Bioactive Sulfated Sesterterpene Alkaloids and Sesterterpene Sulfates from the Marine Sponge *Fasciospongia* sp.

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Two new sulfated sesterterpene alkaloids, 19-oxofasciospongine A (**3**) and fasciospongine C (**4**), and a new sesterterpene sulfate, 25-hydroxyhalisulfate 9 (**5**), along with two known sesterterpene sulfates, halisulfates 7 (**6**) and 9 (**7**), were isolated from an organic extract of the marine sponge *Fasciospongia* sp. The structures of the new compounds were established on the basis of one- and two-dimensional NMR spectroscopic studies as well as by HRESIMS analysis. Compounds **1**−**7** exhibited inhibitory activity against *Streptomyces* 85E in the hyphae-formation inhibition assay. Compounds **1**, **2**, and **4**−**7** were not cytotoxic when tested at 20 μg/mL with MCF-7, LNCaP, and LU cell lines. Only compound **3** demonstrated a moderate cytotoxic effect on the MCF-7 (IC_{50} 13.4 μM), LNCaP (IC_{50} 21.8 μM), and LU-1 cells (IC_{50} 5.0 μM), respectively.

Marine invertebrates have proven to be prolific producers and valuable sources of secondary metabolites.[1] In particular, species belonging to the genus *Fasciospongia* constitute a rich source of novel sesterterpenoids[2−11] that display a vast array of biological activities such as antimicrobial activity, cytotoxicity, and inhibition of human secretory phospholipase A_2. As part of an effort to identify protein phosphorylation inhibitors, we have studied a number of marine extracts.[12−15] The crude organic extract of a marine sponge *Fasciospongia* sp. exhibited significant inhibitory activity against *Streptomyces* 85E in the hyphae-formation inhibition (HFI) assay and was chosen for further study. We earlier reported the first study and biological investigation of this sponge, with the isolation of two rare sulfated sesterterpene alkaloids, fasciospongines A (**1**) and B (**2**).[15] Continued investigation has resulted in the isolation of two additional new sulfated sesterterpene alkaloids, 19-oxofasciospongine A (**3**) and fasciospongine C (**4**), and one new sesterterpene sulfate bearing a γ-hydroxybutenolide moiety, 25-hydroxyhalisulfate 9 (**5**), together with two known sesterterpene sulfates, halisulfates 7 (**6**)[16,17] and 9 (**7**).[18] Herein we report the isolation, structure determination, and biological activities of these compounds.

The sponge *Fasciospongia* sp. (1732 g) was collected in Palau on December 7, 2003, and extracted with water at 4 °C. The aqueous extract was lyophilized and extracted subsequently with MeOH−CH_2Cl_2 (1:1) and MeOH. The organic extracts were combined, concentrated, and subjected to Si gel MPLC (medium-pressure liquid chromatography). Final separation of the active fractions by Sephadex LH-20, Si gel MPLC, and RP-HPLC afforded compounds **1**−**7**. Compounds **6** and **7** were identified as halisulfate 7[16,17] and halisulfate 9,[18] respectively, by comparing their spectroscopic properties with reported data.

19-Oxofasciospongine A (**3**) was isolated as a colorless oil, with the molecular formula $C_{30}H_{44}N_3NaO_6S$ determined from the ^{1}H and ^{13}C NMR data and HRESIMS (*m/z* 620.2771 $[M + Na]^+$, calcd 620.2741; 598.2973 $[M + H]^+$, calcd 598.2921), indicating 10 degrees of unsaturation. The IR data suggested the presence of an amino group (3370 cm^{-1}), an α,β-unsaturated lactam carbonyl (1708, 1665 cm^{-1}), and sulfate (1259, 1214 cm^{-1}) functionalities. In the ^{1}H NMR spectrum of **3** (Table 1), four olefinic protons [δ_H 8.45 (1H, br s); 7.16 (1H, br s); 6.41 (1H, t, J = 1.5 Hz); 5.34 (1H, dd, J = 2.5, 4.5 Hz)], three tertiary methyls [δ_H 1.02 (3H, s); 0.91 (3H, s); 0.86 (3H, s)], and a secondary methyl [δ_H 0.87 (3H, d, J = 7.0 Hz)] were observed. The ^{13}C NMR and DEPT spectra indicated 30 carbon signals, including two carbonyls, four methyls, 12 methylenes, three sp^3 and four sp^2 methines, and three sp^2 and two sp^3 quaternary carbons. One methylene (δ_C 71.6, δ_H 3.80 and 3.95) was assigned as oxygen-bearing, and another methylene (δ_C 38.0, δ_H 3.78) as nitrogen-bearing. Two amide carbonyls and four double bonds from the ^{13}C NMR spectra accounted for six degrees of unsaturation, and the remaining four degrees of unsaturation required the presence of four rings in **3**. Comparison of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) with values for fasciospongine A (**1**) indicated that **3** and **1** were analogous. The primary difference between the NMR spectra of these two compounds was that the C-19 methylene carbon (δ_C 52.5) in **1** was replaced by a carbonyl signal at δ_C 172.3 in **3**. In the HMBC spectrum of **3**, correlations from H-18 (δ_H 6.41, t, J = 1.5 Hz) and H-26 (δ_H 3.78, t, J = 6.5 Hz) to the carbonyl carbon (δ_H 172.3, C-19) revealed that the carbonyl carbon was located at C-19. In addition, the molecular formula, $C_{30}H_{44}N_3NaO_6S$, indicated that **3** was a sodium salt of a sulfonic acid RSO_3H; **3** is not a sulfonic acid, like **1**. The bicyclic decalin moiety of **3** contained stereogenic centers at C-5, C-8, and C-9, and their ^{13}C NMR data were quite similiar to those in **1**, which indicated compound **3** had a relative configuration similar to that of compound **1**. The relative configuration of **3** was determined from analysis of the 2D NOESY data (Figure 1). The configuration of the decalin portion of compound **3** was assumed to be the same as that of known sesterterpenoids bearing the same skeleton such as halisulfate 7,[16] halisulfate 8, halisulfate 9, and halisulfate 10.[18] Its relative configuration was determined from analysis of the 2D NOESY data and on the basis of coupling constants. The large J value of H-5 (J = 13.0 Hz) indicated an axial orientation (β-face). The NOESY correlations among H-5 (δ_H 1.64, br d, J = 13.0 Hz, 1H), H-7β (δ_H 1.52, m, 1H), and H-11a (δ_H 1.75, m, 1H) suggested β-orientations for both H-5 and H_2-11 relative to the cyclohexane ring. In addition, the β-orientation of H_3-22 was deduced from the NOESY correlations of H_3-21 (δ_H 0.86, s, 3H) to H-6α (δ_H 1.08, m, 1H), of H-6α to H-8 (δ_H 1.25, m, 1H), of H-8 to H_3-22 (δ_H 0.87, d, J = 7.0 Hz, 3H), and of H_3-22 to H-11b (δ_H 1.05, m, 1H). The key NOESY correlations of compound **3** are shown in Figure 1. Detailed analysis of the ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments allowed the

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Chart 1

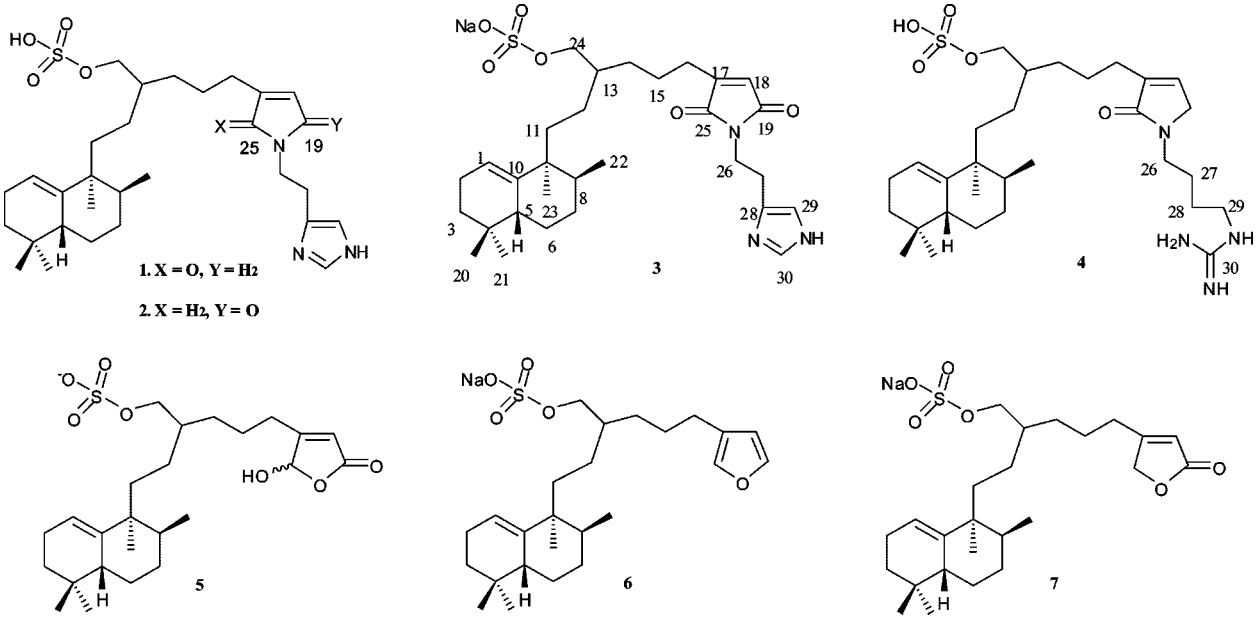

Table 1. ^{1}H NMR Data of Compounds **1**−**5** in CD_3OD[a]

no.	1	2	3	4	5
1	5.34 dd (2.3, 4.8)	5.34 dd (2.3, 4.8)	5.34 dd (2.5, 4.5)	5.34 dd (2.3, 4.8)	5.34 dd (2.3, 4.8)
2α	1.98 m	1.98 m	1.98 m	1.98 m	1.98 m
2*β*	2.07 m	2.07 m	2.07 m	2.07 m	2.07 m
3α	1.39 m	1.39 m	1.39 m	1.39 m	1.39 m
3*β*	1.09 m	1.09 m	1.09 m	1.09 m	1.09 m
5*β*	1.64 dd (2.5, 13.0)	1.64 m	1.64 br. d (13.0)	1.64 m	1.64 dd (2.5, 13.0)
6α	1.08 m	1.08 m	1.08 m	1.08 m	1.08 m
6*β*	1.83 m	1.84 m	1.83 m	1.83 m	1.84 m
7α	1.53 m	1.54 m	1.54 m	1.53 m	1.54 m
7*β*	1.51 m	1.52 m	1.52 m	1.51 m	1.52 m
8α	1.25 m	1.25 m	1.25 m	1.25 m	1.25 m
11a	1.75 m	1.75 m	1.75 m	1.75 m	1.75 m
11b	1.05 m	1.05 m	1.05 m	1.05 m	1.05 m
12a	1.10 m	1.10 m	1.10 m	1.10 m	1.10 m
12b	1.04 m	1.04 m	1.04 m	1.04 m	1.05 m
13	1.59 m	1.59 m	1.59 m	1.58 m	1.62 m
14a	1.44 m	1.44 m	1.44 m	1.44 m	1.52 m
14b	1.29 m	1.31 m	1.29 m	1.29 m	1.40 m
15	1.57 m	1.61 m	1.61 m	1.60 m	1.68 m
16a	2.24 dd (15.0, 7.0)	2.48 dd (15.0, 7.0)	2.43 ddd (15.0, 7.0, 1.5)	2.43 ddd (15.0, 7.0, 1.5)	2.44 m
16b	2.19 dd (15.0, 7.5)	2.42 dd (15.0, 7.5)	2.36 ddd (15.0, 7.5, 1.5)	2.30 ddd (15.0, 7.5, 1.5)	2.39 m
18	6.84 t (1.5)	5.76 s	6.41 t (1.5)	6.91 t (1.5)	5.90 s
19	3.89 d (1.5)			3.98 br. s	
20	0.90 s	0.90 s	0.91s	0.91 s	0.91 s
21	0.85 s	0.86 s	0.86 s	0.86 s	0.86 s
22	0.86 d (7.0)	0.87 d (7.0)	0.87 d (7.0)	0.87 d (7.0)	0.87 d (7.0)
23	1.01 s	1.02 s	1.02 s	1.02 s	1.02 s
24a	3.94 dd (5.0, 9.5)	3.99 dd (4.0, 9.5)	3.95 dd (4.5, 9.5)	3.93 dd (5.0, 9.5)	3.97 dd (4.5, 9.5)
24b	3.81 dd (6.8, 9.5)	3.81 dd (7.0, 9.5)	3.80 dd (6.5, 9.5)	3.79 dd (7.0, 9.5)	3.84 dd (6.8, 9.5)
25a		4.08 d (20.5)			6.02 (br.s)
25b		4.02 d (20.5)			
26a	3.77 dd (14.0, 7.0)	3.75 dd (13.8, 6.8)	3.78 t (6.5);	3.55 dd (14.0, 6.5);	
26b	3.72 dd (14.0, 6.5)	3.70 dd (13,8, 7.0)	3.78 t (6.5)	3.50 dd (14.0, 6.5)	
27	2.94 t (6.8)	3.02 t (6.8)	2.97 t (6.5)	1.68 m	
28				1.52 m	
29	7.00 br. s	7.25 br. s	7.16 br. s	3.22 t (7.5)	
30	8.06 br. s	8.55 br. s	8.45 br. s		

[a] *J* values (Hz) are showed in parentheses.

complete assignment of structure **3**. Therefore, compound **3** was determined to be a new sulfated sesterterpene alkaloid, a 19-oxo derivative of **1**, and was named 19-oxofasciospongine A.

Fasciospongine C (**4**) was obtained as a colorless oil, with the molecular formula $C_{30}H_{52}N_4O_5S$ resulting from ^{1}H NMR, ^{13}C NMR, and HRESIMS data (*m*/*z* 603.3566, calcd for $[M + Na]^+$, 603.3551). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) for **4** were very similar to those of compound **1**, suggesting that they were closely related sesterterpene alkaloids. The major difference of the two compounds was that the imidazole ring (δ_H 7.00 and 8.06; δ_C 118.0, 134.7, and 135.8) in **1** was replaced by an ethyl guanidino group (δ_H 1.52, m, 2H and 3.22, t, J = 7.5 Hz, 2H; δ_C 26.9, 42.1, and 158.7) in **4**. In the ^{1}H−^{1}H COSY spectrum of **4**, the cross-peaks of H-26 to H-27, H-27 to H-28, and H-28 to H-29 indicated the connectivity

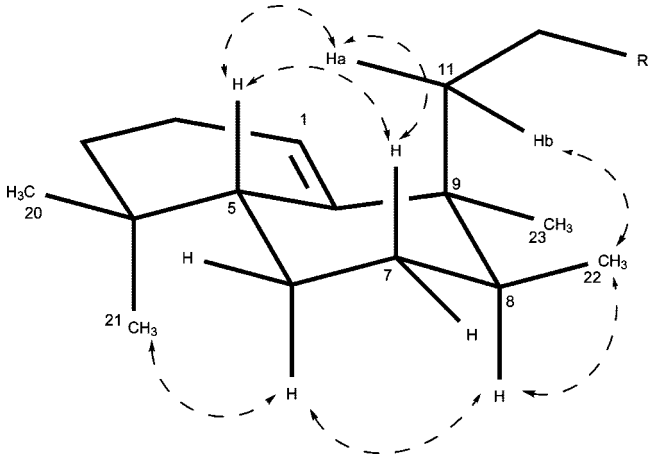

Figure 1. Key NOE correlations for 19-oxofasciospongine A (**3**).

Table 2. ^{13}C NMR Data of Compounds **1**−**5** and **7** in CD_3OD

no.	1	2	3	4	5	7
1	118.0	118.1	118.1	118.0	118.1	118.1
2	24.4	24.4	24.4	24.4	24.4	24.4
3	32.5	32.5	32.5	32.5	32.5	32.5
4	32.4	32.4	32.4	32.4	32.4	32.4
5	44.9	45.0	45.0	44.9	45.0	45.0
6	31.6	31.6	31.6	31.6	31.6	31.6
7	32.6	32.6	32.6	32.6	32.6	32.6
8	46.3	46.3	46.3	46.3	46.3	46.3
9	43.8	43.8	43.8	43.8	43.8	43.8
10	147.8	147.7	147.8	147.8	147.8	147.8
11	29.3	29.5	29.4	29.4	29.3	29.3
12	26.2	26.6	26.3	26.3	26.4	26.4
13	39.9	39.8	39.9	39.9	39.9	39.8
14	32.0	32.1	32.2	31.8	32.4	32.4
15	25.9	25.8	25.8	26.0	25.0	25.6
16	26.8	30.7	26.5	26.7	29.0	29.8
17	140.1	164.3	151.4	140.1	172.7	174.8
18	138.3	122.0	128.1	138.5	117.9	115.6
19	52.5	174.6	172.3	52.2	173.9	177.2
20	28.3	28.2	28.3	28.3	28.2	28.2
21	28.5	28.5	28.5	28.5	28.5	28.5
22	16.9	16.9	16.9	16.9	16.9	16.9
23	23.9	23.9	23.9	23.9	23.9	23.9
24	71.7	71.5	71.6	71.7	71.6	71.6
25	174.0	55.7	172.9	174.4	101.2	75.1
26	43.0	42.1	38.0	42.3		
27	26.3	25.3	25.5	26.8		
28	134.7	133.3	133.2	26.9		
29	118.0	118.1	118.3	42.1		
30	135.8	135.2	135.6	158.7		

of partial structure C-26 to C-29. Furthermore, the HMBC correlation of H-29 (δ_H 3.22, t, $J = 7.5$ Hz, 2H) with C-30, suggested that C-29 (δ_C 42.1, CH_2) was connected to guanidino imino C-30 (δ_C 158.7) through a nitrogen atom. The complete NMR assignments of **4** were deduced from its $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra, and compound **4** was named fasciospongine C.

25-Hydroxyhalisulfate 9 (**5**) was isolated as a colorless oil. It gave a negative HRESIMS molecular ion at *m/z* 483.2419, indicating an organic anion with a molecular formula of $C_{25}H_{39}O_7S^-$ (calcd for $C_{25}H_{39}O_7S^-$, 483.2422). Analysis of ^1H and ^{13}C NMR data suggested that **5** was a sesterterpene sulfate closely related to halisulfate 9 (**7**).[18] The major difference between the NMR spectra of these two compounds was that the C-25 methylene carbon (δ_C 75.1, δ_H 4.59, s, 2H) in **7** was replaced by an oxymethine (δ_C 101.2, δ_H 6.02, s, 1H) in **5**. Comparison of the NMR data of **5** and **7** indicated the presence of an additional hydroxy group at C-25. Its relative location was confirmed by an HMBC experiment, in which correlations were observed for the signal at δ_H 2.44, 2.39 (H-16a and H-16b) and δ_H 5.90 (H-18) with δ_C 101.2 (C-25), and δ_H 6.02 (H-25) with δ_C 117.9 (C-18) and 173.9 (C-19). Thus, the structure of **5** was assigned as the 25-hydroxyl derivative of halisulfate 9 (**7**) and was named 25-hydroxyhalisulfate 9.

Table 3. Biological Activity of Tested Compounds on the Growth and Sporulation of *Streptomyces* 85E[a]

	zone of inhibition observed (mm)[a,b,c]			
compound	20 μg/disk	10 μg/disk	5 μg/disk	2.5 μg/disk
1	18C	16B	14B	NA
2	19C	17B	15B	NA
3	25C	20C	16C	14B
4	14C	13B	NA[d]	NA
5	19C	13B	NA	NA
6	18C	15B	NA	NA
7	16C	14B	NA	NA

[a] Diameter of disk alone is 7 mm. Stock solutions were prepared in either DMSO or methanol. No zones of inhibition were observed with MeOH or DMSO as negative controls. [b] All compounds were tested at 20 μg/disk. Active compounds will be retested again at lower concentrations (20−2.5 μg/disk). B indicates bald phenotype and C indicates clear phenotype. [c] Kinase inhibitory activity of compounds was tested on the growth and sporulation of *Streptomyces* 85 E. [d] NA indicates not active.

Sesterterpene sulfates are rare in nature. To date, 27 sesterterpene sulfates[16−28] have been discovered from natural sources. Sesterterpene sulfates showed cytotoxicity,[18] antimicrobial activity,[25] and inhibitory activity against phospholipase A_2,[25] thrombin,[21] trypsin,[21] and isocitrate lyase,[28] and cell division in fertilized sea urchin eggs.[18,25] This is the first report of a sesterterpene sulfate from the genus *Fasciospongia*.

Compounds **1**−**7** were evaluated for their inhibitory activities against *Streptomyces* 85E in the HFI assay (Table 3), according to an established protocol.[29] Sulfated sesterterpene alkaloids **1**−**3** exhibited significant inhibitory activity against *Streptomyces* 85E and gave a 14−25 mm clear zone of inhibition at 20 μg/disk and a 13 mm bald zone and 20 mm clear zone of inhibition at 10 μg/disk (Table 3). Sesterterpene sulfates **5** and **7** showed moderate activities and gave 13 and 14 mm bald zones of inhibition, respectively, at 10 μg/disk and were inactive at 5 μg/disk. Compounds **1** and **2**, a pair of regioisomers with an imidazolone ring, showed equal/similar potency in the HFI assay. Compound **3**, with two amide carbonyls at C-19 and C-25, exhibited stronger inhibitory on HFI, with a 14 mm bald zone of inhibition at 2.5 μg/disk compared with compounds **1** and **2**. Compound **4**, in which the imidazole ring in **1** was replaced by an ethyl guanidinium group, exhibited weaker inhibition in the HFI assay, with a 13 mm bald zone at 10 μg/disk. In general, sulfated sesterterpene alkaloids (compounds **1**−**3**) were more active compared to nonalkaloidal sesterterpene sulfates (compounds **5**−**7**). The imidazole ring may contribute to the biological activity. Compound **3**, with two amide carbonyls at C-19 and C-25, exhibited the strongest activities. This observation was consistent with the replacement of the imidazole ring in **1** with the ethyl guanidinium group in **4**. It was reported that halisulfate 9 (**7**) inhibited cell division of the fertilized eggs of the sea urchin *Strongylocentrotus intermedius* with an IC_{50} value of 50 μg/mL.[18] Furanosesterterpene sulfate **6** (halisulfate 7) showed clear phenotypes at concentrations of 20 μg/disk, while bald phenotypes at 10 μg/disk were seen. It was cytotoxic against HeLa cells with an IC_{50} value of 16 μg/mL.[16] Compounds **1**−**7** were further evaluated for cytotoxicity in three different cancer cell lines [MCF-7, LNCaP, and LU-1]. Compounds **1**, **2**, and **4**−**7** were not cytotoxic when tested at 20 μg/mL against all three cell lines. Only compound **3** demonstrated moderate cytotoxic effect against the MCF-7 (IC_{50} 13.4 μM), LNCaP (IC_{50} 21.8 μM), and LU-1 cell lines (IC_{50} 5.0 μM), respectively (Figure 2).

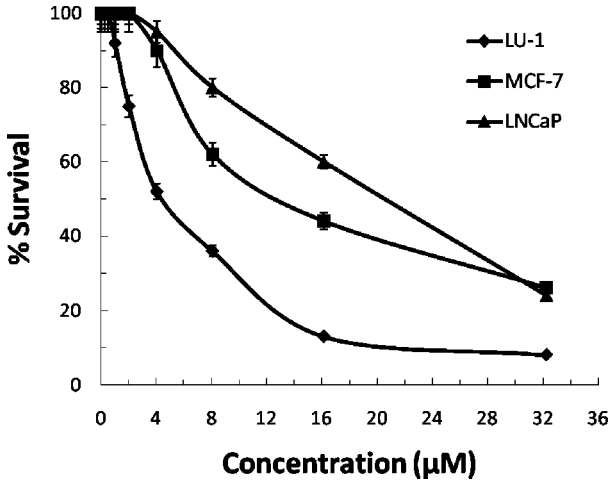

Figure 2. Inhibitory effect of various concentrations of 19-oxofasciospongine A (**3**) on the survival of LU-1, MCF-7, and LNCaP cells. Data points represent averages of triplicate tests.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 automatic polarimeter. UV spectra were recorded on an HP 8453 UV−visible spectrophotometer. The FT-IR spectra were recorded on a Perkin-Elmer BX FT-IR spectrometer. MS and HRMS spectra were taken with a BioTOF II ESI mass spectrometer. 1D and 2D NMR spectra were recorded in methanol-d_4 on INOVA Unity (500 MHz) Varian spectrometers. ^{1}H and ^{13}C NMR chemical shifts were referenced to the central peak of methanol-d_4 ($\delta_H = 3.31$ and $\delta_C = 49.15$). Reversed-phase HPLC was carried out on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech semipreparative Econosil C_{18} column (10 μm, 10 × 250 mm) run with a flow rate of 1.5 mL/min. Chromatographic fractions and pure compounds were monitored by TLC, detected by a color reaction by spraying with a solution of 10% H_2SO_4−EtOH solution followed by 5 min heating at 120 °C. Column chromatography (CC) was carried out using a column of Merck Si gel 60 (70−230 mesh).

Animal Material. The sponge *Fasciospongia* sp. (C012573) was collected at a depth of 1 m in Palau on December 7, 2003. The sponge was frozen immediately upon collection and shipped to NCI, Frederick, MD. After aqueous extraction of the frozen sponge at 4 °C, the extracts were lyophilized and extracted subsequently with CH_2Cl_2−MeOH (1:1) and MeOH. The combined organic extracts were evaporated *in vacuo* and stored at −30 °C. A voucher specimen was deposited at the National Museum of Natural History, Smithsonian Institution, Washington DC.

Extraction and Isolation. A crude organic extract (10.0 g) derived from 1732 g wet weight of the initial collection (provided by the NCI Natural Products Open Repository) showed inhibitory activity in the HFI assay at 80 μg/disk. This extract was chromatographed over Si gel using medium-pressure liquid chromatography (MPLC) eluting with a gradient of *n*-hexane−acetone and then CH_2Cl_2−MeOH, affording 35 fractions (FS-1−FS-35). Fraction FS-31 was fractionated via Si gel MPLC by eluting with $CHCl_3$−MeOH/H_2O (14:1:0.1) to give three major fractions (31-1, 31-2, and 31-3). Each fraction was further chromatographed over Sephadex LH-20 (MeOH) and then Si gel MPLC to afford compounds **1** (10.5 mg), **2** (5.6 mg), and **3** (1.3 mg), respectively. Fraction 27 was fractionated via reversed-phase (RP)-C_{18} MPLC by eluting with a gradient of increasing MeOH (50−100) in H_2O to give two major fractions (27-1 and 27-2). Fraction 27-1 was further purified by RPC-18 HPLC using isocratic MeOH−H_2O (50:50) as the mobile phase to afford compounds **4** (1.1 mg) and **5** (17.2 mg). Fraction 27-2 was further purified by Si gel MPLC by eluting with $CHCl_3$−MeOH (15:1) to afford compounds **6** (30 mg) and **7** (13 mg).

19-Oxofasciospongine A (3): colorless oil; $[\alpha]_D^{23}$ −42.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.37) nm, 217(4.38) nm; IR (AgCl, film) ν_{max} 3370, 3151, 2921, 2852, 2363, 2344, 1708, 1665, 1630, 1458, 1407, 1370, 1259 (strong), 1214 (strong), 1095, 991, 808, 583 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 620.2771 for $[M + Na]^+$ (calcd for $C_{30}H_{44}N_3Na_2O_6S$, 620.2741), 598.2973 for $[M + H]^+$ (calcd for $C_{30}H_{45}N_3NaO_6S$, 598.2921).

Fasciospongine C (4): colorless oil; $[\alpha]_D^{23}$ −51.7 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.05) nm; IR (AgCl, film) ν_{max} 3343, 3187, 2918, 2854, 2360, 2343, 1655, 1627, 1458, 1364, 1259 (strong), 1207 (strong), 1058, 984, 800, 584 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 603.3566 $[M + Na]^+$ (calcd for $C_{30}H_{52}N_4NaO_5S$, 603.3551).

25-Hydroxyhalisulfate 9 (5): colorless oil; $[\alpha]_D^{23}$ −48.5 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.24) nm; IR (AgCl, film) ν_{max} 3469, 2939, 2872, 1752, 1459, 1245 (strong), 1214 (strong), 1062, 952, 587 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; HRESIMS *m/z* 483.2419 $[M]^-$ (calcd for $C_{25}H_{39}O_7S^-$, 483.2422).

Hyphae Formation Inhibition Assay. The inhibition of hyphae formation assay in *Streptomyces* 85E was performed on purified isolates as described previously.[29] The mycelia fragments of *Streptomyces* were spread on minimal medium ISP 4 agar plates for the generation of bacteria lawn. Compounds of known concentration dissolved in MeOH were dispensed onto disks in 20 μL aliquots. The air-dried disks were applied directly onto the plates and incubated at 30 °C. After 30 h of growth (during which the development of hyphae in *Streptomyces* species takes place), the results are identified by a clear zone of inhibition or bald phenotype around the disk. Surfactin, a sporulation inhibitor, and MeOH were employed as positive and negative controls, respectively. An inhibition zone of greater than 9 mm is considered active. Subfractions were tested at 80 μg/disk on 7 mm filter disks. Active compounds were tested at lower concentrations (20, 10, 5, 2.5 μg/disk). The assays were performed in duplicate.

Cytotoxicity Assay. The cytotoxic potential of test compounds with LNCaP (androgen-sensitive human prostate adenocarcinoma), MCF-7 (human breast adenocarcinoma), and LU-1 (human lung carcinoma) cells was determined as described previously.[30,31] Cells were seeded in 96-well plates (106 cells/mL), and six serial dilutions of samples in 10% DMSO (10 μL) were added to each well in triplicate. The plates were incubated for 72 h at 37 °C, after which cell viability was determined with sulforhodamine B staining. IC_{50} values were determined as the concentration of sample required to inhibit cell growth by 50% relative to a control treated with 0.5% DMSO and represent the average of values obtained from two independent experiments.

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Supporting Information Available: HRESIMS and ^{1}H and ^{13}C NMR spectra for compounds **3**−**5** and NOESY spectra for compound **3**. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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