Reversal of P-Glycoprotein-Mediated Multidrug Resistance by Sipholane Triterpenoids

Sandeep Jain,[†] Surat Laphookhieo,[†] Zhi Shi,[‡,§] Li-wu Fu,[§] Shin-ichi Akiyama,[⊥] Zhe-Sheng Chen,[‡] Diaa T. A. Youssef,[∥] Rob W. M. van Soest,[▽] and Khalid A. El Sayed*[,†]

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe, Louisiana 71209, Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, New York 11439, State Key Laboratory for Oncology in South China, Cancer Center, Sun Yat-Sen University, Guangzhou 510060, People's Republic of China, Department of Molecular Oncology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan, Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt, and Zoological Museum, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

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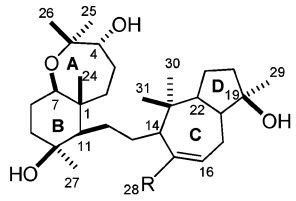
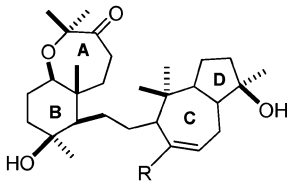
Nineteen triterpenoids, possessing four different skeletons, have been reported so far from the Red Sea sponge *Siphonochalina siphonella*. However, no biological activity of these compounds was ever reported. This study describes the isolation of two new triterpenoids, siphonellinol C (**3**) and sipholenol I (**4**), along with several known sipholane triterpenoids from the Red Sea sponge *Callyspongia* (=*Siphonochalina*) *siphonella*. Allylic oxidation of the major sipholane triterpenoids, sipholenol A (**1**) and sipholenone A (**2**), by selenium dioxide afforded four C-28-oxidized derivatives. Sipholane triterpenoids along with their semisynthetic derivatives were evaluated for their cytotoxicity and effect on reversing P-glycoprotein-mediated MDR to colchicine. Sipholenol A was found to be the most potent, and it increased the sensitivity of resistant KB-C2 cells by 16 times toward colchicine. This is the first report related to reversal of cancer chemotherapy resistance using these triterpenoids.

The marine environment is a rich source of bioactive compounds with significant antitumor, anti-inflammatory, analgesic, immunomodulatory, and antiviral activities.[1] There are significant numbers of preclinical marine natural products or their analogues that are either in or approaching phase II/III clinical trials as anticancer agents, e.g., bryostatin 1, ecteinascidin 743, synthadotin, halichondrin B, and kahalalide F.[1,2] Previously, 19 triterpenoids have been isolated from the Red Sea sponge *Siphonochalina siphonella*, possessing four different skeletons, namely, the sipholane, siphonellane, neviotane, and dahabane.[3−7] Sipholenol A (**1**) and sipholenone A (**2**) are the major sipholane triterpenoids.[4] The sipholanes contain a perhydrobenzoxepine (rings "A" and "B") and a [5,3,0]bicyclodecane system (rings "C" and "D"), linked together through an ethylene bridge.[4,7] The biological activity of these triterpenoids has never been reported. Sodwanones, structurally related polyepoxysqualene-derived triterpenoids, from the Indian Ocean sponge *Axinella weltneri* have been reported to possess cytotoxicity against human tumor cell lines.[8,9] Therefore, we hypothesize that sipholane triterpenoids could be potential anticancer leads or anticancer enhancers.

The ability of cancer cells to develop multidrug resistance (MDR) is one of the major reasons why chemotherapy fails. Various cellular mechanisms that could cause MDR include increased drug efflux, reduced drug uptake, activation of detoxifying proteins, activation of DNA repair, and disruption in apoptotic signaling.[10,11] Among these, P-glycoprotein (P-gp)-mediated increased drug efflux is generally responsible for classical MDR.[10,11] P-gp belongs to a family of ATP-binding cassette (ABC) transporters and acts as an ATP-dependent efflux pump and efficiently removes cytotoxic drugs from cancer cells.[10,11] So far, many agents have been investigated for their ability to reverse P-gp-mediated MDR in cancer patients, e.g., verapamil, the phenothiazines, quinidine, quinacrine, amiodarone, tamoxifen, progesterone, cyclosporin A, dexverapamil, dexniguldipine, GF-902128, PSC-833, and VX-710.[10,11] However, these were found to be either weak inhibitors or associated with serious toxicity at the high doses required for MDR reversal.[10,11]

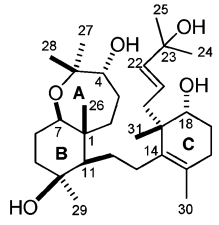
In a search for anticancer metabolites or MDR reversal agents from marine sponges, two new triterpenoids, siphonellinol C (**3**) and sipholenol I (**4**), were isolated from the Red Sea sponge *Callyspongia siphonella*, along with the known sipholenols A and D and sipholenone A. Four new semisynthetic derivatives (**5**−**8**) were prepared by allylic oxidation of **1** and **2** with selenium dioxide/acetic acid to generate structurally diverse analogues. All isolated triterpenoids along with the semisynthetic derivatives were evaluated for their cytotoxicity and reversal effect on P-gp-mediated MDR to the cytotoxicity of colchicine against human epidermoid carcinoma cell lines.





Compound	R
1	CH_3
5	CHO
6	CH_2OH

Compound	R
2	CH_3
7	CHO
8	CH_2OH



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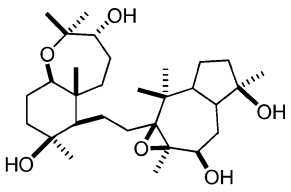

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* To whom correspondence should be addressed. Tel: 318-342-1725. Fax: 318-342-1737. E-mail: elsayed@ulm.edu.
[†] University of Louisiana at Monroe.
[‡] St. John's University.
[§] Sun Yat-Sen University.
[⊥] Kagoshima University.
[∥] Suez Canal University.
[▽] University of Amsterdam.

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

	3[a]		**4**[a]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	42.9, qC		42.9, qC	
2	34.6, CH_2	1.38, 1.52, m	34.5, CH_2	1.46, 1.64, m
3	25.3, CH_2	1.74, 1.98, m	25.3, CH_2	1.68, 2.02, m
4	77.1, CH	3.81, d (6.6)	77.2, CH	3.79, d (6.6)
5	77.8, qC		77.8, qC	
7	76.4, CH	3.52, dd (12.1, 4.4)	76.8, CH	3.48, dd (11.7, 4.4)
8	26.8, CH_2	1.38, 1.71, m	26.8, CH_2	1.37, 1.74, m
9	39.4, CH_2	1.60, m	39.3, CH_2	1.46, 1.64, m
10	72.3, qC		72.7, qC	
11	56.0, CH	0.88, m	55.7, CH	0.83, m
12	26.5, CH_2	1.46, m	21.0, CH_2	1.55, 1.63, m
13	30.1, CH_2	2.04, m	36.7, CH_2	1.50, m 2.22, ddd (14.3, 12.4, 7.3)
14	135.2, qC		70.0, qC	
15	128.9, qC		65.7, qC	
16	32.6, CH_2	2.02, m	69.5, CH	3.99 (dd, 9.9, 6.6)
17	26.4, CH_2	1.70, m	31.3, CH_2	1.28, 2.01, m
18	72.2, CH	3.60, dd (9.6, 2.9)	39.2, CH	1.84, brm
19	43.6, qC		72.2, qC	
20	40.9, CH_2	2.13, dd (14.6, 6.2) 2.27, dd (14.6, 8.1)	30.2, CH_2	1.36, 1.60, m
21	123.6, CH	5.46, ddd (15.8, 8.1, 6.2)	38.0, CH_2	1.21, 1.79, m
22	140.7, CH	5.63, d (15.8)	40.2, CH	2.48, d (4.4)
23	70.8, qC		31.8, qC	
24	29.9, CH_3	1.28, s	13.4, CH_3	1.03, s
25	30.0, CH_3	1.28, s	21.4, CH_3	1.25, s
26	13.2, CH_3	0.98, s	29.2, CH_3	1.12, s
27	21.4, CH_3	1.26, s	29.5, CH_3	1.15, s
28	29.1, CH_3	1.12, s	16.8, CH_3	1.42, s
29	31.0, CH_3	1.23, s	30.4, CH_3	1.14, s
30	20.7, CH_3	1.67, s	25.4, CH_3	1.00, s
31	21.1, CH_3	1.06, s	35.6, CH_3	1.24, s

[a] In $CDCl_3$, 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Coupling constants (J) are in Hz.

Results and Discussion

Two new triterpenoids (**3** and **4**) were isolated along with three known sipholane triterpenoids, sipholenol A (**1**), sipholenone A (**2**), and sipholenol D, from the Red Sea sponge *C. siphonella*. The identification of the known sipholanes was based on extensive analyses of their NMR data and comparison with the literature.[4,12] Siphonellinol C (**3**) contains the siphonellane skeleton and is closely related to the previously reported siphonellinols A and B.[5,7] Sipholenol I (**4**) belongs to the sipholane class and is closely related to sipholenol E.[4]

The HREIMS data of siphonellinol C (**3**) showed a molecular ion peak at m/z 515.3708 $[M + Na]^+$ corresponding to the molecular formula $C_{30}H_{52}O_5$ and suggested five degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Table 1) showed the presence of two double bonds, indicating three rings in the structure. Rings A and B of the molecule were found to be identical to those of siphonellinols A and B.[5,7] The methyl singlet at δ 0.98 was assigned to H_3-26 on the basis of its ^{3}J-HMBC correlation with C-7 (δ 76.4). The methyl singlets H_3-27 (δ 1.26) and H_3-28 (δ 1.12) showed ^{2}J-HMBC correlation with quaternary oxygenated C-5 (δ 77.8). The methyl singlet at δ 1.23 was assigned H_3-29 as evidenced by its ^{2}J-HMBC correlation with C-10 (δ 72.3) and ^{3}J-HMBC correlations with C-9 (δ 39.4) and C-11 (δ 56.0). The right-hand section of the molecule was found to possess a six-membered ring, C, with an attached six-carbon chain. Quaternary olefinic carbons at δ 135.2 and 128.9 were assigned to C-14 and C-15, respectively, through their HMBC correlations with H_3-31 (δ 1.06) and H_3-30 (δ 1.67) in a similar fashion to siphonellinols A and B.[5,7] The methine proton at δ 3.60 (dd, J = 9.6, 2.9 Hz) was assigned to H-18, on the basis of the ^{3}J-HMBC correlation of H_3-31 (δ 1.06) with C-18 (δ 72.2). Olefinic protons resonating at δ 5.46 (ddd, 15.8, 8.1, 6.2 Hz) and 5.63 (d, 15.8 Hz) were assigned as H-21 and H-22, respectively, because both protons were COSY-coupled and showed ^{2}J- and ^{3}J-HMBC correlations with C-23 (δ 70.8). Two methyl singlets at δ 1.28 (H_3-24 and H_3-25) showed ^{2}J-HMBC correlations with C-23 and ^{3}J-HMBC correlations with C-22 (δ 140.7). The stereochemistry of the $\Delta^{21,22}$-system was assigned *E* on the basis of large coupling constant (15.8 Hz) of H-21 and H-22. The splitting pattern and J values of proton H-18 indicated its pseudoaxial orientation. A modeling study of ring C suggested that the pseudoaxially oriented substituent should be in the β-orientation. The H_3-31 singlet showed a NOESY correlation with the β-oriented H-18, suggesting a similar relative stereochemistry.

The HREIMS data of sipholenol I (**4**) suggested the molecular formula $C_{30}H_{52}O_6$ and five degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Table 1) indicated that rings A, B, and D of the molecule were identical to those of sipholenol A. Ring C was found to possess a C-14/C-15 epoxy functionality. The methine protons at δ 3.99 (dd, 9.9, 6.6 Hz) and 2.48 (d, 4.4 Hz) were assigned to H-16 and H-22 and oxygenated quaternary carbons at δ 70.0 and 65.7 were assigned to C-14 and C-15, respectively. This was based on ^{2}J- and ^{3}J-HMBC correlations of H_2-13 (δ 1.50), H-16, H-22, and H_3-28 (δ 1.42) with C-14 and C-15 (Figure 1). It was further supported by strong COSY coupling of H-22 with H-18 (δ 1.84) and ^{3}J-HMBC correlation of H_2-17 (δ 2.01) with C-15. The methyl singlet at δ 1.14 was assigned to H_3-29 on the basis of its ^{2}J- and ^{3}J-HMBC correlations with C-18 (δ 39.2), C-19 (δ 72.2), and C-20 (δ 30.2). Methyl singlets at δ 1.00 and 1.24, assigned to H_3-30 and H_3-31, respectively, showed ^{2}J-HMBC correlation with the quaternary carbon C-23 (δ 31.8) and ^{3}J-HMBC correlation with each other. Further, H-22 showed a ^{3}J-HMBC correlation with the methyl C-30. The relative stereochemistry of the chiral centers C-14 and C-15 is based on NOESY correlations (Figure 1). The methyl singlet H_3-28 showed NOESY correlations with H_3-29 and H_3-30, suggesting its α-orientation. This is further confirmed by NOESY correlation of H_3-28 with the α-oriented H-16. Therefore, the C-14/C-15 epoxide group should be β-oriented, and compound **4** was found to be 14β,15β-epoxysipholenol E.

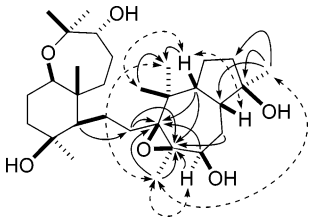

Figure 1. Selected HMBC (plain arrows) and NOESY (dashed arrows) correlations of **4**.

To generate more diverse sipholanes and to test whether semisynthetically oxidized sipholanes would have enhanced biological activities, **1** and **2** were subjected to allylic oxidation reactions. Reaction of **1** with selenium dioxide in acetic acid afforded two new products, **5** and **6**. Compound **5** analyzed for $C_{30}H_{50}O_5$ by HREIMS. The ^{1}H and ^{13}C NMR data suggested it was the aldehyde derivative of **1**. Oxidation at C-28 was evident by replacement of the characteristic H_3-28 singlet (δ 1.75) of **1** with an aldehyde proton singlet (δ 9.39). This signal was correlated with the downfield methine carbon at δ 194.8 in the HETCOR spectrum. Hence, compound **5** was shown to be the C-28 aldehyde of sipholenol A.

The HREIMS data of compound **6** suggested the molecular formula $C_{30}H_{52}O_5$. The ^{1}H and ^{13}C NMR data suggested allylic hydroxylation at C-28 had occurred, as shown by replacement of the characteristic H_3-28 singlet of **1** with a hydroxy methylene group. Two methylene protons at δ 4.00 (br d, 15.5 Hz) and 3.95 (br d, 14.5 Hz) were assigned to H_2-28 on the basis of their ^2J-HMBC correlation with C-15 (δ 145.7) and ^3J-HMBC correlation with C-16 (δ 120.0). Therefore, compound **6** was found to be 28-hydroxysipholenol A.

Similarly, allylic oxidation of **2** by selenium dioxide/acetic acid afforded two C-28-oxidized derivatives (**7** and **8**). Assignments of the structures of **7** and **8** were based on analyses of their ^{1}H and ^{13}C NMR data in a similar fashion to **5** and **6**.

To evaluate the anticancer potential of compounds **1**−**8**, they were first examined for their cytotoxicity against the following cancer cell lines: the parental drug-sensitive cell line KB-3-1, its transfected MRP1 clone cells KB/MRP1, and two drug-selected MDR cell lines, KB-CV60 highly expressing MRP1 and KB-C2 with P-gp overexpression. All compounds showed no cytotoxicity to all four cell lines, with the IC_{50} values being greater than 50 μM.

The ability of compounds **1**−**8** to reverse P-gp- or MRP1-mediated MDR to colchicine in cancer cells was investigated at a noncytotoxic concentration (5 μM). Out of the eight compounds tested, sipholenol A (**1**) showed the best reversal effect and was able to reverse the resistance of KB-C2 cells to colchicine from 578.0-fold to 35.6-fold (Table 2). Sipholenone A (**2**) and siphonellinol C (**3**) showed only 2−3-fold reversal effects, while the other sipholanes were not active. Verapamil was used as a reference and was found to be twice as active as sipholenol A (**1**) in reversing P-gp-mediated MDR. Furthermore, all compounds (**1**−**8**) did not affect the IC_{50} values of colchicine to the parent cell line KB-3-1. Additionally, at similar concentration (5 μM) these compounds had no reversal effect on MRP1-mediated MDR in KB-CV60 cells (data not shown).

First-generation modulators of P-gp-mediated MDR (e.g., verapamil and cyclosporin A) reverse MDR above their therapeutic concentrations, which is associated with enhanced cytotoxicity in normal cells.[11] Sipholenol A (**1**) did not affect the parent cancer cells KB-3-1 (which do not express P-gp) and MRP1-expressing KB-CV60 cells; therefore, it appears to be more selective to P-gp. Substitutions at C-28 with aldehyde (**5** and **7**) or hydroxy (**6** and **8**) groups decreased this MDR-reversing activity. These findings strongly suggest that the sipholane triterpenoid sipholenol A (**1**) has development potential as a P-gp modulator.

Table 2. Effect of Compounds **1**−**8** on Reversal of P-gp-Mediated MDR to the Cytotoxicity of Colchicine[a]

	$IC_{50} \pm SD$[c] (μM) of colchicine	
compound[b]	KB-3-1	KB-C2
control	0.0059 ± 0.0016 (1.0)[d]	3.41 ± 0.47 (578.0)
1	0.0050 ± 0.0017 (0.8)	0.21 ± 0.04 (35.6)
2	0.0052 ± 0.0011 (0.9)	0.93 ± 0.03 (157.6)
3	0.0078 ± 0.0007 (1.3)	1.48 ± 0.27 (250.8)
4	0.0086 ± 0.0005 (1.5)	2.87 ± 1.33 (486.4)
5	0.0050 ± 0.0008 (0.8)	2.85 ± 0.76 (483.1)
6	0.0059 ± 0.0019 (1.0)	5.07 ± 2.45 (859.3)
7	0.0056 ± 0.0012 (0.9)	6.32 ± 3.38 (1071.2)
8	0.0063 ± 0.0008 (1.1)	6.00 ± 1.51 (1016.9)
verapamil	0.0047 ± 0.0021 (0.8)	0.10 ± 0.03 (16.9)

[a] Cell survival was determined by MTT cytotoxicity assay as described in the Experimental Section. [b] Compounds **1**−**8** and verapamil (positive control) were used at a concentration of 5 μM. [c] Data are means ± SD of three independent experiments performed in triplicate. [d] Fold-resistance, shown in parentheses, was calculated by dividing the corresponding IC_{50} value with the IC_{50} value of colchicine for KB-3-1 cells (0.0059 μM).

Experimental Section

General Experimental Procedures. Measurements of optical rotation were carried out on a Rudolph Research Analytical Autopol III polarimeter. IR spectra were recorded on a Varian 800 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, using TMS as an internal standard, on a JEOL Eclipse NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The HREIMS experiments were conducted at the University of Michigan on a Micromass LCT spectrometer. TLC analyses were carried out on precoated silica gel 60 F_{254} 500 μm TLC plates, using the developing systems *n*-hexane/EtOAc (1:4) and $CHCl_3$/MeOH (9:1). For CC, Si gel 60 (particle size 63−200 μm) was used.

Sponge Material. The marine sponge *Callyspongia siphonella* (Levi 1965), formerly known as *Siphonochalina siphonella*, was collected by scuba in June 2003 from Hurghada, at the Egyptian Red Sea coast. The sponge was pink, thin-walled, dichotomously divided, and tubular with a smooth surface. Consistency was compressible, rather soft, but difficult to tear. Height of the voucher was 10 cm, individual tubes 4−5 cm high, diameter 2−2.5 cm, tapering slightly toward the tube openings. Tube walls were approximately 3 mm thick. The ectosomal skeleton was indistinctly double-meshed with primary fibers of 20−30 μm diameter making vaguely delimited meshes of 200−300 μm, and secondary fibers 10−25 μm in diameter enclosing meshes of 50−90 μm. Most fibers were cored by a single spicule, and occasionally two spicules occurred in primary fibers. The choanosomal skeleton was irregularly rectangular, with primary, secondary, and occasionally tertiary fibers of thickness similar to those of the ectosmal skeleton. Primary fibers lie at distances of 150−250 μm and were cored with 1−3 spicules at the periphery. Secondary and tertiary fibers were cored with a single spicule, becoming progressively devoid of coring spicules toward the interior. Choanosomal meshes were 50−150 μm in diameter. Spicules were vestigial strongyles 60−66 μm long and less than 1 μm thick. The voucher specimen could be compared with the type specimen ZMAPOR00198 and conforms in all aspects to it. It is incorporated in the collections of the Zoological Museum of the University of Amsterdam under registration number ZMAPOR16627.

Extraction and Isolation. The frozen sponge (2.95 kg) was extracted with MeOH at room temperature. The MeOH extract was concentrated under vacuum, and the dried extract (88.3 g) was subjected to medium-pressure liquid chromatography (MPLC) using *n*-hexane/EtOAc gradient elution to afford sipholenone A (~1 g), sipholenol A (~1 g), sipholenol D (15 mg), siphonellinol C (**3**, 14 mg, R_f 0.39, $CHCl_3$/MeOH, 9:1), and sipholenol I (**4**, 16 mg, R_f 0.32, $CHCl_3$/MeOH, 9:1). All isolated compounds were purified by MPLC using either fine Si gel 60 (particle size <63 μm) with $CHCl_3$/MeOH gradient elution or C-18 Si gel (Bakerbond, Octadecyl 40 μm) with H_2O/CH_3CN gradient elution.

Preparation of Compounds 5 and 6. To a solution of sipholenol A (100 mg, 0.21 mmol) in acetic acid (5 mL) was added SeO_2 (23 mg, 0.21 mmol), and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was neutralized to pH 7 with 1 M KOH

solution and extracted with $CHCl_3$ (10 mL × 3). The residue obtained after evaporation of the $CHCl_3$ layer was fractionated on a Si gel 60 column using *n*-hexane/EtOAc gradient elution to afford compounds **5** (23 mg, 22.3%, R_f 0.49, $CHCl_3$/MeOH, 9:1) and **6** (17 mg, 16.4%, R_f 0.39, $CHCl_3$/MeOH, 9:1).

Preparation of Compounds 7 and 8. To a solution of sipholenone A (100 mg, 0.21 mmol) in acetic acid (5 mL) was added SeO_2 (23 mg, 0.21 mmol), and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was worked up as described above to afford compounds **7** (21 mg, 20.4%, R_f 0.59, $CHCl_3$/MeOH, 9:1) and **8** (19 mg, 18.4%, R_f 0.46, $CHCl_3$/MeOH, 9:1).

Cell Lines and Cell Culture. KB-3-1, a human epidermoid carcinoma cell line, was the parental drug-sensitive cell line, obtained from Dr. Micheal M. Gottesman at NIH. KB/MRP1 cells were KB-3-1 cells stably transfected with MRP cDNA.[13] An MRP1-mediated MDR mutant, KB-CV60, was also isolated from KB-3-1 cells and maintained in the medium with 1 μg/mL cepharanthine and 60 ng/mL vincristine.[14] KB-C2, a P-gp-mediated MDR mutant, was isolated from KB-3-1 cells and maintained in the medium with 2 μg/mL colchicine.[15] KB/MRP1 cells were kindly provided by Dr. Kazumitsu Ueda (Kyoto University). All the cell lines were grown as adherent monolayers in flasks in DMEM culture medium with 10% bovine serum at 37 °C in a humidified atmosphere of 5% CO_2.

MTT Cytotoxicity Assay. Cells were harvested with trypsin and resuspended in a final concentration of 4×10^4 cells/mL for KB-3-1, 5×10^4 cells/mL for KB/MRP1, and 7.5×10^4 cells/mL for KB-CV60 and KB-C2. Aliquots (180 μL) for each cell suspension were distributed evenly into 96-well multiplates. For cytotoxicity experiments, different concentrations of compounds **1**−**8** (10 μL/well) were added into designated wells, and for MDR reversal experiments, different concentrations of colchicine (10 μL/well) were added into designated wells with or without compounds **1**−**8** (10 μL/well). After 68 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, 20 μL (2 mg/mL), was added to each well, and the plate was further incubated for 4 h, allowing viable cells to change the yellow MTT into dark blue formazan crystal. The medium was discarded, and 100 μL of DMSO was added into each well to dissolve the formazan crystal. The absorbance in individual wells was determined at 570 nm by an OPSYS microplate reader from DYNEX Technologies, Inc. (Chantilly, VA). The concentrations required to inhibit growth by 50% (IC_{50} values) were calculated from survival curves using the Bliss method.[16]

Siphonellinol C (3): colorless oil, $[\alpha]_D^{25}$ −54.2 (*c* 0.28, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3471, 2928, 2856, 1463, 1374, 1082, 908 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 515.3708 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_5Na$, 515.3712).

Sipholenol I (4): colorless oil, $[\alpha]_D^{25}$ −26.5 (*c* 0.63, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3458, 2987, 2927, 2855, 1462, 1377, 1087, 997, 910 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 531.3662 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_6Na$, 531.3662).

Compound 5: amorphous solid, $[\alpha]_D^{25}$ −51.1 (*c* 0.28, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3457, 2985, 2927, 2855, 1682, 1463, 1378, 1084, 910 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.39 (1H, s, H-28), 6.77 (1H, m, H-16), 2.67 (1H, m, H-14), 2.31 (1H, m, H-17a); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.8 (CH, C-28), 152.1 (CH, C-16), 149.8 (C, C-15), 82.0 (C, C-19), 47.2 (CH, C-18), 46.0 (CH, C-14), 26.4 (CH_2, C-17), for detailed ^1H and ^{13}C NMR, see Table 3 in Supporting Information; HREIMS *m*/*z* 513.3553 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3556).

Compound 6: amorphous solid, $[\alpha]_D^{25}$ −59.9 (*c* 0.22, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3447, 2951, 2929, 2858, 1464, 1377, 1083, 910 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.76 (1H, m, H-16), 4.00 (1H, br d, *J* = 15.5 Hz, H-28a), 3.95 (1H, br d, *J* = 14.5 Hz, H-28b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.7 (C, C-15), 120.0 (CH, C-16), 82.2 (C, C-19), 69.3 (CH_2, C-28), 48.8 (CH, C-18), 25.4 (CH_2, C-17), for detailed ^1H and ^{13}C NMR, see Table 3 in Supporting Information; HREIMS *m*/*z* 515.3722 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_5Na$, 515.3712).

Compound 7: amorphous solid, $[\alpha]_D^{25}$ −52.9 (*c* 0.46, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3455, 2983, 2959, 2933, 2858, 1711, 1682, 1463, 1378, 1080, 911 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.37 (1H, s, H-28), 6.77 (1H, m, H-16), 2.64 (1H, m, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.9 (CH, C-28), 152.4 (CH, C-16), 149.6 (C, C-15), 82.0 (C, C-19), 47.1 (CH, C-18), 45.9 (CH, C-14), 26.1 (CH_2, C-17), for detailed ^1H and ^{13}C NMR, see Table 4 in Supporting Information; HREIMS *m*/*z* 511.3398 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_5Na$, 511.3399).

Compound 8: amorphous solid, $[\alpha]_D^{25}$ −28.7 (*c* 0.32, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3447, 2987, 2955, 2931, 2858, 1711, 1463, 1378, 1080, 910 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.71 (1H, m, H-16), 3.88 (1H, br d, *J* = 15.4 Hz, H-28a), 3.80 (1H, br d, *J* = 15.4 Hz, H-28b); ^{13}C NMR ($CDCl_3$, 100 MHz) 145.4 (C, C-15), 119.3 (CH, C-16), 81.9 (C, C-19), 68.6 (CH_2, C-28), 53.4 (CH, C-14), 48.4 (CH, C-18), 24.3 (CH_2, C-17), for detailed ^1H and ^{13}C NMR, see Table 4 in Supporting Information; HREIMS *m*/*z* 513.3559 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3556).

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Supporting Information Available: ^1H, APT, HETCOR, HMBC, COSY, and NOESY NMR spectra of compounds **3** and **4**, detailed NMR data of compounds **5**−**8**, and a representative graph for the reversal of P-gp-mediated MDR to colchicine by sipholenol A and sipholenone A in KB-3-1 and KB-C2 cells are available free of charge via the Internet at http://pubs.acs.org.

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