Sesquiterpene Esters from *Celastrus orbiculatus* and Their Structure−Activity Relationship on the Modulation of Multidrug Resistance

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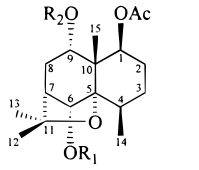
Six new (**1**−**6**) and three known (**7**−**9**) sesquiterpene esters were isolated from the roots of *Celastrus orbiculatus*. The structures of the new compounds were elucidated as 1*β*-acetoxy-6α-furoyloxy-9α-benzoyloxydihydro-*β*-agarofuran (**1**), 1*β*-acetoxy-6α-benzoyloxy-9α-furoyloxydihydro-*β*-agarofuran (**2**), 1*β*-acetoxy-6α,9α-difuroyloxydihydro-*β*-agarofuran (**3**), 1*β*,2*β*-diacetoxy-6α-furoyloxy-9α-benzoyloxydihydro-*β*-agarofuran (**4**), 1*β*-acetoxy-2*β*,6α-difuroyloxy-9α-benzoyloxydihydro-*β*-agarofuran (**5**), and 1*β*-acetoxy-2*β*,6α,9α-tribenzoyloxydihydro-*β*-agarofuran (**6**). Compounds **4**, **5**, and **7**−**9** were shown to be more active than verapamil in reversing vinblastine resistance in multidrug-resistant KB-V1 cells.

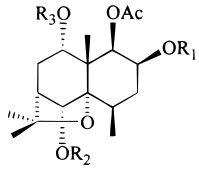
The family Celastraceae is well known for producing various dihydro-*β*-agarofuran derivatives,[1] some of which exhibit insecticidal or insect antifeedant activity,[2] antitumor activity,[3] and antitumor promoting activity.[4] Recently, we reported that several dihydro-*β*-agarofuran compounds reversed multidrug resistance (MDR) in cancer cells.[5]

MDR is one of the major obstacles to cancer chemotherapy. A primary mechanism of classical MDR is attributed to the overexpression of P-glycoprotein (P-gp) in the plasma membranes of resistant cells where the P-gp acts as an energy-dependent efflux pump, reducing intracellular accumulation of anticancer drugs.[6] Although many drugs such as verapamil, nicardipine, and cyclosporins are known to reverse MDR *in vitro* and *in vivo*, they have not been used clinically because of high toxicity.[7] Thus, there appears to be a need to develop new classes of MDR-reversing agents with less toxicity to the host.

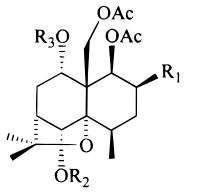
In continuation of a search for MDR-reversing compounds, we isolated six new (**1**−**6**) and three known (**7**−**9**) sesquiterpene esters from the roots of *Celastrus orbiculatus* Thunb. (Celastraceae). Structure elucidation of these sesquiterpene esters, along with the relationship between structure and MDR-reversing activity, are described herein.



1	R_1=Fu	R_2=Bz	
2	R_1=Bz	R_2=Fu	
3	R_1=Fu	R_2=Fu	
10	R_1=Bz	R_2=Bz	
11	R_1=Cin	R_2=Bz	



4	R_1=Ac	R_2=Fu	R_3=Bz
5	R_1=Fu	R_2=Fu	R_3=Bz
6	R_1=Bz	R_2=Bz	R_3=Bz
7	R_1=Ac	R_2=Ac	R_3=Bz
12	R_1=Ac	R_2=Bz	R_3=Bz



8	R_1=H	R_2=Ac	R_3=Bz
9	R_1=OBz	R_2=Ac	R_3=Bz

Results and Discussion

Repeated column chromatography of the dichloromethane-soluble fraction from a methanol extract of the roots of *C. orbiculatus* yielded six new sesquiterpene esters, orbiculin B−G (**1**−**6**) and three known compounds (**7**−**9**).

Orbiculin B (**1**) had molecular formular $C_{29}H_{34}O_8$ by HRMS. Its EIMS and NMR spectra (Tables 1 and 2) suggested the presence of one acetyl ester, one *β*-furoyl ester, and one benzoyl ester. The ^{13}C NMR spectrum revealed four methyls, three methylenes, three oxygenated methines, and three quaternary carbons. The ^1H NMR signals observed at *δ* 5.47 (1H, dd, J = 12.0, 4.2 Hz), 5.49 (1H, s), and 5.04 (1H, d, J = 6.9 Hz) were assigned to the three protons attached to carbons bearing secondary esters. These facts agreed well with the molecular formula $C_{29}H_{34}O_8$. Thus, compound **1** was indicated to be a 1,6,9-triesterified dihydro-*β*-agarofuran compound, as dihydro-agarofuran sesquiterpene esters are commonly found in the Celastraceae family.[1] Assignments of the proton and carbon signals of **1** were confirmed by $^1H-^1H$ and $^{13}C-^{13}C$ COSY spectra. Generally, in this class of compounds, H-1 and H-6 are axial.[1] The stereochemistry of H-6 and H-9 was determined from the NOESY spectrum, which showed cross peaks between H-9 and H-15 and between H-6 and H-14/H-15, indicating that H-6 and H-9 are axial and equatorial, respectively. H-1 (*δ* 5.47) was assigned to be axial from the splitting patterns and coupling constants. The HMBC spectrum demonstrated that the carbonyl signal at *δ* 170.0 correlated with the proton signals at *δ* 5.47 (H-1) and *δ* 1.61, the carbonyl signal at *δ* 165.5 with the signals at *δ* 5.04 (H-9) and *δ* 8.06, and the carbonyl signal at *δ* 162.2 with the signals at *δ* 5.49 (H-6) and *δ* 6.76. From these data, **1** was elucidated to be 1*β*-acetoxy-6α-furoyloxy-9α-benzoyloxydihydro-*β*-agarofuran.

Orbiculin C (**2**) analyzed for molecular formula $C_{29}H_{34}O_8$ by HRMS. The EIMS, ^1H NMR, and ^{13}C NMR spectra indicated that **2** also contained one acetyl, one furoyl, and one benzoyl ester. The ^1H and ^{13}C NMR spectra showed chemical shifts, multiplicity, and coupling constants very similar to those of **1**, except that the proton signals of H-1 and H-9 were shifted upfield (Δ*δ* = 0.11 and 0.16). This

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Table 1. ^{1}H NMR (300 MHz) Data of Compounds **1**−**6** ($CDCl_3$)[a]

position	1	2	3	4	5	6
1	5.47 dd (12.0, 4.2)	5.36 dd (12.0, 3.9)	5.39 dd (11.7, 3.9)	5.53 s	5.61 d (3.6)	5.75 d (3.6)
2	1.87 m	1.80 m	1.85 m	5.53 s	5.73 dd (6.3, 3.3)	5.91 dd (6.6, 3.3)
	1.58 m	1.57 m	1.58 m			
3	2.18 m	2.17 m	2.18 m	2.42 m	2.47 m	
	1.48 m	1.46 m	1.45 m	1.75 dd (13.5, 2.7)	1.86 dd (13.0, 3.0)	2.01 dd (13.8, 3.0)
4	2.30 m	2.35 m	2.31 m	2.36 m	2.4 m	2.60 m
6	5.49 s	5.45 s	5.45 s	5.52 s	5.56 s	5.73 s
7	2.31 dd (3.0, 3.0)	2.29 dd (3.0, 3.0)	2.29 m	2.28 dd (3.0, 3.0)	2.3 m	2.43 dd (3.0, 3.0)
8	2.48 ddd (16.8, 6.9, 3.0)	2.41 ddd (16.5, 7.2, 3.3)	2.43 m	2.5 m	2.47 m	2.59 m
	2.23 m	2.13 m	2.18 m	2.15 dd (16.5, 3.3)	2.20 dd (16.5, 3.3)	2.25 dd (16.5, 3.5)
9	5.04 d (6.9)	4.88 d (6.9)	4.91 d (6.9)	4.93 d (6.6)	4.94 d (7.2)	5.05 d (6.6)
12	1.42 s	1.36 s	1.39 s	1.36 s	1.37 s	1.47 s
13	1.42 s	1.36 s	1.39 s	1.36 s	1.37 s	1.49 s
14	1.02 d (7.2)	0.94 d (7.8)	0.99 d (7.2)	1.16 d (8.1)	1.21 d (7.8)	1.34 d
15	1.36 s	1.29 s	1.33	1.44 s	1.50 s	1.61
Ac	1.61	1.68 s	1.72	1.54 s	1.54 s	1.66
Ac				1.94 s		

[a] **1**: 1× Fu: δ 8.03 (1H, s), 7.46 (1H, d, 1.5), 6.76 (1H, d, 1.8), 1× Bz: 8.06 (2H, m), 7.54 (1H, m), 7.43 (2H, m). **2**: 1× Fu: δ 7.97 (1H, s), 7.34 (1H, dd, 1.8, 1.8), 6.69 (1H, d, 1.2), 1× Bz: 7.97 (2H, m), 7.53 (1H, m), 7.40 (2H, m). **3**: 2× Fu: δ 8.01 (2H, s), 7.45 (1H s), 7.39 (1H, s), 6.74 (2H, s). **4**: 1× Fu: δ 7.97 (1H, s), 7.40 (1H, d, 1.8), 6.70 (1H, d, 1.8), 1× Bz: 7.98 (2H, m), 7.49 (1H, m), 7.37 (2H, m). **5**: 2× Fu: δ 8.00 (2H, s) 7.85 (1H, s), 7.40 (1H, d, 1.8), 6.70 (1H, d, 1.8), 6.60 (1H, d, 1.8), 1× Bz: 7.98 (2H, m), 7.36 (3H, m). **6**: 3× Bz: δ 8.03 (6H, m), 7.54 (9H, m).

Table 2. ^{13}C NMR (75 MHz) Data of Compounds **1**−**6** ($CDCl_3$)

position	1	2	3	4	5	6
1	73.7 d	73.6 d	73.5 d	71.1 d	71.2 d	71.3 d
2	21.5 t	21.6 t	21.6 t	69.9 d	70.2 d	70.7 d
3	26.8 t	26.8 t	26.8 t	31.0 t	31.2 t	31.2 t
4	34.3 d	34.4 d	34.3 d	34.1 d	34.0 d	34.1 d
5	90.0 s	90.0 s	89.9 s	89.8 s	89.7 s	90.0 s
6	79.6 d	80.3 d	79.6 d	79.3 d	79.3 d	79.9 d
7	49.0 d	49.0 d	49.0 d	48.9 d	49.0 d	49.9 d
8	32.1 t	32.2 t	32.1 t	31.6 t	31.7 t	31.8 t
9	73.6 d	72.9 d	72.8 d	73.1 d	73.0 d	73.1 d
10	50.6 s	50.5 s	50.4 s	50.0 s	49.9 s	50.9 s
11	82.6 s	82.5 s	82.5 s	82.9 s	83.0 s	83.0 s
12	26.0 q	25.9 q	25.9 q	26.0 q	26.1 q	26.1 q
13	30.7 q	30.8 q	30.7 q	30.8 q	30.8 q	30.9 q
14	17.5 q	17.6 q	17.5 q	18.6 q	18.9 q	19.1 q
15	18.8 q	18.8 q	18.8 q	20.4 q	20.4 q	20.4 q
C=O	170.0 s	170.2 s	170.2 s	170.0 s	169.7 s	169.7 s
C=O	165.5 s	165.7 s	162.2 s	169.6 s	165.5 s	166.1 s
C=O	162.2 s	162.2 s	162.1 s	165.5 s	162.3 s	165.7 s
C=O				162.1 s	162.1 s	165.5 s
Ac	20.8 q	21.0 q	21.0 q	20.4 q	20.6 q	20.8 q
Ac				21.3 q		

suggested that the orientation of ester groups in **2** was different from that in **1**, and that the relative configuration of **2** was the same as that of **1**. The HMBC spectrum of **2** showed cross peaks between the acetate carbonyl signal (δ 170.2) and H-1 (δ 5.36), between the benzoate carbonyl signal (δ 165.7) and H-6 (δ 5.45), and between the furoate carbonyl signal (δ 162.2) and H-9 (δ 4.88). Therefore, compound **2** was elucidated to be 1β-acetoxy-6α-benzoyloxy-9α-furoyloxydihydro-β-agarofuran.

Orbiculin D (**3**), $C_{27}H_{32}O_9$, was determined to contain one acetyl and two furoyl ester groups from the MS, ^{1}H NMR, and ^{13}C NMR spectra. The ^{1}H and ^{13}C NMR spectra showed very parallel chemical shifts, multiplicities, and coupling constants to those of **1** except that the benzoyl ester was substituted for another furoyl ester. The HMBC spectrum showed cross peaks between the acetate carbonyl signal (δ 170.2) and H-1 (δ 5.39) and between the two furoate carbonyl signals (δ 162.1 and 162.2) and H-6 (δ 5.45) and H-9 (δ 4.91). Therefore, **3** was elucidated to be 1β-acetoxy-6α,9α-difuroyloxy-dihydro-β-agarofuran.

Orbiculin E (**4**), $C_{31}H_{36}O_{10}$, contained two acetyl, one furoyl, and one benzoyl ester groups (MS, ^{1}H NMR, and ^{13}C NMR spectra). The ^{13}C NMR spectrum revealed four methyls, two methylenes, six methines, and three quaternary carbons. The ^{1}H NMR signals at δ 5.53 (2H, br s), 5.52 (1H, s), and 4.93 (1H, d, J = 6.6 Hz) were assigned to the four protons attached to the carbons bearing secondary esters. The NMR spectra of **4** were almost identical with those of **12** (orbiculin A).[5] Thus, **4** was deduced to be 1β,2β,6α,9α-tetra-esterifed dihydro-β-agarofuran with the same stereochemistry as that of **12**. In the NOESY spectrum the signal at δ 5.53 (H-1) was correlated with that of H-3ax, and the signal at δ 4.93 (H-9) was correlated with that of H-15. The signal at δ 5.52 (H-6) was correlated with those of H-14/H-15. The HMBC spectrum demonstrated that acetate groups were attached to C-1 and C-2, a furoate was attached to C-6, and a benzoate was attached to C-9. Therefore, **4** was determined to be 1β,2β-diacetoxy-6α-furoyloxy-9α-benzoyloxy-dihydro-β-agarofuran.

Orbiculin F (**5**), $C_{34}H_{36}O_{11}$, contained one acetyl, two furoyl, and one benzoyl ester groups. The HMBC spectrum demonstrated that one acetate was attached to C-1, two furoates to C-2 and C-6, and one benzoate to C-9, respectively. Therefore, **5** was determined to be 1β-acetoxy-2β,6α-difuroyloxy-9α-benzoyloxydihydro-β-agarofuran.

Orbiculin G (**6**), $C_{38}H_{40}O_9$, contained one acetyl and three benzoyl ester groups. The HMBC spectrum demonstrated that one acetate was attached to C-1, and three benzoates to C-2, C-6, and C-9, respectively. Therefore, **6** was determined to be 1β-acetoxy-2β,6α,9α-tribenzoyloxydihydro-β-agarofuran. Compounds **7**−**9** were identified to be triptogelin C-1, Ejap-2, and 1β,6α,15-triacetoxy-2β,9α-dibenzoyloxydihydro-β-agarofuran, respectively, by comparison of their physical and spectral data with those of previous reports.[8−10]

The cytotoxicities of **1**−**9** and sesquiterpene esters previously isolated from *C. orbiculatus* (**10**−**12**) were measured in both drug sensitive KB-3-1 and multidrug-resistant KB-V1 cells. As shown in Table 3, tri-esters **2, 3**, **10**, and **11** (except **1**) showed moderate toxicity, with IC_{50} values of 8.6−17.8 μM, although **1** showed no toxicity partly because of low solubility. Tetra-esters **4**−**8** and **12** and penta-ester **9** exhibited weak cytotoxicity, with IC_{50} values of 26.4−85.3 μM. All compounds tested exhibited no discernible difference in cytotoxicity between sensitive and resistant cells. However, in the presence of 100 nM of vinblastine (VLB), IC_{50} values of these compounds toward KB-V1 cells were markedly reduced to the range of 0.4−4.8 μM. The

Table 3. Effects of **1**−**12** on the Growth of KB-3−1 and KB−V1 Cell Lines

compounds	IC_{50} (μM)[a] KB-3-1	KB-V1	KB-V1 (+VLB)	EF[b]
1	268.0 ± 26.7	246.0 ± 32.3	19.6 ± 0.7	12.5
2	8.6 ± 0.1	9.6 ± 0.3	1.0 ± 0.2	9.6
3	16.8 ± 1.4	17.8 ± 0.9	1.2 ± 0.2	14.8
4	28.3 ± 2.3	33.1 ± 1.9	0.5 ± 0.2	66.2
5	32.4 ± 6.3	39.3 ± 3.2	1.1 ± 0.1	35.7
6	26.4 ± 1.5	28.6 ± 2.2	4.8 ± 0.1	5.9
7	41.3 ± 2.0	34.8 ± 1.2	0.4 ± 0.0	87.0
8	85.3 ± 6.2	79.4 ± 8.1	1.0 ± 0.1	79.4
9	27.3 ± 2.5	28.6 ± 1.6	0.5 ± 0.0	57.2
10	10.5 ± 0.4	12.3 ± 0.3	1.9 ± 0.0	6.4
11	12.4 ± 0.4	12.4 ± 0.2	0.7 ± 0.0	17.7
12	31.6 ± 0.8	32.0 ± 0.6	0.4 ± 0.0	80.0
VRP	45.2 ± 0.3	39.2 ± 0.5	1.6 ± 0.0	24.5

[a] Data are mean ± S.D. from two separate experiments. [b] EF (enhancement factor) of VLB (100 nM) cytotoxicity by each compound was calculated as follows: IC_{50} of KB-V1 cells without VLB/IC_{50} of KB-V1 cells with VLB.

concentration of VLB added was lethal to drug-sensitive KB-3-1 cells, but had no effect on the growth of drug-resistant KB-V1 cells. Similar effects were observed when each compound was tested against KB-V1 cells in the presence of 10 nM of taxol (TX). These results clearly demonstrated that orbiculins reversed MDR in drug-resistant KB-V1 cells partially or completely to the level of sensitive cells.

MDR-reversing activity of each compound was compared (Table 3) by the cytotoxicity enhancement factor (EF), the ratio of the IC_{50} values in the KB-V1 cells in the absence or presence of VLB. The tri-esters showed relatively weak activity with an EF of 6.4−17.7. Tri-ester compounds, which had an acetoxy group at C-1 and benzoyloxy, furoyloxy, or cinnamoyloxy groups at C-6 and C-9, showed no significant differences in activity. The tetra-esters, except **6**, and the penta-ester exhibited strong reversal activity with EFs of 35.7−87.0. All tetra-ester compounds tested had an acetoxy group at C-1, benzoyloxy groups at C-9, and various groups (acetoxy, furoyloxy, or benzoyloxy) at C-2 and C-6. Of the tetra-esters, **4**, **7**, and **12**, which had an additional acetoxy group at C-2, showed strong reversal activity with EFs of 66.2−87.0. The reversal activity of **5**, with a furoyloxy at C-2, was reduced to about 50% in comparison with **4**. The reversal activity of **6**, with a benzoyloxy at C-2, was markedly reduced to EF 5.9, which was the weakest activity among the compounds tested. Compounds **8** and **9**, which had two acetoxy groups at C-1 and C-15, exhibited strong activity irrespective of the presence of ester group at C-2. These results suggest that the polarity of C-1/C-2 or C-1/C-15 is an important factor in MDR-reversal activity.

To confirm this result, KB-V1 and its drug-sensitive parent KB-3-1 cells were treated with various concentrations of Adriamycin (ADR), VLB, and TX in the presence of tetra- or penta-esters. Compared to KB-3-1 cells, KB-V1 cells were 439.6-fold more resistant to ADR, 942.8-fold to VLB, and 719.1-fold to TX. When KB-V1 cells were treated with various concentrations of ADR, VLB, or TX in the presence of 10 μM of **4**−**9**, **12**, or verapamil (VRP), the relative resistance of KB-V1 cells to each drug was remarkably reduced, as shown in Table 4. These results demonstrated that **4**, **5**, **7**−**9**, and **12** are more active than VRP in reversing MDR in KB-V1 cells. In particular, compounds **4** and **12** restored the sensitivities of KB-V1 cells to the anticancer drugs to the level of drug-sensitive KB-3-1 cells.

Table 4. Effects of Tetra-ester (**4**−**8**, **12**) and Penta-ester (**9**) Compounds on the Resistance of KB-V1 Cells to ADR, VLB, and TX Expressed as Relative Resistance (RR) to KB-3-1 Cells

cell line	compounds	RR[a] ADR	VLB	TX
KB-3-1	none	1	1	1
KB-V1	none	440.0 ± 18.4	943.0 ± 123.4	719.0 ± 54.3
	4	1.8 ± 1.4	1.6 ± 0.9	2.6 ± 0.2
	5	4.4 ± 2.3	8.9 ± 1.9	11.0 ± 0.2
	6	153.0 ± 9.3	168.0 ± 13.2	143.0 ± 21.0
	7	8.3 ± 1.5	2.3 ± 0.3	3.7 ± 0.1
	8	5.5 ± 2.0	12.9 ± 1.2	10.0 ± 0.2
	9	1.4 ± 0.7	2.7 ± 0.1	3.9 ± 0.1
	12	3.0 ± 6.2	0.9 ± 0.1	1.2 ± 0.1
	VRP	7.6 ± 1.9	9.7 ± 1.8	20.0 ± 2.7

[a] Drug-sensitive and -resistant cells were treated with various concentrations of ADR, VLB, and TX in the presence of 10 μM of each compound. Cell growth was measured and RR was calculated as follows: IC_{50} of drug resistant cells/IC_{50} of sensitive parent cells.

Several groups have performed systematic and statistically significant structure−activity relationships of individual classes of MDR-reversing agents such as phenothiazines, thioxanthenes, and indole alkaloids.[11] It was pointed out that most MDR inhibitors apparently interact with P-gp and share some common chemical properties, such as hydrophobicity, conjugated planar rings, and substituted tertiary amino groups. However, compounds such as cyclic peptides and scytophycins have also been reported as MDR-reversing agents.[12] One important factor for MDR-reversing activity is weak polar interaction, including aromatic−aromatic, oxygen−aromatic, and amino−aromatic interactions between P-gp and MDR-reversing agents.[13] The number of phenyl groups in the molecule and charged nitrogen atoms did not seem to be necessarily associated with high MDR-reversal activity. Weak polar interactions are believed to play an important role in stabilizing protein structures and drug−protein binding. Our study also suggests that the polarity of substituents at C-1 and C-2 or C-1 and C-15, which would enhance weak polar interaction with aromatic residues in P-gp, is an important factor for MDR-reversal activity of sesquiterpene esters.

Experimental Section

General Experimental Procedures. Melting points were measured without correction on a Electrothermal model 9100 instrument. Optical rotations were determined on JASCO DIP-181 polarimeter. UV spectra were obtained on a Milton Roy 3000 spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian Unity NMR spectrometer using $CDCl_3$ as a solvent. HMBC spectra were determined on a Bruker AMX 500 NMR spectrometer. EIMS were measured on a Hewlett−Packard 5989A mass spectrometer, and HRMS on a JEOL HX 110 mass spectrometer. Kieselgel 60 (Merck nos. 9385 and 7729) and LiChroprep RP_{18} were used for column chromatography. Preparative HPLC was carried out on a Delta Pak C_{18} column (19 mm × 300 mm, Waters) with UV detection at 230 nm. Fetal calf serum, media, and supplement materials for cell culture were purchased from GIBCO−BRL (Grand Island, NY). Standard anticancer drugs (Adriamycin, vinblastine, and taxol) and verapamil were obtained from Sigma Chemical Co. (St. Louis, MO).

Plant Material. The roots of *C. orbiculatus* were collected at Cheongju, Chungbuk Province, Korea, in October 1995, and identified by Dr. Kyong Soon Lee, a plant taxonomist at Chungbuk National University. A voucher specimen (no. 950821) is deposited in our institute.

Extraction and Isolation. Air-dried plants (4.0 kg) were extracted with MeOH at room temperature. The extract (230 g) was concentrated, diluted in H_2O, and extracted with CH_2-

Cl_2. The CH_2Cl_2-soluble layer (58 g) was chromatographed on a Si gel column using CH_2Cl_2, CH_2Cl_2−MeOH (25:1) as eluents to give six fractions. Fraction 4 (24 g), which contained crude sesquiterpene esters, was subjected to RP_{18} column chromatography (eluent; 75−85% MeOH) to yield fractions 7−18. Compounds **1**−**9** were obtained from preparative HPLC and/or preparative TLC; compound **1** (30 mg) from fraction 10 by preparative HPLC (65% MeOH), compounds **2** (15 mg), **5** (17 mg), and **9** (5 mg) from fraction 11 by successive preparative HPLC (70% MeOH) and preparative TLC [hexane−EtOAc (3:1)], compound **3** (348 mg) from fraction 8 by preparative HPLC (63% MeOH), compound **4** (21 mg) from fraction 9 by preparative HPLC (65% MeOH), compound **6** (14 mg) from fraction 18 by preparative TLC [hexane−EtOAc (3:1)], and compounds **7** (22 mg) and **8** (8 mg) from fraction 7 by preparative HPLC (60% MeOH) and preparative TLC [hexane−EtOAc (2:1)].

Compound 1: white amorphous powder; mp 244−246 °C; $[\alpha]^{25}_D$ +18.67° (*c* 1.66, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.57), 230 (4.27), 275 (3.29) nm; EIMS *m*/*z* 510 $[M]^+$ (18), 294 (31), 234 (25), 159 (18), 105 (81), 95 (100), 77 (35); HRFABMS *m*/*z* 510.2256 (calcd for $C_{29}H_{34}O_8$, 578.2254).

Compound 2: white amorphous powder; mp 68−70 °C; $[\alpha]^{25}_D$ +19.73° (*c* 0.76, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.86), 229 (4.49), 280 (3.63) nm; EIMS *m*/*z* 510 $[M]^+$ (16), 294 (22), 234 (18), 159 (35), 105 (100), 95 (82), 77 (9); HRFABMS *m*/*z* 510.2248 (calcd for $C_{29}H_{34}O_8$, 510.2254).

Compound 3: white amorphous powder; mp 76−78 °C; $[\alpha]^{25}_D$ +16.87° (*c* 2.49, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.56), 230 (4.435), 275 (3.22) nm; EIMS *m*/*z* 500 $[M]^+$ (5), 294 (13), 206 (17), 159 (18), 95 (100), 67 (14); HRFABMS *m*/*z* 500.2053 (calcd for $C_{27}H_{32}O_9$, 500.2046).

Compound 4: white amorphous powder; mp 134−136 °C; $[\alpha]^{25}_D$ +34.67° (*c* 1.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.48), 227 (4.08), 280 (3.20) nm; EIMS *m*/*z* 568 $[M]^+$ (10), 352 (26), 292 (14), 237 (41), 217 (20), 175 (29), 105 (100), 95 (100), 77 (34); HRFABMS *m*/*z* 568.2302 (calcd for $C_{31}H_{36}O_{10}$, 568.2308).

Compound 5: white amorphous powder; mp 98−100 °C; $[\alpha]^{25}_D$ +45.0° (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.81), 229 (4.45), 275 (3.55) nm; EIMS *m*/*z* 620 $[M]^+$ (18), 404 (16), 237 (25), 175 (18), 105 (100), 95 (100) 77 (42); HRFABMS *m*/*z* 620.2248 (calcd for $C_{34}H_{36}O_{11}$, 620.2258).

Compound 6: white amorphous powder; mp 110−112 °C; $[\alpha]^{25}_D$ +47.06° (*c* 0.85, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (5.09), 229 (4.84), 275 (3.97) nm; EIMS *m*/*z* 640 $[M]^+$ (1), 414 (5), 354 (1.8), 237 (8), 204 (5), 105 (100), 77 (29); HRFABMS *m*/*z* 640.2666 (calcd for $C_{38}H_{40}O_9$, 640.2672).

***In Vitro* Cytotoxicity.** Human oral epidermal cancer KB-3-1 and its VLB-selected multidrug-resistant KB-V1 cells were obtained from Dr. M. Gottesman (NCI, Bethesda, MD). KB-3-1 and KB-V1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 2 mM L-glutamine and 10% heat-inactivated fetal calf serum. KB-V1 cells were maintained in the presence of 1 mM VLB. All cells were grown at 37 °C in humidified atmosphere with 5% CO_2. Cell growth was measured using the SRB method.[14] Cells in exponential growth were trypsinized, dispersed in single-cell suspension, and dispensed in 100-mL volumes into 96-well plates. Cells, 2.5−5.0 × 10^3/well, were inoculated in 100 mL of medium containing 5% fetal calf serum and allowed to attach and grow overnight. Medium containing anticancer drug and/or reversing compound (100 μL) was added and further incubated for 48 h. Drugs were dissolved in small amounts of DMSO or MeOH before dilution with medium (final concentration of solvent < 0.5%). Controls were exposed to vehicle-containing medium. Cells were fixed by gently layering 50 mL of cold 50% trichloroacetic acid (final concentrations 10%) on the top of the growth medium in each well, incubated at 4 °C for 1 h, and then washed five times with tap water. Plates were air-dried, stained with 0.4% (w/v) sulforhodamine B in 1% HOAc for 15−30 min, and rinsed four times with 1% HOAc to remove unbound dye. Plates were air-dried, and bound dye was solubilized with 10 mM of unbuffered Tris base on a shaker for 5 min. Absorbance was read with a microtiter plate reader at 570 nm. IC_{50} was the concentration of drug that reduced absorbance to 50% of vehicle-treated controls. The cytotoxicity of each compound was determined in KB-V1 cells in the absence or presence of 100 nM of VLB. The cytotoxicity enhancement of VLB by each compound was expressed as EF, which was calculated as: IC_{50} of KB-V1 cells without VLB/IC_{50} of KB-V1 cells with VLB.

In another set of experiments, the effects of compounds on MDR were studied by exposing cells to a range of concentrations of anticancer drugs, ADR, VLB, and TX in the absence or presence of MDR-reversing compounds. Drug resistance of MDR cells to various anticancer drugs and MDR-reversing effects of each compound were expressed as relative resistance (RR), which was calculated as: IC_{50} of drug-resistant cells/IC_{50} of sensitive parent cells.

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