Supporting information

Apoptolidin A

^1H NMR (CD_3OD)

For previously reported NMR spectra of apoptolidin A including ^1H , ^{13}C , COSY, HMQC, HMBC, NOESY and 1D-COSY, see reference 2 (Seto et al, *J. Am. Chem. Soc.* **1998**, *120*, 3524-3525).

Apoptolidin B

^1H -NMR (600 MHz, CD_3OD)

IR (NaCl Thin Film) ν = 3408, 2974, 2932, 1668, 1245, 1080, 1010 cm^{-1} ;

MS (ESI-MS) m/z 1136.1 (1135.7 calcd for $\text{C}_{58}\text{H}_{96}\text{O}_{20} + \text{Na}$);

HRMS m/z 1135.6448 Δ +5.4 mmu (1135.6387 calcd for $\text{C}_{58}\text{H}_{96}\text{O}_{20} + \text{Na}$);

$[\alpha]_D = +9.3$ ($c = 0.29$, MeOH);

HPLC (30 - 80%, 254 nm) 7.31 min, (10 - 90%, 254 nm) 8.53 min.

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

^{13}C -NMR (125 MHz, CD_3OD)

DQF-COSY (600 MHz, CD₃OD)

TOCSY (600 MHz, CD₃OD)

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

HMQC (600 MHz, CD₃OD)

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

HMBC (600 MHz, CD₃OD)

Apoptolidin C

¹H-NMR (600 MHz, CD₃OD)

IR (NaCl Thin Film) ν = 3409, 2974, 2931, 1674, 1254, 1080, 1012 cm^{-1} ;

MS (ESI-MS) m/z 1120.1 (1119.7 calcd for $\text{C}_{58}\text{H}_{96}\text{O}_{19} + \text{Na}$);

HRMS m/z 1119.6462 Δ +2.1 mmu (1119.6438 calcd for $\text{C}_{58}\text{H}_{96}\text{O}_{19} + \text{Na}$);

$[\alpha]_{\text{D}} = -41.1$ ($c = 0.17$, MeOH);

HPLC (30 - 80%, 254 nm) 8.33 min, (10 - 90%, 254 nm) 9.21 min.

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

^{13}C -NMR (125 MHz, CD_3OD)

DQF-COSY (500 MHz, CD₃OD)

TOCSY (600 MHz, CD₃OD)

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

HMQC (600 MHz, CD₃OD)

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

HMBC (600 MHz, CD₃OD)

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity

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Table 1. ^{13}C (125 MHz) and ^1H (600 MHz) NMR data for apoptolidin A, B and C in CD_3OD .

no.	apoptolidin A (1)		apoptolidin B (2)		apoptolidin C (3)	
	$\delta_{\text{H}} (J = \text{Hz})$	δ_{C}	$\delta_{\text{H}} (J = \text{Hz})$	δ_{C}	$\delta_{\text{H}} (J = \text{Hz})$	δ_{C}
1		172.7		172.5		170.9
2		123.7		124.0		125.0
3	7.41	149.2	7.35	148.8	7.16	146.7
4		133.1		133.1		132.9
5	6.23	147.0	6.18	146.3	6.08	145.4
6		133.4		133.4		133.3
7	5.27	142.9	5.21 (9.6)	142.3	5.18 (9.6)	141.3
8	2.79	38.9	2.76	38.8	2.73	38.7
9	3.87	84.2	3.84 (9.0, 9.0)	84.2	3.84 (9.0, 9.6)	84.3
10	5.26	126.4	5.22 (9.0, 15.6)	126.1	5.21 (9.6, 16.0)	126.0
11	6.21	141.2	6.17 (15.6)	141.1	6.17 (16.0)	141.0
12		134.8		134.2		134.3
13	5.71	133.3	5.58 (5.6, 9.6)	133.9	5.58 (5.8, 9.6)	133.8
14	2.09 / 2.50	24.7	1.96 / 2.31	28.5	1.96 / 2.31	28.6
15	1.44 / 1.52	36.4	1.20 / 1.37	27.7	1.20 / 1.34	27.7
16	3.47	74.6	1.37 / 1.63	33.7	1.33 / 1.60	34.1
17	2.75	83.8	2.86	79.9	2.92	80.1
18	1.78 / 2.20	38.4	1.54 / 2.21 (1.6, 11.4, 14.4)	39.6	1.74 / 1.96	42.6
19	5.32	72.4	5.27 (1.4, 2.5, 11.4)	72.3	5.18	71.0
20	3.57	75.4	3.52 (1.4)	75.2	1.80 / 2.10 (5.5, 14.4)	45.4
21		101.3		101.2		100.4
22	2.08	36.4	2.04 (6.6, 11.2)	36.3	1.81	38.5
23	3.76	73.8	3.71 (11.2, 5.2)	73.8	3.75	73.4
24	1.76	40.6	1.73	40.5	1.74	40.5
25	3.99	69.4	3.95 (2.2, 4.0, 8.4)	69.2	4.07 (2.1, 2.5, 9.6)	68.2
26	1.49 / 1.62	37.2	1.46 (4.0, 9.6, 14.4) / 1.58	37.0	1.43 / 1.67	36.8
27	3.48	76.8	3.44	76.7	3.74	75.8
28	3.36	76.8	3.33	76.6	3.41	76.9
2-Me	2.14	14.2	2.10	14.0	2.06	14.0
4-Me	2.21	18.0	2.17	17.8	2.12	17.7
6-Me	1.97	16.6	1.93	16.4	1.89	16.7
8-Me	1.17	18.4	1.14 (6.6)	18.1	1.13 (6.6)	18.2
12-Me	1.71	12.2	1.66	11.9	1.67	12.1
17-OMe	3.40	61.4	3.26	57.3	3.26	57.3
22-Me	1.06	12.4	1.02 (6.6)	12.0	1.05 (6.6)	12.1
24-Me	0.92	5.3	0.89 (6.6)	5.0	0.90 (6.6)	5.7
28-OMe	3.30	59.5	3.27	59.2	3.34	49.2
1'	4.85	96.0	4.82 (3.8)	95.9	4.82 (3.9)	95.9
2'	3.44	73.6	3.39 (3.8, 9.6)	73.5	3.40	73.4
3'	3.76	74.9	3.72 (9.0, 9.6)	74.8	3.72	74.8
4'	2.76	87.4	2.72 (9.0, 9.0)	87.2	2.72 (9.0, 9.0)	87.3
5'	3.78	68.2	3.74 (6.6, 9.6)	68.1	3.74	67.7
6'	1.29	18.4	1.26 (6.6)	18.0	1.26 (6.6)	18.3
4'-OMe	3.61	61.1	3.58	60.8	3.58	60.8
1''	4.97	99.5	4.93 (4.0)	99.3	4.98 (4.2)	99.0
2''	1.84 / 1.96	45.5	1.80 / 1.92 (4.0, 13.8)	45.3	1.80 / 1.95	45.1
3''		73.0		73.0		73.2
4''	3.37	85.5	3.33 (9.6)	85.6	3.33	85.7
5''	3.70	67.4	3.67 (6.6, 9.6)	67.3	3.74	67.7
6''	1.25	19.0	1.22 (6.6)	18.8	1.24 (6.6)	18.7
3''-Me	1.36	22.9	1.32	22.7	1.34	22.7
1'''	4.86	101.9	4.84 (1.9, 10.2)	101.7	4.83 (2.0, 10.2)	101.6
2'''	1.32 / 2.47	37.2	1.29 / 2.44 (1.9, 5.1, 12.0)	37.1	1.29 / 2.44 (2.0, 5.0, 12.0)	36.9
3'''	3.21	82.0	3.17 (5.1, 9.0, 11.4)	81.8	3.17	81.9
4'''	3.01	77.1	2.97 (9.0, 9.0)	77.0	2.97 (9.6, 9.6)	77.1
5'''	3.24	73.2	3.21 (9.0, 6.6)	73.1	3.21	73.0
6'''	1.31	18.4	1.28 (6.6)	18.0	1.28 (6.6)	18.3
3'''-OMe	3.46	57.4	3.43	57.1	3.43	57.3

Table 2. ROESY correlations used in apoptolidin C solution structure calculations:

proton	proton	distance [Å]
3-H	5-H	2.33 ± 0.25
3-H	17-H	3.34 ± 0.35
3-H	18b-H	3.88 ± 0.40
5-H	7-H	2.43 ± 0.25
7-H	8-Me	2.86 ± 0.30
7-H	9-H	2.54 ± 0.25
8-H	6-Me	2.98 ± 0.30
8-H	10-H	2.58 ± 0.25
8-Me	5'-H	2.94 ± 0.30
9-H	8-Me	2.91 ± 0.30
9-H	11-H	2.33 ± 0.25
9-H	1'-H	2.80 ± 0.30
10-H	12-Me	2.92 ± 0.30
11-H	13-H	2.22 ± 0.25
13-H	14a-H	2.62 ± 0.25
13-H	16a-H	2.78 ± 0.30
14b-H	15a-H	2.56 ± 0.25
14b-H	12-Me	2.66 ± 0.30
15a-H	17-H	2.71 ± 0.30
15b-H	17-H	3.17 ± 0.35
17-H	19-H	2.80 ± 0.30
18a-H	19-H	2.67 ± 0.30
19-H	20a-H	2.79 ± 0.30
19-H	22-H	2.28 ± 0.25
19-H	22-Me	3.80 ± 0.40
19-H	24-Me	4.24 ± 0.45
20a-H	22-Me	3.14 ± 0.30
23-H	24-H	2.25 ± 0.25
23-H	25-H	2.28 ± 0.25
24-H	25-H	2.35 ± 0.25
25-H	1''-H	2.65 ± 0.30
25-H	2a''-H	3.24 ± 0.35
25-H	26a-H	2.64 ± 0.25
26a-H	24-Me	3.66 ± 0.40
26b-H	24-Me	3.10 ± 0.35
26b-H	27-H	2.71 ± 0.25
27-H	1''-H	2.32 ± 0.25
2'-H	4'-H	2.66 ± 0.25
1''-H	2a''-H	2.79 ± 0.30

Apoptolidins B and C: Isolation, Structure Determination, and Biological Activity
Wender, Sukopp, Longcore

1''-H	2b''-H	2.45 ± 0.25
2''-H	4''-H	2.72 ± 0.30
4''-H	1'''-H	2.56 ± 0.25
4''-H	6''-H	2.91 ± 0.30
5''-H	3''-Me	2.92 ± 0.30
1'''-H	5'''-H	2.23 ± 0.25
2a'''-H	3'''-H	2.48 ± 0.25
4'''-H	6'''-H	2.79 ± 0.30

14-Ha: $\delta = 1.96$, 14-Hb: $\delta = 2.31$, 15a-H: $\delta = 1.20$, 15b-H: $\delta = 1.34$, 16a-H: $\delta = 1.33$,
16b-H: $\delta = 1.60$, 18a-H: $\delta = 1.96$, 18b-H: $\delta = 1.74$, 20a-H: $\delta = 2.10$, 26a-H: $\delta = 1.43$,
26b-H: $\delta = 1.67$, 2a''-H: $\delta = 1.95$, 2b''-H: $\delta = 1.80$, 2a'''-H: $\delta = 2.44$.

H292 lung cancer cell growth inhibition assay

H292 cells were cultured in RPMI medium 1640 (Gibco) supplemented with FBS (10% v/v), glutamine (2 mM), penicillin (100 units/ml) and streptomycin (50 units/ml) at 37°C, 5% CO₂ in air in a humidified incubator. To assay for cell proliferation, cells were plated in plasma-treated polystyrene 96-well plates at a density of 3000 cells/well in 0.1 ml of culture media, and allowed to settle and adhere for 24 hours. Drug additions were performed in triplicate. Stock solutions in DMSO were diluted in culture media so that the final DMSO concentration after drug addition (0.1 ml) was less than 0.5%. The number of viable cells in each well was quantified after 2 days using Calcein AM, Pluronic F-127 (both Molecular Probes) and a fluorescence plate reader (Tecan).

Isolation and HPLC methods

Apoptolidin A isolation procedures reported by Seto et al (reference 1, *J. Antibiot.* **1997**, 50, 628-630) were followed with slight modifications. After centrifugation and extraction of the culture broth with EtOAc, the crude material was subjected to silica gel column chromatography (5% MeOH/CHCl₃). Fractions containing material that was less polar than apoptolidin A were further purified by preparative RP-HPLC.

RP-HPLC purifications were performed on a Varian Pro Star instrument using Altima C18 columns (preparative: 22.0 mm × 250 mm, 10 µm C₁₈, 20 mL/min) with a 30-80% linear gradient in 20 min from water and CH₃CN and detection at 220 nm.

Apoptolidin A: 12.0 min

Apoptolidin B: 15.8 min

Apoptolidin C: 17.7 min

RP-HPLC analyses were conducted on a Agilent 1100 instrument using Agilent Sorbax SB-C18 columns (analytical: 3.0 mm × 150 mm, 5 µm C₁₈, 1 mL/min) with different 15 min linear gradients from water and CH₃CN and detection at 254 nm.