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Isolation, Structure Determination, and Anti-Cancer Activity of Apoptolidin D

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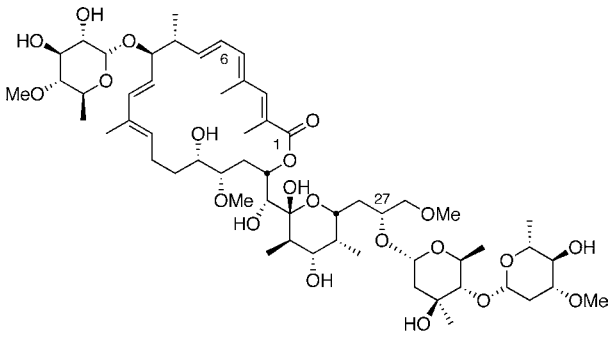
ABSTRACT



Apoptolidin D

The isolation, characterization, and preliminary biological activity of apoptolidin D, a new apoptolidin that exhibits anti-proliferative activity against H292 human lung carcinoma cells at nanomolar concentrations, are reported. Its equilibration with isoapoptolidin D and characterization of the latter are also described.

Apoptolidin A (**1**, Figure 1) is a glycosylated macrolactone first isolated by Seto and co-workers in 1997 from the fermentation of *Nocardiopsis* sp. using a screen designed to identify compounds that exhibit selective activity against cancer cells. Significantly, normal glial cells are found to be insensitive to apoptolidin A at concentrations up to 88 μM, while the corresponding oncogene-containing E1A-transformed glial cells undergo apoptosis when exposed to apoptolidin A at concentrations as low as 10 nM.[1] Such selectivity for transformed cells over normal cells is a major therapeutic goal in cancer treatment and key to minimizing the undesired side effects associated with many currently available chemotherapeutic agents. Potentially, such compounds could also be used to prevent disease progression. Due to its highly selective biological activity, apoptolidin A has drawn significant attention from the synthetic community[2−4] as well as from laboratories interested in elucidating its mode of action.[5,6] Biological studies have led to a proposed mechanism involving inhibition of mitochondrial F_1F_0-ATPase; however, due in part to the difficulty of accessing analogues through total synthesis, relatively little is known about the structural basis for apoptolidin A's ATPase activity and its potential interaction with other targets.

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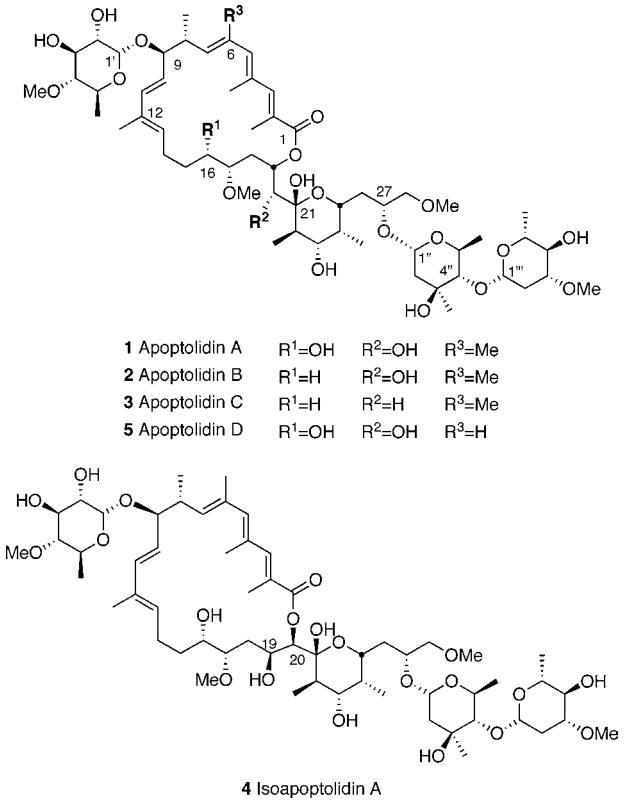

Figure 1. Structures of apoptolidins A−C (**1**−**3**), isoapoptolidin A (**4**), and apoptolidin D (**5**).

The availability of apoptolidin A (>100 mg/L) from fermentation of the producing organism allows for an exceptionally efficient approach to semisynthetic analogues through direct modification of the natural product.[6] Toward this end, our laboratory has been involved with the isolation and characterization of naturally occurring apoptolidins and their conversion to semisynthetic derivatives for advancement of mode of action studies. Recently, we reported the structures and biological activities of two new members of this family, apoptolidins B (**2**) and C (**3**) (Figure 1).[7] While isolated in much lower yields than apoptolidin A (**1**), these new analogues showed improved biological activity in a cell-based assay using H292 cells (human lung carcinoma). Importantly, apoptolidin C (**3**) lacks the C-20 alcohol involved in the isomerization of apoptolidin A to the ring-expanded isomer, isoapoptolidin A (**4**).[8] Due to the promising biological activity of the apoptolidins and the indication that the producing organism generates other minor metabolites, we have continued our efforts to isolate, characterize, and evaluate these other apoptolidins that might not be readily accessed synthetically. Herein, we describe the isolation, characterization, isomerization, and preliminary biological evaluation of a new apoptolidin, apoptolidin D (**5**).

Apoptolidin D (**5**), originally overlooked due to its coelution with apoptolidin A (**1**) during silica gel column chromatography, was subsequently isolated after further purification by preparative RP-HPLC with an isolation yield of 7 mg/L fermentation medium. The molecular formula for apoptolidin D (**5**) was determined to be $C_{57}H_{94}O_{21}$ [*m*/*z* 1137.6154 $(M + Na)^+$] by high-resolution mass spectrometric analysis. The primary structure (Figure 1) was determined through extensive NMR studies (^{1}H, COSY, TOCSY, ROESY, HSQC, HMBC).

Proton NMR spectroscopy clearly shows that apoptolidin A (**1**) and apoptolidin D (**5**) have many features in common but differ in the olefinic region due to the absence of the C-6 methyl group in the latter (Table 1). This structural variation is consistent with the biosynthetic incorporation of a malonyl extender unit in place of the methylmalonyl extender unit that leads to the synthesis of apoptolidin A. Such flexibility in polyketide biosynthesis is known, and analogous substitutions lead to the production of many of the naturally occurring epothilones by the producing organism.[9]

The connectivities of the carbohydrate appendages of apoptolidin D were determined using HMBC data. In particular, correlations between H-9/C-1′, H-1″/C-27, and H-1‴/C-4″ confirmed the attachment of the 6-deoxy-4-*O*-methyl-L-glucose, L-olivomycose, and D-oleandrose groups to C-9, C-27, and C-4″, respectively.

The all-*E*-geometry of the triene portion of apoptolidin D (C-2 through C-7) was established through ROESY analysis, which showed strong correlations between H-3/H-5 and H-5/H-7. Furthermore, H-7 is a doublet-of-doublets (J_1 = 13.2 Hz, J_2 = 10.2 Hz) supporting the assigned *E*-geometry of the C-6/C-7 olefin. Finally, this geometry is consistent with UV/vis spectroscopy that shows that the trienoate chromophore of apoptolidin D is unchanged from that of apoptolidin A (λ_{max} = 320 and 321 nm for apoptolidins A and D, respectively). The *E*-geometries of both olefins of the diene (C-10 through C-13) are consistent with the large coupling constant observed (J_{10-11} = 15.6 Hz) as well as

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Table 1. Comparison of ^{1}H NMR Data for Apoptolidin A (**1**) and Apoptolidin D (**5**) (ppm, CD_3OD, 600 MHz)

	δ_H			δ_H			δ_H			δ_H	
carbon	**5**	**1**	carbon	**5**	**1**	carbon	**5**	**1**	carbon	**5**	**1**
1			16	3.40	3.47	6-Me	–	1.97	1″	4.93	4.97
2			17	2.66	2.75	8-Me	1.20	1.17	2″	1.93, 1.80	1.96, 1.84
3	7.41	7.41	18	2.14, 1.66	2.20, 1.78	12-Me	1.65	1.71	3″		
4			19	5.28	5.32	22-Me	1.03	1.06	4″	3.33	3.37
5	6.26	6.23	20	3.53	3.57	24-Me	0.90	0.92	5″	3.66	3.70
6	6.30		21			17-OMe	3.38	3.40	6″	1.21	1.25
7	5.48	5.27	22	2.05	2.08	28-OMe	3.24	3.30	3″-Me	1.32	1.36
8	2.52	2.79	23	3.72	3.76	1′	4.81	4.85	1‴	4.83	4.86
9	3.84	3.87	24	1.71	1.76	2′	3.39	3.44	2‴	2.43, 1.28	2.47, 1.32
10	5.21	5.26	25	3.95	3.99	3′	3.71	3.76	3‴	3.16	3.21
11	6.08	6.21	26	1.59, 1.43	1.62, 1.49	4′	2.72	2.76	4‴	2.97	3.01
12			27	3.42	3.48	5′	3.74	3.78	5‴	3.20	3.24
13	5.62	5.71	28	3.28	3.36	6′	1.26	1.29	6‴	1.27	1.31
14	2.48, 1.98	2.50, 2.09	2-Me	2.14	2.14	4′-OMe	3.58	3.61	3‴-OMe	3.42	3.46
15	1.43, 1.33	1.52, 1.44	4-Me	2.08	2.21						

with ROESY correlations (H-10/C-12-Me and H-11/H-13). The stereochemistry assigned to apoptolidin D (**5**) is fully consistent with ROESY data and coupling constants and is analogous to that found for apoptolidin A (**1**).

Due to the presence of a hydroxyl at C-20, apoptolidin D (**5**) would be expected to undergo ring expansion to produce an equilibrium mixture of apoptolidin D (**5**) and isoapoptolidin D (**6**, Figure 2). This was indeed observed under

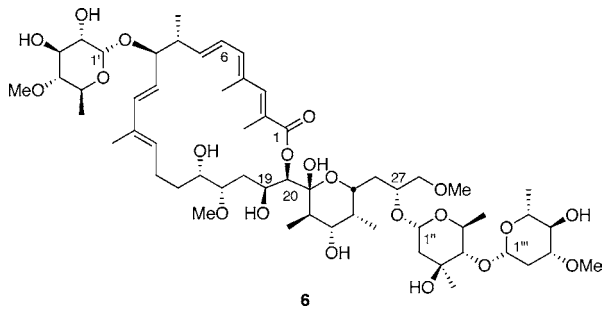

Figure 2. Isoapoptolidin D (**6**).

simulated biological assay conditions (phosphate buffered saline, pH 7.4, 37 °C) leading to the formation of isoapoptolidin D (**6**). This equilibrium is relevant to biological studies because it is established during the 48-hour time course of a typical cell-based assay. Using the integrated rate expression for a simple equilibrium, the kinetic parameters for the equilibrium of apoptolidin D (**5**) and isoapoptolidin D (**6**) were established (Table 2). In addition, it was shown that this same equilibrium could be achieved starting with a dilute aqueous solution of **6**.

The ^{1}H NMR spectrum of isoapoptolidin D exhibits the characteristic isoapoptolidin peaks. Specifically, H-19 appears at 4.39 ppm (J_1 = 11.4 Hz, J_2 = 3.6 Hz, J_3 = 1.9 Hz) as compared to isoapoptolidin A with H-19 at 4.41 ppm (J_1 = 11.3 Hz, J_2 = 3.7 Hz, J_3 = 2.1 Hz). In addition, H-20 in isoapoptolidin D is at 5.04 ppm (J = 3.9 Hz) as compared to isoapoptolidin A with H-20 at 4.98 ppm (J = 3.7 Hz).

Table 2. Kinetic Parameters for Equilibrations of Apoptolidin A (**1**) and Isoapoptolidin A (**4**) and for Apoptolidin D (**5**) and Isoapoptoldin D (**6**)

apoptolidin $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ isoapoptolidin

	k_1 (h^{-1})	k_{-1} (h^{-1})
apoptolidin A (**1**)[a]	0.0656	0.106
apoptolidin D (**5**)	0.0542	0.151

[a] Reference 8a.

In agreement with the structural similarity of apoptolidins A and D, apoptolidin D (**5**) inhibits proliferation of H292 cells with EC_{50} = 110 nM (95% confidence interval 60–

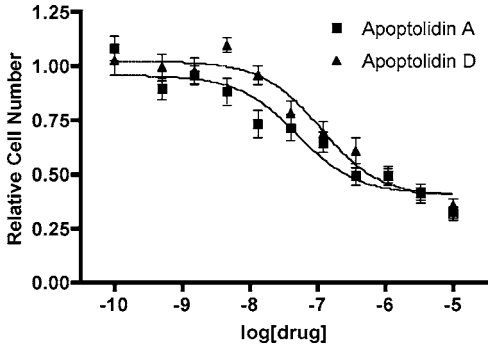

Figure 3. Growth inhibition of H292 cells treated with apoptolidin A (**1**) or with apoptolidin D (**5**).

210 nM), which is comparable to the effect seen in cells treated with apoptolidin A (EC_{50} = 50 nM with 95% confidence interval 25−100 nM). The inhibition curves for the growth of human lung cancer cells (H292) are closely related (Figure 3).

The isolation of apoptolidin D from the producing organism, *Nocardiopsis* sp., demonstrates that analogues that would otherwise require significant synthetic effort to access are readily available through fermentation and provide a superb starting point for further mode of action and SAR studies. Thus far, apoptolidins A−D represent variations of the macrocyclic core of apoptolidin. In all cases examined, the modifications are tolerated and the compounds retain antiproliferative activity *in vitro*. Apoptolidin D is the first derivative with a different carbon skeleton than the parent compound, arising from incorporation of a modified building block during biosynthesis. Further studies on the chemistry, biology, and activity of these new apoptolidins will be reported in due course.

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Supporting Information Available: Procedures for the isolation and purification of **5** and **6** as well as complete spectroscopic data including ^{1}H NMR, COSY, TOCSY, HSQC, HMBC, ROESY, IR, and HRMS. This material is available free of charge via the Internet at http://pubs.acs.org.

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