513.3567 [M − H]$^-$ (calcd for $C_{32}H_{49}O_5$, 513.3580).

3β,6β-Dihydroxy-24-noroleana-12,4(23)-dien-27-oic acid (3): white, amorphous powder; mp 130−131 °C; $[\alpha]^{25}_D$ +62.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.00) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 455.3143 [M − H]$^-$ (calcd for $C_{29}H_{43}O_4$, 455.3161).

3α-Hydroxyolean-5,12-dien-27-oic acid (4): white, amorphous powder; mp 128−129 °C; $[\alpha]^{25}_D$ +20.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.10) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 453.3373 [M − H]$^-$ (calcd for $C_{30}H_{45}O_3$, 453.3369).

3β-Acetoxyurs-12-en-27-oic acid (5): white, amorphous powder; mp 240−241 °C; $[\alpha]^{25}_D$ +103.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.80) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 497.3639 [M − H]$^-$ (calcd for $C_{32}H_{49}O_4$, 497.3631).

3β,6β,24-Trihydroxyurs-12-en-27-oic acid (6): white, amorphous powder; mp 190−191 °C; $[\alpha]^{25}_D$ +104.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.90) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 487.3375 [M − H]$^-$ (calcd for $C_{30}H_{47}O_5$, 487.3423).

Cytotoxicity Assay. HL-60 (human promyelocytic leukemia), SNU-1 (gastric carcinoma), A549 (lung carcinoma), HepG2 (hepatocellular carcinoma), and K562 (chronic myelogenous leukemia) cells were obtained from the American Type Culture Collection (ATCC). HL-60 cells were cultured in IMDM with 20% fetal bovine serum (FBS) in a CO_2 incubator at 37 °C. A549, SNU-1, K562, and HepG2 cells were cultured in RPMI1640 and MEM with 10% FBS in a CO_2 incubator at 37 °C. All isolates were evaluated against a small panel of human cancer cell lines (Table 3), according to established protocols.[21]

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Supporting Information Available: NMR spectra of compounds **1**−**6**. This information is available free of charge via the Internet at http://pubs.acs.org.

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