Supporting Information for

Novel Sulfated Sesterterpene Alkaloids from the Marine Sponge *Fasciospongia* sp.

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Hyphae Formation Inhibition Assay

The inhibition of hyphae formation assay in *Streptomyces* 85E was performed on purified isolates as described previously.¹ 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 μ L 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 a positive and negative controls, respectively. An inhibition zone > 9mm is acceptable and considered active. Subfractions were tested at 80 μ g/disk on 7 mm filter disks. Pure compounds will be retested again at lower concentrations (20, 10, 5, 2.5 μ g/disk). The assays were performed in duplicate.

Table 1. Biological Activities of Tested Compounds on the Growth and Sporulation of *Streptomyces* 85 E

Samples	Concentration (μ g/disk)				
	80	20	10	5	2.5
1	36C	23C	28C	19C	15C
2	34C	24C	22C	20C	14C

*Diameter of disk alone is 7 mm; Stock solutions were prepared in methanol.

No zones of inhibition were observed with MeOH as negative controls.

1 Waters, B. D.; Saxena, G.; Wanggui, Y.; Kau, D.; Wrigley, S.; Stokes, R.; Davies, J. J. *Antibiotics* **2002**, *55*, 407-416.

Table 2. ¹H (500 MHz) and ¹³C NMR (125 MHz) Assignments of **1** in CD₃OD

No.	δ_H (mult, <i>J</i> , Hz)	¹ H- ¹ H COSY	NOESY	δ_C	Key HMBC (H→C)
1	5.34 dd (2.3, 4.8)	H-2 α , H-2 β	H-2 α , H-23	118.0 d	C-2, C-3, C-5, C-9
2 α	1.98 m	H-1, H-3 α , H-3 β	H-1, H-3 α	24.4 t	C-1, C-3, C-4, C-10
2 β	2.07 m	H-1, H-3 α , H-3 β	H-3 β		C-1, C-3, C-4, C-10
3 α	1.39 m	H-2 α , H-2 β	H-2 α , H-21	32.5 t	C-1, C-2, C-4, C-5, C-20, C-21
3 β	1.09 m	H-2 α , H-2 β	H-2 β , H-5 β , H-20		C-1, C-2, C-4, C-5, C-20, C-21
4				32.4 s	
5 β	1.64 dd (2.5, 13.0)	H-6 α , H-6 β	H-3 β , H-11a, H-20, H-22	44.9 d	C-1, C-4, C-6, C-7, C-10, C-20, C-21
6 α	1.08 m	H-6 β , H-7 α , H-7 β	H-7 α , H-23	31.6 t	C-5, C-7, C-8, C-10
6 β	1.83 dddd (11.7, 3.9, 3.9, 3.5)	H-6 α , H-7 α , H-7 β	H-5 β , H-7 α		C-5, C-7, C-8, C-10
7 α	1.53 m	H-6 α , H-6 β , H-8 α	H-8 α	32.6 t	C-5, C-6, C-8, C-9, C-22
7 β	1.51 m	H-6 α , H-6 β , H-8 α	H-22		C-5, C-6, C-8, C-9, C-22
8 α	1.25 m	H-7 α , H-7 β , H-22,	H-7 α , H-23	46.3 d	C-6, C-7, C-8, C-9, C-11, C-22, C-23
9				43.8 s	
10				147.8 s	
11a	1.75 m	H-11b, H-12a, H-12b,	H-5 β	29.3 t	C-8, C-9, C-10, C-12, C-13
11b	1.05 m	H-11a, H-12a, H-12b,			C-8, C-9, C-10, C-12, C-13
12a	1.10 m	H-11a, H-11b, H-12b, H-13		26.2 t	C-11, C-13, C-14, C-24
12b	1.04 m	H-11a, H-11b, H-12a, H-13			C-11, C-13, C-14, C-24
13	1.59 m	H-12a, H-12b, H-14a, H-14b, H-24a, H-24b		39.9 d	C-11, C-24
14a	1.44 m	H-13, H-14b, H-15		32.0 t	C-12, C-13, C-15, C-16, C-24
14b	1.29 m	H-13, H-14a, H-15			C-12, C-13, C-15, C-16, C-24
15	1.57 m	H-14a, H-14b, H-16a, H-16b		25.9 d	C-13, C-14, C-16, C-17
16a	2.24 dd (15.0, 7.0)	H-15, H-18 (W type)	H-18	26.8 t	C-14, C-15, C-17, C-18, C-25
16b	2.19 dd (15.0, 7.5)	H-15, H-18(W type)	H-18		C-14, C-15, C-17, C-18, C-25
17				140.1 s	
18	6.84 t (1.5)	H-16a, H-16b (W type), H-19	H-16	138.3 d	C-16, C-17, C-19, C-25
19	3.89 d (1.5)	H-18		52.5 t	C-17, C-18, C-25
20	0.90 s			28.3 q	C-2 (W type), C-4, C-5, C-21
21	0.85 s			28.5 q	C-2 (W type), C-4, C-5, C-20
22	0.86 d (7.0)	H-8 α		16.9 q	C-8, C-9, C-23
23	1.01 s			23.9 q	C-1, C-8, C-9, C-10, C-11
24a	3.94 dd (5.0, 9.5)	H-13, H-24b		71.7 t	C-12, C-13, C-14
24b	3.81 dd (6.8, 9.5)	H-13, H-24a			C-12, C-13, C-14
25a				174.0 s	
25b					
26a	3.77 dd (14.0, 7.0)	H-26b, H-27, H-19 (W type)		43.0 t	C-19, C-25, C-27, C-28
26b	3.72 dd (14.0, 6.5)	H-26a, H-27, H-19 (W type)			C-19, C-25, C-27, C-28
27	2.94 t (6.8)	H-30 (W type), H-26a, H-26b	H-29	26.3 t	C-26, C-28, C-29
28				134.7 s	
29	7.00 br. s	H-27 (W type), H-30 (W type)	H-27	118.0 d	C-28, C-30
30	8.06 br. s	H-29 (W type)		135.8 d	C-28, C-29

Table 3. ^1H (500 MHz) and ^{13}C NMR (125 MHz) Assignments of **2** in CD_3OD

No.	δ_{H} (mult, J , Hz)	^1H - ^1H COSY	NOESY	δ_{C}	Key HMBC (H $\rightarrow$ C)
1	5.34 dd (2.3, 4.8)	H-2 α , H-2 β	H-2 α , H-23	118.1 d	C-2, C-3, C-5, C-9
2 α	1.98 m	H-1, H-3 α , H-3 β	H-1, H-3 α	24.4 t	C-1, C-3, C-4, C-10
2 β	2.07 m	H-1, H-3 α , H-3 β	H-3 β		C-1, C-3, C-4, C-10
3 α	1.39 m	H-2 α , H-2 β	H-2 α , H-21	32.5 t	C-1, C-2, C-4, C-5, C-20, C-21
3 β	1.09 m	H-2 α , H-2 β	H-2 β , H-5 β , H-20		C-1, C-2, C-4, C-5, C-20, C-21
4				32.4 s	
5 β	1.64 m	H-6 α , H-6 β	H-3 β , H-11 α , H-20, H-22	45.0 d	C-1, C-4, C-6, C-7, C-10, C-20, C-21
6 α	1.08 m	H-6 β , H-7 α , H-7 β	H-7 α , H-23	31.6 t	C-5, C-7, C-8, C-10
6 β	1.84 dddd (11.7, 3.9, 3.9, 3.5)	H-6 α , H-7 α , H-7 β	H-5 β , H-7 α		C-5, C-7, C-8, C-10
7 α	1.54 m	H-6 α , H-6 β , H-8 α	H-8 α	32.6 t	C-5, C-6, C-8, C-9, C-22
7 β	1.52 m	H-6 α , H-6 β , H-8 α	H-22		C-5, C-6, C-8, C-9, C-22
8 α	1.25 m	H-7 α , H-7 β , H-22,	H-7 α , H-23	46.3 d	C-6, C-7, C-8, C-9, C-11, C-22, C-23
9				43.8 s	
10				147.7 s	
11 α	1.75 m	H-11 β , H-12 α , H-12 β ,	H-5 β	29.5 t	C-8, C-9, C-10, C-12, C-13
11 β	1.05 m	H-11 α , H-12 α , H-12 β ,			C-8, C-9, C-10, C-12, C-13
12 α	1.10 m	H-11 α , H-11 β , H-12 β , H-13		26.6 t	C-11, C-13, C-14, C-24
12 β	1.04 m	H-11 α , H-11 β , H-12 α , H-13			C-11, C-13, C-14, C-24
13	1.59 m	H-12 α , H-12 β , H-14 α , H-14 β , H-24 α , H-24 β		39.8 d	C-11, C-24
14 α	1.44 m	H-13, H-14 β , H-15		32.1 t	C-12, C-13, C-15, C-16, C-24
14 β	1.31 m	H-13, H-14 α , H-15			C-12, C-13, C-15, C-16, C-24
15	1.61 m	H-14 α , H-14 β , H-16 α , H-16 β		25.8 d	C-13, C-14, C-16, C-17
16 α	2.48 dd (15.0, 7.0)	H-15, H-16 β , H-18 (W type)	H-18	30.7 t	C-14, C-15, C-17, C-18, C-25
16 β	2.42 dd (15.0, 7.5)	H-15, H-16 β , H-18 (W type)	H-18		C-14, C-15, C-17, C-18, C-25
17				164.3 s	
18	5.76 s	H-16 α , H-16 β (W type)	H-16	122.0 d	C-16, C-17, C-19, C-25
19				174.6 s	
20	0.90 s			28.5 q	C-2 (W type), C-4, C-5, C-21
21	0.86 s			28.2 q	C-2 (W type), C-4, C-5, C-20
22	0.87 d (7.0)	H-8 α		16.9 q	C-8, C-9, C-23
23	1.02 s			23.9 q	C-1, C-8, C-9, C-10, C-11
24 α	3.99 dd (4.0, 9.5)	H-13, H-24 β	H-13	71.5 t	C-12, C-13, C-14
24 β	3.81 dd (7.0, 9.5)	H-13, H-24 α	H-13		C-12, C-13, C-14
25 α	4.08 d (20.5)	C-25 β		55.7 t	C-17, C-18, C-19
25 β	4.02 d (20.5)	C-25 α			C-17, C-18, C-19
26 α	3.75 dd (13.8, 6.8)	H-26 β , H-27		42.1 t	C-19, C-25, C-27, C-28
26 β	3.70 dd (13.8, 7.0)	H-26 α , H-27			C-19, C-25, C-27, C-28
27	3.02 t (6.8)	H-30 (W type), H-26 α , H-26 β	H-29	25.3 t	C-26, C-28, C-29
28				133.3 s	
29	7.25 br. s	H-27 (W type), H-30 (W type)	H-27	118.1 d	C-28, C-30
30	8.55 br. s	H-29 (W type)		135.2 d	C-28, C-29

MM2 Energy Minimized 3D Structure for Fasciospongine A (1) by Chem3D Ultra 10.0

Fasciospongine A (1)

The most stable 3D structure of fasciospongine A (1) was obtained using the molecular modeling energy minimization program (MM2) of Chem3D Ultra 10.0. The procedure is as follows:

1. The chemical structure is drawn in ChemDraw Ultra 10.0.
2. The structure is imported into Chem3D Ultra 10.0
3. The MM2 energy minimization program in the menu of calculations is run.

The relative stereochemistry of the 3D structure of fasciospongine A from molecular modeling was in agreement with the 2D and the NOESY correlations.

Figure S1. LRESI-MS spectrum of Fasciospongine A (**1**)

Figure S2. HRESI-MS spectrum of Fasciospongine A (**1**)

Figure S3. IR (AgCl film) spectrum of Fasciospongine A (**1**)

Figure S4. UV (MeOH) spectrum of Fasciospongine A (**1**)

Figure S5. ^1H NMR spectrum of Fasciospongine A (**1**; 500 MHz, CDCl_3)

Figure S6. ^1H NMR spectrum of Fasciospongine A (**1**; 500 MHz, CD_3OD)

Figure S8. DEPT NMR spectrum of Fasciospongine A (**1**; 125 MHz, CD₃OD)

[illegible]

Figure S10. HMBC spectrum of Fasciospongine A (**1**; CD₃OD)

Figure S11. ^1H - ^1H COSY spectrum of Fasciospongine A (**1**; CD_3OD)

Figure S12. NOESY spectrum of Fasciospongine A (**1**; CD₃OD)

Figure S13. LRESI-MS spectrum of Fasciospongine B (**2**)

Figure S14. HRESI-MS spectrum of Fasciospongine B (**2**)

IR Spectrum of Fasciospongine B (2) showing transmittance (%) versus wavenumber (cm⁻¹). The spectrum displays characteristic absorption bands for the compound, including a broad peak around 3400 cm⁻¹ (O-H stretch), a sharp peak at 1706 cm⁻¹ (C=O stretch), and a strong peak at 1210 cm⁻¹ (C-N stretch). The chemical structure of Fasciospongine B (2) is shown below the spectrum.

CC1=C(C(C2=CC=CC=C2C3=C(C(C4=CC=CC=C4)C5=CC=CC=C5)C6=CC=CC=C6)C7=CC=CC=C7)C(=O)N1CC2=CC=CC=C2

Fasciospongine B (2)

Figure S16. UV (MeOH) spectrum of Fasciospongine B (**2**)

Figure S17. ^1H NMR spectrum of Fasciospongine B (**2**; 500 MHz; CDCl_3)

Figure S18. ^1H NMR spectrum of Fasciospongine B (**2**; 500 MHz, CD_3OD)

Figure S19. ^{13}C NMR spectrum of Fasciospongine B (**2**; 125 MHz, CD_3OD)

Figure S20. DEPT NMR spectrum of Fasciospongine B (**2**; 125 MHz, CD₃OD)

Figure S21. HMQC spectrum of Fasciospongine B (**2**; CD₃OD)

Figure S22. HMBC spectrum of Fasciospongine B (**2**; CD₃OD)

Figure S23. ^1H - ^1H COSY spectrum of Fasciospongine B (**2**; CD_3OD)

Figure S24. NOESY spectrum of Fasciospongine B (**2**; CD₃OD)