Sipholane Triterpenoids: Chemistry, Reversal of ABCB1/P-Glycoprotein-Mediated Multidrug Resistance, and Pharmacophore Modeling

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This study reports the isolation of nine new terpenoids (**2**−**10**), possessing two novel skeletons, from the Red Sea sponge *Callyspongia* (=*Siphonochalina*) *siphonella.* The identity of these novel skeletons was based on X-ray crystallography and extensive spectral analyses. These compounds were evaluated for their ability to reverse P-glycoprotein (P-gp)-mediated multidrug resistance in human epidermoid cancer cells. Sipholenone E (**3**) was better than sipholenol A (**1**), a known P-gp modulator from this sponge, in reversing the P-gp-mediated multidrug resistance. Sipholenol L (**6**) and siphonellinol D (**8**) were nearly as active as sipholenol A. On the basis of X-ray crystallographic data and the established identity of **3**−**7**, the structure of sipholenol I (**11**) is revised. A pharmacophore model of three hydrophobic points and two H-bond acceptors was generated for the active sipholane P-gp modulators.

The overexpression of ABCB1/P-glycoprotein (P-gp) is one of the most common mechanisms for the development of multidrug resistance (MDR) in cancer cells (e.g., acute myelogenous leukemia, colon, kidney, adrenocortical, hepatocelluar, breast, and lung cancers).[1,2] The P-gp acts as an efflux pump that derives the energy from ATP hydrolysis to transport the cytotoxic drug molecules out of the cells.[1−3] The P-gp modulators, when coadministered with chemotherapeutic agents, reverse MDR by inhibiting P-gp-mediated drug efflux and resensitize the resistant cancer cells to chemotherapeutic agents.[1−6] The first generation P-gp modulators (e.g., verapamil, cyclosporine A, tamoxifen) require higher doses than their therapeutic doses to reverse P-gp-mediated MDR, resulting in serious side effects.[2−5] The second-generation modulators (e.g., dexverapamil, valspodar, biricodar) are more potent and less toxic than the first generation P-gp modulators.[2−5] However, they exhibit pharmacokinetic interactions with chemotherapeutic agents, increasing the plasma concentration levels of those chemotherapeutic agents beyond acceptable limits.[2−5] The third-generation modulators (e.g., tariquidar, laniquidar, zosuquidar, elacridar, ontogen) modulate the P-gp activity at nanomolar concentrations and exhibit minimal pharmacokinetic interactions with chemotherapeutic drugs.[2−5] Recently, tariquidar has been withdrawn from phase III clinical trials for the treatment of lung cancer due to its toxicity.[4−6] Many third-generation modulators are currently in clinical trials, and their clinical efficacy is yet to be fully evaluated.[4−6]

The Red Sea sponge *Callyspongia* (=*Siphonochalina*) *siphonella* is a rich source of triterpenoids. So far, 21 triterpenoids have been isolated from this sponge, possessing four different skeletons, namely, sipholane, siphonellane, neviotane, and dahabane.[7−12] Among these four types, sipholane triterpenoids are the major group and include sipholenol A (**1**) and sipholenone A.[7,8,11,12] In a previous study, the potential of sipholane triterpenoids as P-gp modulators to reverse MDR in human epidermoid cancer cells was investigated.[12] Sipholenol A was found to potently reverse P-gp-mediated MDR to colchicine, paclitaxel, and vinblastine in the resistant KB-C2 and KB-V1 cells overexpressing P-gp.[12,13] Moreover, sipholenol A was shown to have no effect on cells lacking P-gp expression or cells overexpressing other P-gp-like ATP binding cassette (ABC) transporters, e.g., multidrug resistance associated protein-1 (ABCC1/MRP1) and breast cancer resistance protein/mitoxantrone resistance protein (ABCG2/BCRP/MXR).[13] Sipholenol A potently reversed P-gp-mediated MDR in MDR cancer cells by directly inhibiting the drug efflux function of P-gp, resulting in an increase in the intracellular accumulation of the drug.[13] Sipholenol A stimulated the activity of ATPase of P-gp and inhibited the photoaffinity labeling of the transporter with [^{125}I]-IAAP.[13] The promising activity of sipholenol A as a P-gp modulator encouraged reinvestigation of the source sponge *C. siphonella*, in an attempt to isolate and test additional related analogues and to establish a preliminary structure−activity relationship. This study reports nine new terpenoids (**2**−**10**), possessing two novel skeletons, which were evaluated for their P-gp modulating activity in human epidermoid cancer cells. A pharmacophore modeling study was carried out using the most active sipholanes **1**, **3**, **6**, and **8**.

Results and Discussion

Nine new terpenoids (**2**−**10**) were isolated from a new collection of Red Sea sponge *C. siphonella*, along with the known triterpenoids sipholenols A, G, and sipholenone A.[7,8] The structures of **2**−**10** were elucidated using detailed NMR and HRMS techniques and X-ray crystallography. Compound **2** was found to possess a novel skeleton in which octahydro-2*H*-chromene (rings "A" and "B") and octahydroazulene (rings "C" and "D") systems are connected through an ethylene bridge. It is biogenetically related to **1** possessing identical rings B, C, and D and differs only in ring A, lacking C-5, C-25, and C-26 of **1**. Compounds **3**−**7** were also found to contain a novel skeleton, a perhydrobenzoxepine (rings

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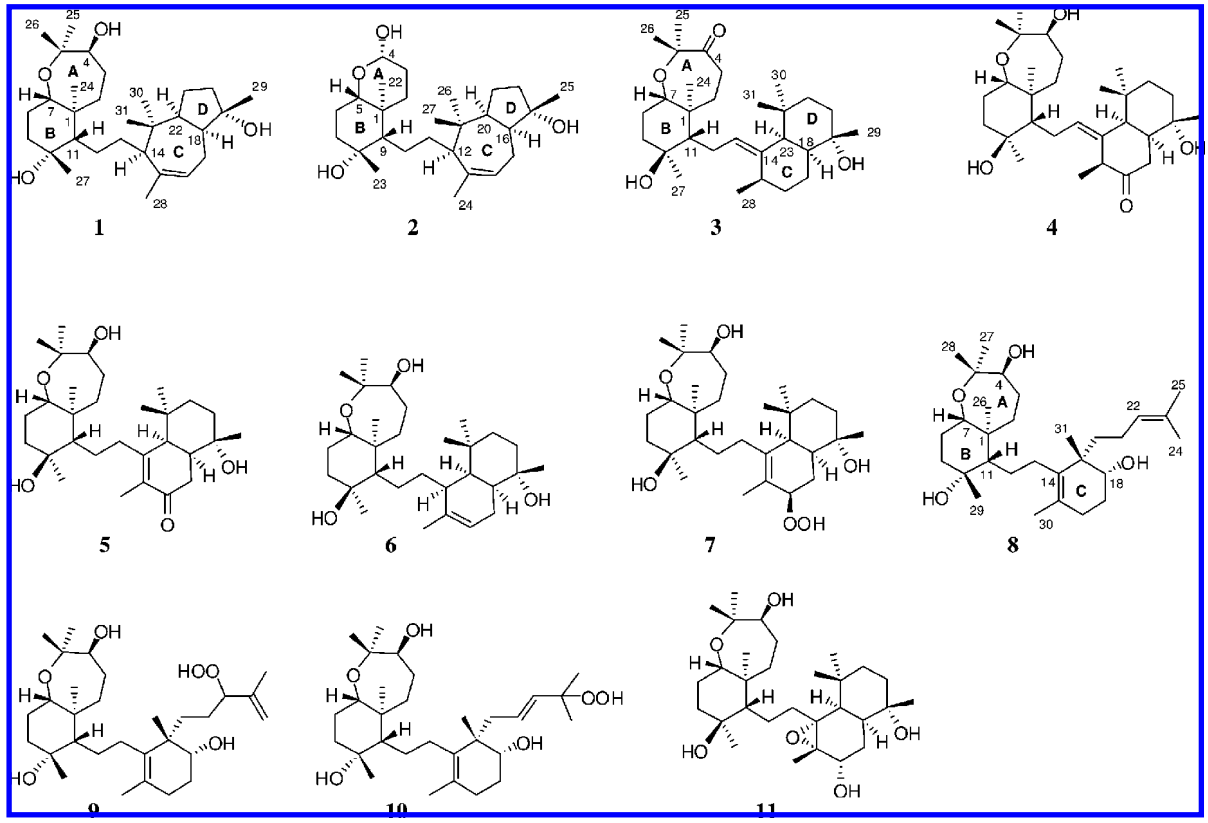

"A" and "B") and a *cis*-decalin (rings "C" and "D") system connected through an ethylene bridge. The new sipholanes **8**−**10** were found to possess the known siphonellane skeleton.[9,11,12]

The HRESIMS data of **2** suggested the molecular formula $C_{27}H_{46}O_4$. The ^{1}H and ^{13}C NMR data (Tables 1 and 3) showed the presence of a double bond, suggesting four rings in the structure to account for five degrees of unsaturation. The oxygenated methine protons at δ 5.23 (d, $J = 9.2$ Hz) and δ 3.31 (dd, $J = 11.9$, 3.9 Hz) were assigned to H-4 and H-5, respectively. The downfield shift of the methine H-4 and its carbon C-4 (δ 97.6) should be due to the two immediate neighboring oxygen atoms. The methyl singlet H_3-22 (δ 1.56) showed ^2J- and ^3J-HMBC correlations with C-1 (δ 37.7), C-2 (δ 30.5), C-5 (δ 82.2), and C-9 (δ 55.3). The methyl singlet at δ 1.43 was assigned to H_3-23 based on its ^2J- and ^3J-HMBC correlations with C-7 (δ 40.3), C-8 (δ 71.9), and C-9. The rings C and D were similar to those of sipholenol A and possessed

Table 1. ^{1}H NMR Data of Compounds **2**−**5**[a]

position	**2**[b]	**3**	**4**	**5**
2	2.01, m	1.25, 1.86, ddd (13.9, 6.4, 2.9)	1.48, 1.62 m	1.44 m
3	2.12, m	2.10, ddd (10.3, 5.9, 2.2) 3.13, ddd (13.6, 11.0, 2.6)	1.73, m, 2.00 m	1.70, m, 2.00 m
4	5.23, d (9.2)		3.80, dd (6.6, 3.0)	3.81, dd (6.6)
5	3.31, ddd (11.9, 3.9)			
6	1.74, m, 2.36, m			
7	1.63, m, 1.95, m	2.95, ddd (10.2, 5.5)	3.53, ddd (11.9, 4.4)	3.53, ddd (11.9, 4.4)
8		1.57, m	1.37, m, 1.71, m	1.39, m, 1.71, m
9	0.85, m	1.44, m, 1.76, m	1.54, m	1.52, m, 1.60, m
10	1.35, m, 2.13, m			
11	1.97, m, 2.32, m	1.29, m	1.07, m	0.99, m
12	1.76, m	2.26, m	2.02, m, 2.51 m	1.48, m, 1.63, m
13		5.25, ddd (7.0, 7.0)	5.28, ddt (9.6, 2.6)	2.15, m, 2.73, m
14	5.49, m			
15	1.96, m, 2.35, m	2.85, m	3.39, m	
16	2.18, m	1.49, m, 1.58, m		
17		1.42, m	2.40, ddd (14.3, 5.5) 2.47, m	2.33, m
18	1.40, m, 1.89, m	1.36, m	1.79, m	2.07, m
19	2.03, −2.25, m			
20	3.06, m	1.39, m, 1.61, m	1.49, m, 1.79, m	1.41, m
21		1.17, m, 1.73, m	1.30, m, 1.92, m	1.26, m 1.81, ddd (13.6, 3.3)
22	1.56, s			
23	1.43, s	2.44, dd (5.5)	3.17, dd (5.1)	2.69, dd (4.8)
24	1.72, s	1.01, s	0.99, s	0.97, s
25	1.46, s	1.23, s	1.12, s	1.11, s
26	1.16, s	1.30, s	1.25, s	1.25, s
27	1.21, s	1.23, s	0.93, s	1.20, s
28		1.11, dd (7.7)	1.15, dd (6.6)	1.87, s
29		1.20, s	1.16, s	1.16, s
30		0.87, s	1.00, s	0.98, s
31		0.94, s	1.16, s	0.73, s

[a] In $CDCl_3$, 400 MHz, J in Hz. [b] In C_5D_5N.

Table 2. ^{1}H NMR Data of Compounds **6**−**10**[a]

position	6	7[b]	8	9	10
2	1.44, 1.59 m	1.56, m	1.56 m	1.54, m	1.52, m
3	1.70, m, 1.99, m	1.67, m, 1.96, m	1.71, m, 2.01, m	1.73, m, 2.01, m	1.72, m, 2.01, m
4	3.80, d (6.6)	3.70, d (7.0)	3.81, d (7.0)	3.81, d (6.6)	3.81, d (6.6)
7	3.49, dd (11.9, 4.4)	3.57, dd (11.7, 4.0)	3.51, dd (12.1, 4.4)	3.52, dd (11.7, 4.4)	3.52, dd (11.9, 4.4)
8	1.38, m, 1.71, m	1.29, m 1.76, m	1.38, m, 1.71, m	1.38, m, 1.71, m	1.38, m, 1.71, m
9	1.52, m	1.48, m, 1.62, m	1.56, m	1.56, m, 1.64, m	1.60, m
11	0.91, m	0.83, m	0.86, m	0.87, m	0.88, m
12	1.48, m	1.45, m	1.27, m, 1.45, m	1.26, m, 1.46, m	1.46, m
13	1.76, m	2.05, m, 2.49, m	1.99, 2.09, m	1.95, 2.12, m	2.07, m
14	2.44, br s				
16	5.24, br s	4.43, dd (7.7, 7.7)	2.03, m	2.02, m	2.01, m
17	1.80, m, 2.10 m	1.88, m, 2.04, m	1.68, m	1.69, m	1.68, m
18	1.86, m	1.64, m	3.70, dd (9.5, 3.3)	3.60, dd (9.7, 2.6)	3.61, dd (10.3, 3.3)
20	1.30, m, 1.73, m	1.40, m, 1.58, m	1.36, m, 1.53, m	1.48, m	2.13, dd (14.3, 6.2), 2.35, dd (14.3, 7.3)
21	1.03, m, 1.73, m	1.16, m, 1.82, m	1.67, m, 1.87 m	1.26, m, 1.39, m	5.61, m
22			5.04, m	4.22, m	5.53, m
23	2.04, m	2.49, d (4.8)			
24	0.98, s	0.98, s	1.56, s	4.99, 5.01, m	1.29, s[c]
25	1.11, s	1.09, s	1.65, s	1.71, s	1.30, s[c]
26	1.25, s	1.22, s	0.97, s	0.98, s	0.98, s
27	1.13, s	1.22, s	1.12, s	1.12, s	1.12, s
28	1.75, s	1.82, s	1.26, s	1.26, s	1.26, s
29	1.15, s	1.17, s	1.22, s	1.24, s	1.23, s
30	1.07, s	0.99, s	1.66, s	1.65, s	1.67, s
31	0.87, s	0.90, s	1.04, s	1.04, s	1.07, s

[a] In $CDCl_3$, 400 MHz, *J* in Hz. [b] In CD_3OD. [c] Interchangeable in the same column.

Table 3. ^{13}C NMR Data of Compounds **2**−**5**[a]

position	2[b]	3	4	5
1	37.7, qC	42.1, qC	42.7, qC	42.9, qC
2	30.5, CH_2	40.8, CH_2	34.6, CH_2	34.7, CH_2
3	37.8, CH_2	35.3, CH_2	25.4, CH_2	25.3, CH_2
4	97.6, CH	217.6, qC	77.0, CH	77.0, CH
5	82.2, CH	82.6, qC	77.9, qC	77.9, qC
6	25.2, CH_2			
7	40.3, CH_2	81.3, CH	76.3, CH	76.3, CH
8	71.9, qC	28.6, CH_2	26.7, CH_2	26.6, CH_2
9	55.3, CH	40.5, CH_2	39.7, CH_2	39.4, CH_2
10	26.4, CH_2	73.2, qC	72.4, Qc	72.3, qC
11	34.4, CH_2	57.9, CH	56.3, CH	56.2, CH
12	57.9, CH	24.0, CH_2	25.7, CH_2	24.7, CH_2
13	143.2, qC	132.6, CH	131.0, CH	40.0, CH_2
14	121.9, CH	140.8, qC	136.5, qC	163.1, qC
15	25.2, CH_2	28.9, CH	49.9, CH	133.0, qC
16	49.1, CH	33.5, CH_2	211.0, qC	199.7, qC
17	80.5, qC	21.2, CH_2	42.4, CH_2	38.2, CH_2
18	36.9, CH_2	50.9, CH	47.6, CH	45.3, CH
19	25.6, CH_2	72.4, qC	71.9, qC	71.6, qC
20	52.9, CH	32.0, CH_2	31.2, CH_2	30.5, CH_2
21	35.6, qC	37.2, CH_2	37.1, CH_2	35.9, CH_2
22	13.3, CH_3	33.3, qC	35.3, qC	35.6, qC
23	30.6, CH_3	50.0, CH	41.5, CH	46.6, CH
24	30.1, CH_3	12.5, CH_3	13.3, CH_3	13.1, CH_3
25	26.0, CH_3	26.6, CH_3	29.2, CH_3	29.1, CH_3
26	29.6, CH_3	20.6, CH_3	21.5, CH_3	21.4, CH_3
27	31.9, CH_3	23.6, CH_3	30.7, CH_3	30.5, CH_3
28		22.4, CH_3	12.3, CH_3	11.8, CH_3
29		30.1, CH_3	29.1, CH_3	28.7, CH_3
30		33.9, CH_3	32.9, CH_3	34.5, CH_3
31		26.4, CH_3	26.4, CH_3	22.7, CH_3

[a] In $CDCl_3$,100 MHz, carbon multiplicities were determined by APT experiments, qC = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons. [b] In C_5D_5N.

the remaining four methyl groups.[7,8] The methine proton H-20 (*δ* 3.06) was coupled with H-16 (*δ* 2.18) and H_2-19 (*δ* 2.03, 2.25) in ^{1}H−^{1}H COSY experiment. Finally, the structure and configuration of **2** was unambiguously established based on X-crystallography data (Figure 1). The absolute configuration of all nine chiral centers was established as 1*S*, 4*S*, 5*S*, 8*R*, 9*S*, 12*R*, 16*S*, 17*S*, and 20*S*. The absolute configuration was determined based on the anomalous X-ray scattering from the oxygen atoms, with the refinement of

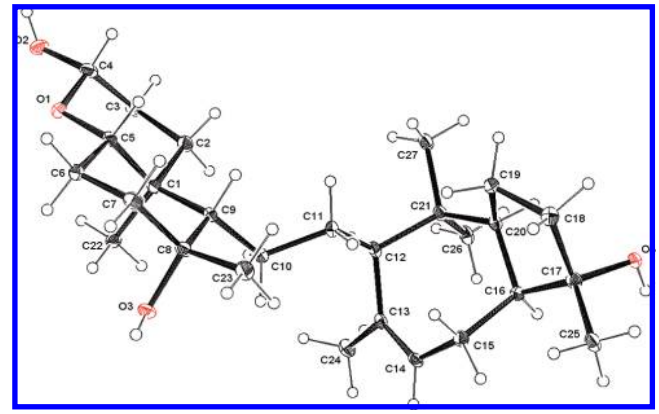

Figure 1. ORTEP-3 projection of compound **2**, with the displacement ellipsoids drawn at the 50% probability level. The cocrystallized molecule of solvent (chloroform) was omitted for clarity.

2110 Bijvoet pairs. The Flack absolute configuration parameter was 0.066(10),[14] where a value close or equal to zero represents the correct structure. Also, the deviation parameter *u*, being 0.0010, satisfies the requirement that "$u < 0.04$ implies a strong inversion-distinguishing power".[15]

The HRESIMS data of sipholenone E (**3**) suggested the molecular formula $C_{30}H_{50}O_4$ and six degrees of unsaturation. The IR spectrum showed a strong peak at 1712 cm^{-1}, corresponding to a ketone group. The ^{1}H and ^{13}C NMR data (Tables 1 and 3) indicated the presence of a double bond and a ketone group, and therefore a tetracyclic structure was proposed. The rings A and B were similar to those of sipholenone A,[7,8] while rings C and D were fused to form a *cis*-decalin system. The olefinic proton at *δ* 5.25 (dd, *J* = 7.0, 7.0 Hz) was assigned to H-13, based on its ^{3}*J*-HMBC correlations (Figure 2) with C-11 (*δ* 57.9), C-15 (*δ* 28.9), and C-23 (*δ* 50.0). This was further supported by the strong COSY couplings of the methylene protons H_2-12 (*δ* 2.26, m) with H-13 and H-11 (*δ* 1.29, m). The unambiguous identity of the latter proton (H-11) was confirmed through the ^{3}*J*-HMBC correlations of the methyl singlets H_3-24 and H_3-27 (*δ* 1.01 and 1.23, respectively) with C-11 (*δ* 57.9). The methyl doublet H_3-28 (*δ* 1.11, *J* = 7.7 Hz) further

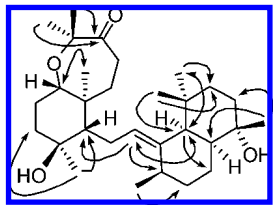

Figure 2. Selected HMBC (plain arrows) correlations of **3**.

supported the assignment of $\Delta^{13,14}$ system via a ^3J-HMBC correlation with C-14 (δ 140.8). The methyl H_3-28 also showed ^2J- and ^3J-HMBC correlations with C-15 and C-16 (δ 33.5), respectively, aiding in assigning ring C. The methyl singlet H_3-29 (δ 1.20) showed ^2J- and ^3J-HMBC correlations with C-18 (δ 50.9), C-19 (δ 72.4), and C-20 (δ 32.0). The methine proton doublet at δ 2.44 was assigned to H-23 because it showed ^3J-HMBC correlations with C-13 (δ 132.6), C-17 (δ 21.2), and C-30 (δ 33.9). Moreover, H-23 showed only one COSY correlation with the methine proton H-18 (δ 1.36, m). Further, both methyl singlets H_3-30 (δ 0.87) and H_3-31 (δ 0.94) showed ^2J-HMBC correlations with the quaternary C-22 (δ 33.3) and ^3J-HMBC correlations with C-23 and C-21 (δ 37.2). This connected ring D with ring C and supported the proposed *cis*-decalin system, though it does not agree with the usual isoprene rule. Finally, X-ray crystallography of **3** (Figure 3) confirmed the proposed unique *cis*-decalin system, connected through a two-carbon linker to a perhydrobenzoxepine system. The absolute configuration of all eight chiral centers was established as 1*S*, 7*S*, 10*S*, 11*S*, 15*R*, 18*S*, 19*S*, and 23*R*. The absolute configuration was determined based on the anomalous X-ray scattering from the oxygen atoms, with the refinement of 2164 Bijvoet pairs. The Flack absolute configuration parameter was determined as $-0.01(13)$,[14] where a value close or equal to zero represents the correct structure. Also, as is the case with **2**, the deviation parameter *u*, being 0.013, satisfies the requirement that "$u < 0.04$ implies a strong inversion-distinguishing power".[15]

The HREIMS data of sipholenol J (**4**) showed a molecular ion peak at *m/z* 490.3650 [M^+] corresponding to the molecular formula $C_{30}H_{50}O_5$. The IR spectrum showed a strong peak at 1707 cm^{-1}, indicating the presence of a ketone group. The six degrees of unsaturation were accounted by one double bond, one ketone group, and four rings, based on ^{1}H and ^{13}C NMR data (Tables 1 and 3). The four rings, A−D, were found to be identical to those of sipholenone E (**3**). The oxygenated methine protons H-4 (δ 3.80, dd, J = 6.6, 3.0 Hz) and H-7 (δ 3.53, dd, J = 11.9, 4.4 Hz) were assigned in a similar fashion to sipholenol A.[7,8] The ketone carbon at δ 211.0 was assigned to C-16, based on its ^3J-HMBC correlation with the methyl doublet H_3-28 (δ 1.15) and ^2J-HMBC correlation with H_2-17 (δ 2.47 and 2.40). The $\Delta^{13,14}$ system was assigned in a similar fashion to **3**. Sipholenone E (**3**) was used as a reference to assign the relative configuration of **4**−**7**. The β-oriented methine proton H-7 showed a strong NOESY correlation with methyl singlet H_3-26 (δ 1.25), which suggested α-orientation for methyl singlet H_3-25 (δ 1.12). The methine proton H-4 showed a strong NOESY correlation with H_3-25, suggesting α-orientation for H-4.

The molecular formula of sipholenol K (**5**) was found to be $C_{30}H_{50}O_5$. The IR spectrum showed a strong peak at 1651 cm^{-1}, suggesting the presence of an α,β-unsaturated ketone group. The ^{1}H and ^{13}C NMR data (Tables 1 and 3) suggested a closely related structure to **4** with a $\Delta^{14,15}$ instead of a $\Delta^{13,14}$ system. The methyl singlet H_3-28 (δ 1.87) showed ^3J- and ^2J-HMBC correlations with the olefinic carbons C-14 (δ 163.1) and C-15 (δ 133.0) and the ketone carbon C-16 (δ 199.7). The methine proton doublet H-23 (δ 2.69) further confirmed this assignment through its ^2J- and ^3J-HMBC correlations with C-14 and C-15, respectively.

The HRESIMS data of sipholenol L (**6**) suggested a molecular formula of $C_{30}H_{52}O_4$. The ^{1}H and ^{13}C NMR data (Tables 2 and 4) indicated a closely related structure to **4**, without any ketone group. The assignment of a $\Delta^{15,16}$ system was based on ^2J- and ^3J-HMBC correlations of the methyl singlet H_3-28 (δ 1.75) with C-15 (δ 135.7) and C-16 (δ 121.8). This was further supported by ^3J-HMBC correlations of the olefinic proton H-16 (δ 5.24) with C-14 (δ 47.2) and C-28 (δ 22.1). Proton H-23 (δ 2.04) showed COSY correlations with protons H-14 (δ 2.44) and H-18 (δ 1.86). Proton H-18 also showed COSY couplings with H_2-17 (δ 2.10 and 1.80), which in turn was COSY-coupled to the olefinic proton H-16. The relative configuration of the new chiral center C-14 was established using a NOESY experiment. Proton H-14 showed strong NOESY correlations with the α-oriented protons H-18 and H-23, suggesting a similar orientation.

The ^{1}H NMR, ^{13}C NMR (Tables 2 and 4), and HRESIMS data of sipholenol M (**7**) suggested a closely related structure to **5**, with a hydroperoxy instead of a ketone group at C-16 (δ 84.9). The downfield oxymethine proton doublet of doublets at δ 4.43 (J = 7.7 and 7.7 Hz) was assigned H-16. This was based on its ^2J- and ^3J-HMBC correlations with C-14 (δ 142.5), C-15 (δ 127.0), and C-28 (δ 15.3). This was further supported by ^2J- and ^3J-HMBC correlations of H_3-28 (δ 1.82) with C-14, C-15, and C-16. The relative configuration of C-16 was deduced using NOESY data. Proton H-16 showed a NOESY correlation with the α-oriented proton H-18 (δ 1.64), indicating a similar orientation.

Siphonellinol D (**8**) was found to possess the siphonellane skeleton with two double bonds, based on its ^{1}H and ^{13}C NMR data (Tables 2 and 4).[9,11] The rings A, B, and C of the molecule were identical to those of siphonellinol C.[12] The olefinic quaternary carbons at δ 135.7 and 128.6 were assigned C-14 and C-15, respectively, in a similar fashion to siphonellinol C.[12] The oxymethine proton H-18 (δ 3.70, J = 9.5, 3.3 Hz) showed ^3J-HMBC correlations with C-16 (δ 30.5) and C-31 (δ 21.5). The ^3J-HMBC correlations of H_3-31 (δ 1.04) with C-14, C-18 (δ 71.6), and C-20 (δ 37.9) further confirmed the assignment of the segment C-14, C-18−C-20. The assignment of a $\Delta^{22,23}$ system was based on ^2J- and ^3J-HMBC correlations of C-22 (δ 124.9) and C-23 (δ 131.4) with H_2-21 (δ 1.67 and 1.87), H_3-24 (δ 1.56), and H_3-25 (δ 1.65). The olefinic proton H-22 (δ 5.04) also showed COSY couplings with H_2-21.

The HRESIMS data of siphonellinol E (**9**) suggested a molecular formula $C_{30}H_{52}O_6$ and five degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 2 and 4) indicated a structure similar to **8**, with a hydroperoxy group at C-22 (δ 90.2). A $\Delta^{23,24}$ system was suggested based on NMR data. The olefinic H_2-24 exomethylene protons (δ 4.99 and 5.01) showed ^3J-HMBC correlations with C-22 and C-25 (δ 17.3). The methyl singlet H_3-25 (δ 1.71) showed ^2J- and ^3J-HMBC correlations with C-22, C-23 (δ 143.5), and C-24 (δ 114.6). There was inadequate spectral evidence to assign the configuration of the C-22 hydroperoxy functionality.

Compound **10** also exhibited the molecular formula $C_{30}H_{52}O_6$. The ^{1}H and ^{13}C NMR data (Tables 2 and 4) suggested an identical structure to siphonellinol C with a C-23 hydroperoxy instead of a hydroxy group.[12] The quaternary carbon C-23 (δ 82.1) was assigned based on its ^2J- and ^3J-HMBC correlations with H-21 (δ 5.61), H-22 (δ 5.53), H_3-24 (δ 1.29), and H_3-25 (δ 1.30). The relative configuration of rings A and B of **8**−**10** was established based on the configuration of **1**, while the relative configuration of ring C was established in a similar fashion to that of siphonellinol C.[12] The natural identity of the hydroperoxide-containing triterpenes **7**, **9**, and **10** could not be confirmed in the fresh extract of *C. siphonella* via the use of TLC. This may be due to their scarce yield in the sponge. Although there are several documented natural plant-derived triterpene hydroperoxides in the literature,[16−18] it is also plausible that compounds **7**, **9**, and **10** are artifactual oxidation byproducts generated during the extraction and isolation process.

Sipholenol I was previously reported as 14β,15β-epoxysipholenol E.[12] In the prior article, the proton doublet at δ 2.48 assigned as H-22 was split as a sharp doublet (J = 4.4 Hz) although it is

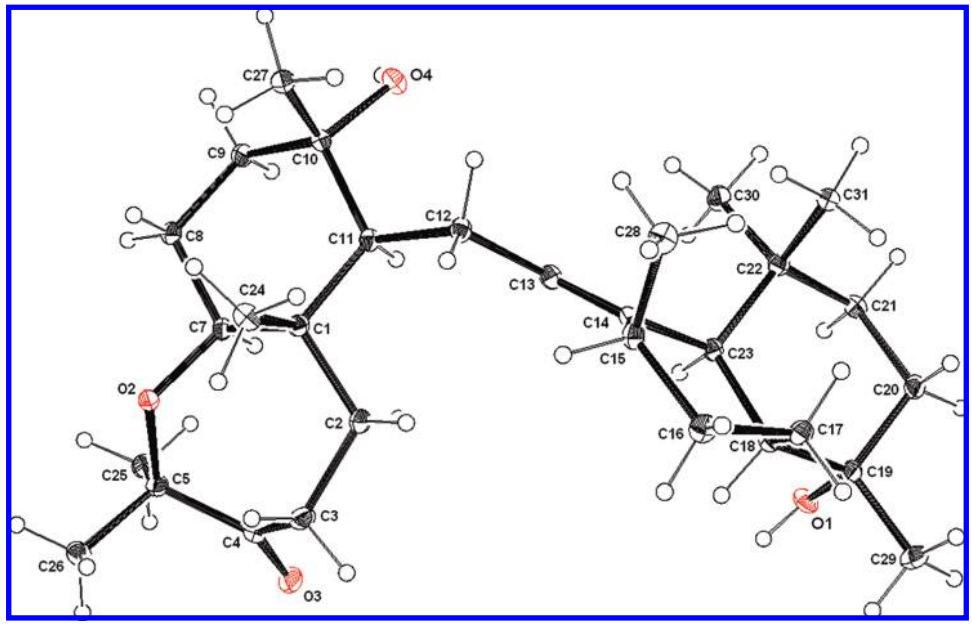

Figure 3. ORTEP-3 projection of compound **3**, with the displacement ellipsoids drawn at the 50% probability level.

Table 4. ^{13}C NMR Data of Compounds **6**−**10**[a]

position	**6**	**7**[b]	**8**	**9**	**10**
1	42.8, qC	42.9, qC	42.9, qC	43.0, qC	43.0, qC
2	34.3, CH_2	34.4, CH_2	34.5, CH_2	34.5, CH_2	34.6, CH_2
3	25.2, CH_2	25.2, CH_2	25.3, CH_2	25.3, CH_2	25.3, CH_2
4	77.0, CH	76.0, CH	77.1, CH	77.1, CH	77.1, CH
5	77.9, qC	78.5, qC	77.8, qC	77.9, qC	77.9, qC
7	76.5, CH	76.7, CH	76.5, CH	76.5, CH	76.4, CH
8	26.7, CH_2	26.5, CH_2	26.7, CH_2	26.9, CH_2	26.7, CH_2
9	39.3, CH_2	38.8, CH_2	39.4, CH_2	39.4, CH_2	39.5, CH_2
10	72.5, qC	71.6, qC	72.3, qC	72.3, qC	72.4, qC
11	56.7, CH	55.4, CH	56.0, CH	55.9, CH	56.0, CH
12	27.6, CH_2	23.8, CH_2	26.6, CH_2	26.7, CH_2	26.5, CH_2
13	36.4, CH_2	36.5, CH_2	32.5, CH_2	32.2, CH_2	32.6, CH_2
14	47.2, CH	142.5, qC	135.7, qC	135.4, qC	135.6, qC
15	135.7, qC	127.0, qC	128.6, qC	129.0, qC	129.0, qC
16	121.8, CH	84.9, CH	30.5, CH_2	30.5, CH_2	30.6, CH_2
17	29.1, CH_2	29.4, CH_2	26.7, CH_2	26.8, CH_2	26.9, CH_2
18	47.7, CH	46.4, CH	71.6, CH	71.6, CH	72.8, CH
19	73.0, qC	71.8, qC	43.4, qC	43.1, qC	44.0, qC
20	30.5, CH_2	30.3, CH_2	37.9, CH_2	33.2, CH_2	41.5, CH_2
21	39.7, CH_2	38.4, CH_2	22.9, CH_2	25.2, CH_2	128.9, CH
22	34.8, qC	33.3, qC	124.9, CH	90.2, CH	135.7, CH
23	42.0, CH	44.2, CH	131.4, qC	143.5, qC	82.1, qC
24	13.3, CH_3	12.3, CH_3	17.8, CH_3	114.6, CH_2	24.6, CH_3
25	29.2, CH_3	28.1, CH_3	25.8, CH_3	17.3, CH_3	24.6, CH_3
26	21.5, CH_3	20.8, CH_3	13.1, CH_3	13.2, CH_3	13.2, CH_3
27	30.4, CH_3	29.3, CH_3	29.2, CH_3	29.2, CH_3	29.2, CH_3
28	22.1, CH_3	15.3, CH_3	21.4, CH_3	21.4, CH_3	21.4, CH_3
29	28.6, CH_3	27.9, CH_3	31.1, CH_3	31.0, CH_3	31.1, CH_3
30	35.6, CH_3	35.2, CH_3	20.7, CH_3	20.7, CH_3	20.7, CH_3
31	24.8, CH_3	23.6, CH_3	21.5, CH_3	21.6, CH_3	20.4, CH_3

[a] In $CDCl_3$, 100 MHz, carbon multiplicities were determined by APT experiments, qC = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons. [b] In CD_3OD.

adjacent to three other protons (H-18 and H_2-21).[12] Proton H-22 also showed a strange ^4J- HMBC correlation with the other epoxy carbon C-15.[12] It was also unusual that both H_3-30 and H_3-31 methyl singlets did not show the expected ^3J-HMBC correlations with the epoxy carbon C-14.[12] Because the structure of **3** was supported by X-ray crystallography, the structure of sipholenol I should be revised to **11**. The presence of a *cis*-decalin system as in **3**−**7** instead of *cis*-octahydroazulene as in sipholenol A (**1**) or the old structure of sipholenol I[12] should be now easily distinguishable by observing the marker proton doublet H-23 in *cis*-decalin-containing sipholanes, while the corresponding proton in the *cis*-octahydroazulene-containing sipholanes (H-22) splits as a complex multiplet. On the basis of the similarity of NMR data (Table S1, Supporting Information), the relative configuration of **11** was reassigned based on the configuration of **3**.

There is a discrepancy in the literature concerning the configuration of sipholenol A (**1**). The original report of sipholenol A by Kashman and co-workers included the 1*R*, 7*R* configuration for the A/B rings, while the configuration of the C/D rings was left ambiguous.[7,8,19] Later, sipholenol A was revised to have the absolute configuration of 1*S*, 7*S* and 18*S*, 22*S* for the A/B and C/D rings, respectively, as determined by the application of high-field NMR and Mosher's ester method.[20,21] However, the older configuration for sipholenol A has occasionally still been used.[11,12] On the basis of X-ray crystallographic data of **2** and **3**, rings A/B and C/D of sipholenol A (**1**) should have the configurations of 1*S*, 7*S* and 18*S*, 22*S*, respectively. These results are consistent with Ohtani and co-workers' Mosher ester-revised configurations[20,21] and should eliminate any future ambiguity concerning the configuration of sipholenol A and related compounds.

The sipholanes **1**−**10** were evaluated for their cytotoxicity against human epidermoid cancer cells, KB-3-1 and KB-C2. The KB-3-1 is a parent, drug-sensitive cell line, while KB-C2 is a MDR cell line overexpressing P-gp. The IC_{50} values of **1**−**10** were found to be greater than 50 μM for both cell lines (data not shown). Later, the ability of these triterpenoids to reverse P-gp-mediated MDR to colchicine was investigated in KB-C2 cells (Table 5). At a noncytotoxic concentration of 10 μM, sipholenone E (**3**) displayed better activity than sipholenol A (**1**) in reversing P-gp-mediated MDR to colchicine. Sipholenol L (**6**) and siphonellinol D (**8**) also showed P-gp modulatory activity comparable to **1**. The terpenoids **2**, **7**, and **9** were less active than **1**, while compounds **4**, **5**, and **10** were inactive. The KB-3-1 cell line was used as a control to check the effect of these compounds on the cells that lack P-gp overexpression. The triterpenoids **3**, **6**, and **8** had a minimal effect on the sensitivity of the parent KB-3-1 cells to colchicine, similar to **1**.

P-gp is one of the most characterized ABC transporters with a variety of substrates and modulators, including anticancer agents, calcium channel blockers, immunosuppressants, neuroleptics, antimalarials, antiarrhythmics, antifungals, and antibacterial agents.[22] P-gp is a pseudosymmetrical molecule consisting of twelve transmembrane domains (TMD) and two nucleotide-binding domains (NBD).[1] The hydrophobic drug substrates bind to TMD, while ATP

Table 5. Effect of Compounds **1**−**10** on Reversing P-gp-Mediated MDR to Colchicine[a]

	$IC_{50} \pm SD$[c] (nM) of colchicine	
compound[b]	KB-3-1	KB-C2
control	5.6 ± 0.7 (1.0)[d]	1300 ± 100 (230)
verapamil[e]	2.8 ± 0.3 (0.5)	30 ± 3 (5.4)
1[e]	4.8 ± 0.1 (0.9)	140 ± 30 (25)
2	5.6 ± 0.5 (1.0)	390 ± 40 (70)
3	4.7 ± 0.4 (0.8)	62 ± 11 (11)
4	4.7 ± 0.3 (0.8)	1700 ± 200 (300)
5	7.1 ± 0.4 (1.3)	1600 ± 200 (290)
6	5.1 ± 0.3 (0.9)	150 ± 10 (27)
7	4.7 ± 0.3 (0.8)	780 ± 60 (140)
8	4.2 ± 0.1 (0.7)	180 ± 10 (32)
9	4.6 ± 0.6 (0.8)	560 ± 50 (100)
10	3.9 ± 0.6 (0.7)	1500 ± 100 (270)

[a] Cell survival was determined by MTT cytotoxicity assay as described in the Experimental section. [b] Compounds were used at a concentration of 10 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 without compound (5.6 nM). [e] Sipholenol A (**1**) and verapamil are the positive controls for reversing P-gp-mediated MDR to colchicine.

binds to the NBD to transport the molecule across the cell membrane.[1,3] The lack of high-resolution 3D structure of P-gp has slowed down the process of discovering new P-gp modulators, to a certain extent.[23] Therefore, pharmacophore mapping and QSAR studies have emerged as very important tools for the development of P-gp modulators.[23] A highly effective P-gp modulator should possess a log $P > 2.92$, an 18-atom long or longer molecular axis, nucleophilicity, and at least one tertiary basic nitrogen atom as suggested by Wang et al.[4,24] Two types (type I and type II) of structural elements required for interaction with P-gp were proposed by Seelig, based on the comparison of various P-gp substrates, inducers, and nonsubstrates.[25] The proposed pharmacophore models contain two electron donor groups separated by either 2.5 ± 0.3 Å (type I) or 4.6 ± 0.6 Å (type II) or three electron donor groups with the outer two groups separated by 4.6 ± 0.6 Å (type II).[25] Pajeva and Wiese proposed a general pharmacophore model of two hydrophobic points, three H-bond acceptors, and one H-bond donor, for P-gp substrates and modulators that bind to the verapamil binding site of P-gp.[22]

A pharmacophore modeling study was carried out using sipholanes **1**, **3**, **6**, and **8** that modulated the function of P-gp and reversed its mediated resistance to colchicine. The pharmacophore modeling program DISCOtech[26] was used to generate a model that suggests common features among active sipholanes crucial for P-gp modulation. A total of 10 models with superimposed conformations of active sipholanes were generated by DISCOtech. The best model was selected based on maximum structural overlap as reflected by its highest score (Figure 4). The model has three hydrophobic points (HY-1 to HY-3) and two H-bond acceptors (AL-1 and AL-2). The spatial distance between AL-1 and AL-2 was 11.11 ± 0.25 Å.

These findings strongly suggest that the sipholane triterpenoids sipholenone E (**3**), sipholenol L (**6**), and siphonellinol D (**8**) reversed P-gp-mediated MDR to colchicine in resistant KB-C2 cells overexpressing P-gp in a similar fashion to sipholenol A (**1**). Sipholane triterpenoids are novel promising P-gp modulators.

Experimental Section

General Experimental Procedures. Melting points were determined on a TA Instruments DSC 2920 differential scanning calorimeter and were uncorrected. A Rudolph Research Analytical Autopol III polarimeter was used to measure optical rotation. The UV spectrum was obtained on a Cintra 20 UV−visible spectrophotometer. The IR spectra were recorded on a Varian 800 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD, or C_5D_5N 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 and HRESIMS experiments were conducted at the University of Michigan on a Micromass LCT spectrometer. For CC, Si gel 60 (EMD Chemicals, 63−200 μm), fine Si gel 60 (EM Science, <63 μm), and C-18 Si gel (Bakerbond, Octadecyl 40 μm) were used. The TLC analyses were carried out on precoated Si gel 60 F_{254}-500 μm TLC plates (EMD Chemicals), using the developing system $CHCl_3$/MeOH (9:1). HyQ DMEM-RS medium was purchased from Hyclone (Logan, UT, USA). Bovine serum was purchased from PML Microbiologicals, Inc. (Wilsonville, OR, USA). Colchicine was purchased from Sigma Chemical Co. (ST. Louis, MO, USA). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution was purchased from EMD Bioscience, Inc. (La Jolla, CA, USA).

Animal Material. The marine sponge *Callyspongia siphonella* (Levi 1965), formerly known as *Siphonochalina siphonella*, was collected from Hurghada, at the Egyptian Red Sea coast by SCUBA in June 2003. A voucher specimen is incorporated in the collections of the Zoological Museum of the University of Amsterdam under registration number ZMAPOR19765.

Extraction and Isolation. The frozen sponge (1.85 kg) was successively extracted with *n*-hexane, EtOAc, and isopropyl alcohol at room temperature.[27] The EtOAc extract was concentrated under vacuum, and the dried extract (36 g) was subjected to CC on Si gel 60 using *n*-hexane−EtOAc gradient elution to afford the known sipholenol A (~1 g) and sipholenone A (~1 g), along with the new sipholenone E (**3**, 19 mg, R_f 0.80) and siphonellinol D (**8**, 253 mg, R_f 0.65). Compound **3** was purified on C-18 Si gel using H_2O−CH_3CN gradient elution, while **8** was purified on fine Si gel 60 using MeOH−$CHCl_3$ gradient elution. The polar fractions from the first column were pooled together and fractionated on a Si gel 60 column using MeOH−$CHCl_3$ gradient elution. The fractions obtained were further purified by repeated chromatography on fine Si gel 60 using toluene−EtOAc or $CHCl_3$−MeOH gradient elution and C-18 Si gel using H_2O−CH_3CN gradient elution to afford seven new compounds, **2** (10 mg, R_f 0.57), sipholenol J (**4**, 14 mg, R_f 0.56), sipholenol K (**5**, 15 mg, R_f 0.48), sipholenol L (**6**, 35 mg, R_f 0.58), sipholenol M (**7**, 5 mg, R_f 0.44), siphonellinol E (**9**, 6 mg, R_f 0.52), and siphonellinol C-23-hydroperoxide (**10**, 10 mg, R_f 0.52), along with the known sipholenol G (60 mg). All R_f measurements were carried out with a solvent system of $CHCl_3$/MeOH, 9:1.

Compound 2: colorless blocks ($CHCl_3$/*n*-hexane); mp 175.4 °C; $[\alpha]_D^{25}$ −49.6 (*c* 0.41, $CHCl_3$); IR υ_{max} ($CHCl_3$) 3379, 2958, 2928, 2859, 1462, 1376, 1090, 1056, 909 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m/z* 457.3287 $[M + Na]^+$ (calcd for $C_{27}H_{46}O_4Na$, 457.3294).

Sipholenone E (3): colorless blocks (EtOH); mp 198.3 °C; $[\alpha]_D^{25}$ −12.2 (*c* 0.37, $CHCl_3$); IR υ_{max} ($CHCl_3$) 2988, 2947, 2929, 2856, 1712, 1459, 1379, 1085, 1001 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m/z* 497.3595 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

Sipholenol J (4): colorless oil; $[\alpha]_D^{25}$ −6.0 (*c* 0.31, $CHCl_3$); IR υ_{max} ($CHCl_3$) 3379, 2987, 2959, 2929, 2871, 1707, 1457, 1364, 1084, 909 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HREIMS *m/z* 490.3650 $[M^+]$ (calcd for $C_{30}H_{50}O_5$, 490.3658).

Sipholenol K (5): colorless oil; $[\alpha]_D^{25}$ −34.4 (*c* 0.43, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 256 (5.88) nm; IR υ_{max} ($CHCl_3$) 3478, 2989, 2936, 2872, 1651, 1462, 1378, 1082, 908 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m/z* 513.3545 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3556).

Sipholenol L (6): colorless oil; $[\alpha]_D^{25}$ −22.2 (*c* 1.76, $CHCl_3$); IR υ_{max} ($CHCl_3$) 3437, 2989, 2930, 2856, 1462, 1377, 1082, 908 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 4; HRESIMS *m/z* 499.3763 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_4Na$, 499.3763).

Sipholenol M (7): amorphous solid; $[\alpha]_D^{25}$ −18.4 (*c* 0.22, MeOH); IR υ_{max} (ATR) 3431, 2957, 2923, 2854, 1458, 1373, 1083, 1038, 909 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 4; HRESIMS *m/z* 531.3665 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_6Na$, 531.3662).

Siphonellinol D (8): amorphous solid; $[\alpha]_D^{25}$ −52.4 (*c* 0.85, $CHCl_3$); IR υ_{max} ($CHCl_3$) 3459, 2987, 2935, 2861, 1462, 1454, 1377, 1084, 908 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 4; HREIMS *m/z* 476.3866 $[M^+]$ (calcd for $C_{30}H_{52}O_4$, 476.3865).

Siphonellinol E (9): colorless oil; $[\alpha]_D^{25}$ −27.4 (*c* 0.23, $CHCl_3$); IR υ_{max} ($CHCl_3$) 3370, 2957, 2927, 2856, 1463, 1377, 1083, 908 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 4; HRESIMS *m/z* 531.3665 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_6Na$, 531.3662).

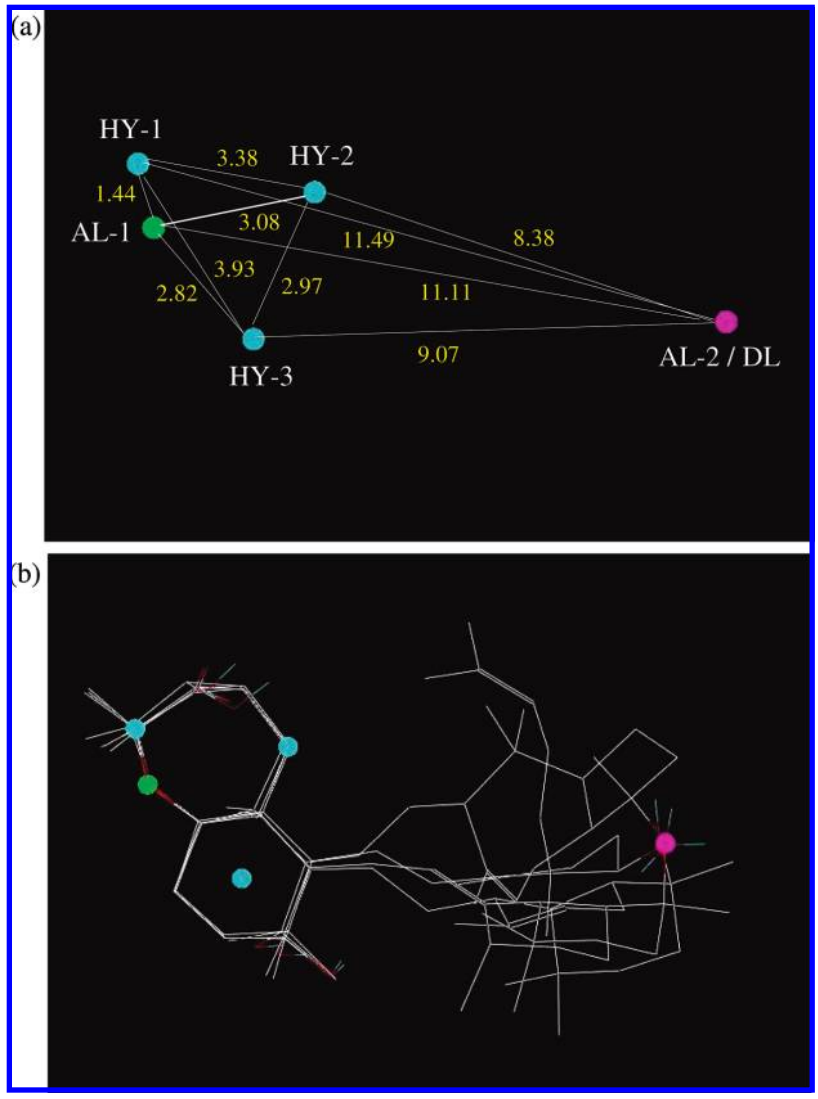

Figure 4. DISCOtech pharmacophore model generated for sipholanes **1**, **3**, **6**, and **8**. Pharmacophore features are color-coded: blue, hydrophobic point; green, H-bond acceptor; pink, H-bond acceptor and/or donor. (a) Pharmacophore features with separation distances in Å with a tolerance of 0.25 Å: AL, H-bond acceptor ligand; HY, hydrophobic point; DL, H-bond donor ligand. (b) Pharmacophore model overlapped with **1**, **3**, **6**, and **8**.

Siphonellinol C-23-hydroperoxide (10): colorless oil; $[\alpha]_D^{25}$ −53.8 (*c* 0.26, $CHCl_3$); IR υ_{max} ($CHCl_3$) 3365, 2928, 2857, 1457, 1378, 1363, 1083, 908 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 4; HRESIMS *m*/*z* 531.3658 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_6Na$, 531.3662).

X-ray Crystal Data of 2 and 3. A single-crystal X-ray diffraction study was conducted for (2*S*,4a*S*,5*S*,6*R*,8a*S*)-5-(2-((1*S*,3a*S*,5*R*,8a*S*,*Z*)-1-hydroxy-1,4,4,6-tetramethyl-1,2,3,3a,4,5,8,8a-octahydroazulen-5-yl)-ethyl)-4a,6-dimethyloctahydro-2*H*-chromene-2,6-diol (**2**). Colorless blocks were obtained with the vapor diffusion of *n*-hexane into a solution in $CHCl_3$. $C_{27}H_{46}O_4$, $M = 434.65$, crystal dimensions 0.12 × 0.16 × 0.21 mm^3, crystallizes in the triclinic space group *P*1, with two molecules in the asymmetric unit, being one of cocrystallized solvent, $CHCl_3$, and one molecule per unit cell ($Z = 1$). Cell dimensions were: $a = 8.32080(10)$ Å, $b = 9.56710(10)$ Å, $c = 10.1649(2)$ Å, $\alpha = 68.3920(10)°$, $\beta = 79.8950(10)°$, $\gamma = 80.5150(10)°$, $V = 736.202(19)$ $Å^3$. The final cell parameters were determined from least-squares refinement on 2899 reflections, and the absolute configuration was determined from the anomalous X-ray scattering from the oxygen atoms, with the refinement of 2110 Bijvoet pairs. The refined *R* values for the structural refinement were $R = 0.039$, and $wR(F^2) = 0.075$.

A single-crystal X-ray diffraction study was conducted for (5a*S*,6*S*,7*S*,9a*S*)-7-hydroxy-6-((*E*)-2-((2*R*,4a*S*,5*S*,8a*R*)-5-hydroxy-2,5,8,8-tetramethyloctahydronaphthalen-1(2*H*)-ylidene)-ethyl)-2,2,5a,7-tetramethyloctahydrobenzo(b)oxepin-3(2*H*)-one (**3**). Colorless blocks were obtained with the slow evaporation of a solution in EtOH. $C_{30}H_{50}O_4$, $M = 474.72$, crystal dimensions 0.09 × 0.12 × 0.17 mm^3, crystallizes in the monoclinic space group *C*2, with one molecule in the asymmetric unit, and four molecules per unit cell ($Z = 4$). Cell dimensions were: $a = 26.1853(7)$ Å, $b = 8.6009(2)$ Å, $c = 12.7015(3)$ Å, $\alpha = \gamma = 90°$, $\beta = 104.8110(10)°$, $V = 2765.55(12)$ $Å^3$. The final cell parameters were determined from least-squares refinement on 9969 reflections, and the absolute configuration was determined from the anomalous X-ray scattering from the oxygen atoms, with the refinement of 2164 Bijvoet pairs. The refined *R* values for the structural refinement were $R = 0.032$ and $wR(F^2) = 0.082$.

Data collection of **2** and **3** was performed on a Bruker Smart Apex II system, using Cu Kα radiation with a graphite monochromator, fine-focus sealed tube. The crystals were kept at 100 K under a stream of cooled nitrogen gas from a KRYO-FLEX low-temperature device. Data collection, indexing, and initial cell refinements were all carried out using APEX II software.[28] Frame integration and final cell refinements were done using SAINT software.[29]

Structure solution, refinement, graphics, and generation of publication materials were performed using SHELXTL V6.12 software.[30] Hydrogen atoms were placed in their expected chemical positions using the HFIX command and were included in the final cycles of least-squares with isotropic U_{ij}'s related to the atom's ridden upon. Crystallographic data of **2** and **3**, reported in this paper, have been deposited with The Cambridge Crystallographic Data Centre, deposit No. CCDC 684583 (**2**) and CCDC 684584 (**3**). Copies of the data can be obtained, free of charge, either via www.ccdc.cam.ac.uk/data_request/cif or on applica-

tion to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44-(0)-1223−336033 or e-mail: deposit@ccdc.cam.ac.uk].

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 (NIH). KB-C2, a P-gp-mediated MDR mutant, was isolated from KB-3-1 cells and maintained in the medium with 2 μg/mL of colchicine.[31] Both 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. The cells were harvested with trypsin and resuspended to a final concentration of 2.5×10^4 cells/mL for KB-3-1 and 4.0×10^4 cells/mL for KB-C2. Aliquots (180 μL) for each cell suspension were evenly distributed into 96-well multiplates. For cytotoxicity experiments, different concentrations of **1**−**10** (20 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 **1**−**10** (10 μL/well). After 68 h, 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 crystals. The medium was discarded, and 100 μL of DMSO was added into each well to dissolve the formazan crystals. The absorbance in individual wells was determined at 570 nm by an OPSYS microplate reader from DYNEX Technologies, Inc. (Chantilly, VA, USA). The concentrations required to inhibit growth by 50% (IC_{50} values) were calculated from survival curves using the Bliss method.[32]

Pharmacophore Modeling. The molecular modeling study was performed using SYBYL 8.0[26] on a Dell precision workstation T5400 equipped with a 2.0 GHz Quad Core Intel Xeon E5405 processor, 4 GB RAM, and Red Hat Enterprise Linux v5 operating system. The structures used in the present study were built and minimized within the SYBYL software package. The various conformations of each molecule were generated by a random search method. The number of maximum cycles was kept at 5000 and the energy cutoff was set at 7 kcal/mol in the random search details. The ring conformations were not searched. The number of conformers found within 7 kcal/mol of the energy minimum for 1, 3, 6, and 8 were 96, 27, 38, and 81, respectively. The DISCOtech module in SYBYL 8.0 was used to build pharmacophore models based on common chemical features. Initial standard pharmacophore features generated by DISCOtech were edited to remove the interaction sites of the receptor, e.g., H-bond donor and acceptor sites present in the receptor. The distance tolerance for a feature match among the molecules was kept from 0.25 to 2.5 Å in increments of 0.25 Å. All other parameters used were kept at their default settings. The selection of appropriate models was based on overall alignment score and the associated pharmacophore features (hits).

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR or Attached Proton Test (APT) spectra of compounds **2**−**10** and ^{1}H and ^{13}C NMR data of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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