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Correlation of F_0F_1-ATPase Inhibition and Antiproliferative Activity of Apoptolidin Analogues

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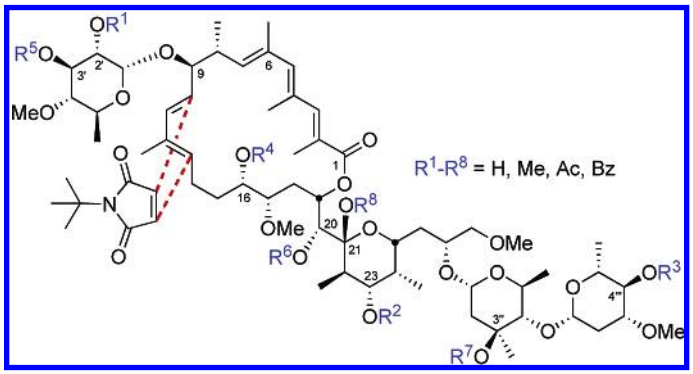
ABSTRACT



Apoptolidin (1) exhibits potent and highly selective apoptosis inducing activity against sensitive cancer cell lines and is hypothesized to act by inhibition of mitochondrial F_0F_1-ATP synthase. A series of apoptolidin derivatives, including a new intermolecular Diels–Alder adduct, were analyzed for antiproliferative activity in E1A-transformed rat fibroblasts. Potent F_0F_1-ATPase inhibition was not a sufficient determinant of antiproliferative activity for several analogues, suggesting the existence of a secondary biological target or more complex mode of action for apoptolidin.

Apoptolidin (**1**) was isolated in 1997 by Seto and co-workers as the result of a biological activity-guided screening program to identify new natural products that selectively and potently induce apoptosis, or programmed cell death, in E1A-transformed cells.[1] Apoptolidin was shown to induce apoptosis at nanomolar concentrations in E1A transformed rat fibroblasts (Ad12-3Y1) even when the E1B family of anti-apoptotic gene products (E1B19k and E1B54k) are coexpressed. Significantly, normal cells are unaffected by apoptolidin even at micromolar concentrations. The ability of apoptolidin to selectively induce apoptosis in oncogenically modified cells without affecting normal cells is a highly sought after trait of fundamentally new leads for the treatment of cancer.

In 2000, Khosla and co-workers demonstrated that apoptolidin is an inhibitor of mitochondrial F_0F_1-ATPase and provided evidence that interruption of this metabolic pathway might be the basis of the observed biological effects of apoptolidin.[2] Significantly, they found that apoptolidin was among the top 0.1% most selective agents tested in the National Cancer Institute's 60-cell line screening panel, with

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(1) (a) Kim, J. W.; Adachi, H.; Shin-ya, K.; Hayakawa, Y.; Seto, H. J. Antibiotics **1997**, *50*, 628. (b) Hayakawa, Y.; Kim, J. W.; Adachi, H.; Shin-ya, K.; Fujita, K.; Seto, H. *J. Am. Chem. Soc.* **1998**, *120*, 3524.

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an activity profile that closely matched other known F_0F_1-ATPase inhibitors, including cytovaricin, ossamycin, and oligomycin.

The promising activity of apoptolidin warrants a detailed investigation of its structure activity relationship and biological mechanism(s) of action. Although apoptolidin has been accessed via total synthesis[3] and is the target of impressive synthetic efforts,[4] its availability in substantial quantities by fermentation (~130 mg/L) provides the basis for the expedient investigation of its mode of action and improvement of its therapeutic potential through direct chemical modification. Toward this end, we have reported a series of compounds derived by the direct chemical modification of **1**, including derivatives arising from a selective ester/ether "scan" of hydroxyl groups in **1**[5] (**2**−**9**, Figure 1), a new ring expanded

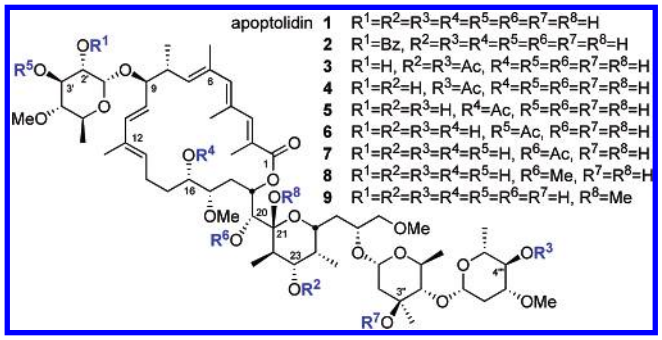


Figure 1. Structures of apoptolidin and hydroxyl derivatives.

isomer of **1**, isoapoptolidin[6] (**10**, Figure 2), and a series of

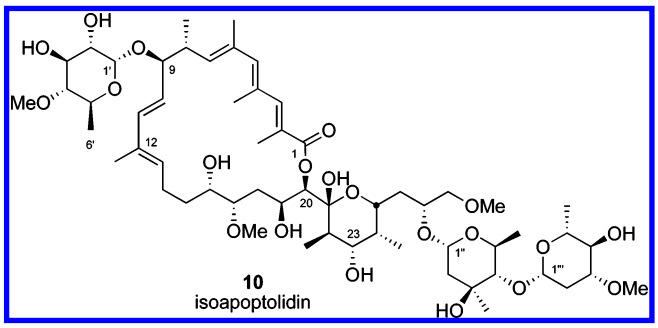


Figure 2. Structure of isoapoptolidin.

compounds derived from the oxidative cleavage of **1**[7] (**11**−**14**, Figure 3). Recently, we also reported the isolation, characterization, and preliminary activity of two new apoptolidins (B and C),[8] compounds lacking oxygenation at C-16 and at C-16 and C-20, respectively.

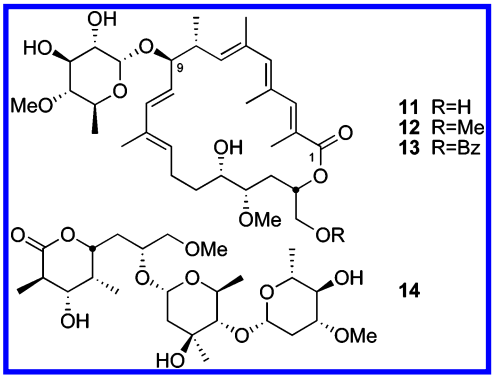


Figure 3. Structures of apoptolidin fragmentation products.

As a preliminary evaluation of the biological activities of new apoptolidin derivatives, the potency with which they inhibit F_0F_1-ATPase in isolated yeast mitochondria was measured (Table 1).[5−7] Significantly, modifications of the hydroxyl groups in **1** do not substantially affect activity in this assay, with the largest effect (~4-fold decrease) seen with C-20 (**8**) or C-21 (**9**) hydroxyl group derivatives. This apparent insensitivity of activity to widely varied structural changes is unusual. Isoapoptolidin (**10**), which possesses a macrocycle core conformation different from apoptolidin,[6a] is over 24-fold less potent than apoptolidin.

Compounds derived from the macrolide core of apoptolidin[7] (**11**−**13**, Figure 3) also retain activity against mitochondrial F_0F_1-ATPase comparable to isoapoptolidin. The C-21−C-28 fragment (**14**), however, is significantly less active.

To complement studies in which derivatives are obtained through covalent modifications of the polyol array or through truncations, our attention was directed at probing changes to apoptolidin that would preserve the existing functionality. The dienyl subunit (C-10 through C-13) in the macrocycle core of **1** suggested that apoptolidin might function as the

(2) (a) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14766. (b) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Chem. Biol.* **2001**, *8*, 71. (c) Salomon, A. R.; Zhang, Y.; Seto, H.; Khosla, C. *Org. Lett.* **2001**, *3*, 57.

(3) (a) Wehlan, H.; Dauber, M.; Fernaud, M. T. M.; Schuppan, J.; Mahrwald, R.; Ziemer, B.; Garcia, M. E. J.; Koert, U. *Angew. Chem., Int. Ed.* **2004**, *43*, 2597. (b) Nicolaou, K. C.; Fylaktakidou, K. C.; Monenschein, H.; Li, Y. W.; Weyershausen, B.; Mitchell, H. J.; Wei, H. X.; Guntupalli, P.; Hepworth, D.; Sugita, K. *J. Am. Chem. Soc.* **2003**, *125*, 15433. (c) Nicolaou, K. C.; Li, Y. W.; Sugita, K.; Monenschein, H.; Guntupalli, P.; Mitchell, H. J.; Fylaktakidou, K. C.; Vourloumis, D.; Giannakakou, P.; O'Brate, A. *J. Am. Chem. Soc.* **2003**, *125*, 15443.

(4) (a) Bouchez, L. C.; Vogel, P. *Chem. Eur. J.* **2005**, *11*, 4609. (b) Crimmins, M. T.; Christie, H. S.; Chaudhary, K.; Long, A. *J. Am. Chem. Soc.* **2005**, *127*, 13810. (c) Jin, B.; Liu, Q.; Sulikowski, G. A. *Tetrahedron* **2005**, *61*, 401. (d) Wu, B.; Liu, Q. S.; Sulikowski, G. A. *Angew. Chem., Int. Ed.* **2004**, 6673−6675. (e) Abe, K.; Kato, K.; Arai, T.; Rahim, M. A.; Sultana, I.; Matsumura, S.; Toshima, K. *Tetrahedron Lett.* **2004**, *45*, 8849. (f) Paquette, W. D.; Taylor, R. E. *Org. Lett.* **2004**, *6*, 103. (g) Chng, S. S.; Xu, J.; Loh, T. P.; *Tetrahedron Lett.* **2003**, *44*, 4997. (h) Chen, Y.; Evarts, J. B.; Torres, E.; Fuchs, P. L. *Org. Lett.* **2002**, *4*, 3571.

(5) Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 487.

(6) (a) Wender, P. A.; Gulledge, A. V.; Jankowski; O. D.; Seto, H. *Org. Lett.* **2002**, 4, 3819. (b) Pennington, J. D.; Williams, H. J.; Salomon, A. R.; Sulikowski, G. A. *Org. Lett.* **2002**, 4, 3823.

(7) Wender, P. A.; Jankowski O. D.; Tabet, E. T.; Seto, H. *Org. Lett.* **2003**, *5*, 2299.

(8) Wender, P. A.; Sukopp, M.; Longcore, K. *Org Lett.* **2005**, *7*, 3025.

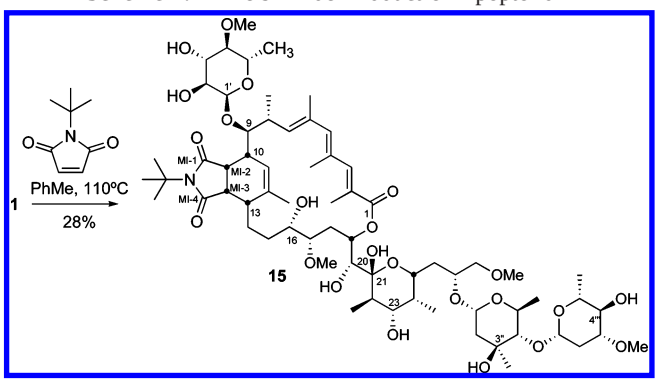
Table 1. Activities of Apoptolidin Analogs **1**−**15** and Oligomycin (**16**) in F_0F_1-ATP Inhibition and Cell Proliferation (Ad12-3Y1) Assays

compd	GI_{50} (μM) Ad12−3Y1[a]	GI_{50} (μM) 3Y1[a]	IC_{50} (μM) F_0F_1-ATPase[a]
1 (apoptolidin)	0.0065	>1.0	0.7
2 (C2′-OBz)	0.0036	>1.0	0.3
3 (C4‴-OAc, C23-OAc)	0.0095	>0.6	0.4
4 (C4‴-OAc)	0.0098	>1.0	0.8
5 (C16-OAc)	0.056	>1.0	0.8
6 (C3′-OAc)	0.0027	>1.0	0.4
7 (C20-OAc)	0.011	>1.0	1.1
8 (C20-OMe)	0.012	>1.0	2.8
9 (C21-OMe)	0.016	>1.0	2.3
10 (isoapoptolidin)	0.009	>1.0	17
11 (macrolide, C20-OH)	5.4	>7.0	13
12 (macrolide, C20-OMe)	1.4	>5.0	16
13 (macrolide, C20-OBz)	2.4	>16	32
14 (δ-lactone)	>12	>12	190
15 (Diels−Alder adduct)	3.2	>5.0	2.3
16 (oligomycin)	0.0002[b]	3.3[b]	1.0[b]

[a] All values are ±25%. [b] Assay performed on a mixture (75:25) of oligomycin A and oligomycin B.

diene component in an intermolecular Diels−Alder cycloaddition (Scheme 1). Cycloaddition reactions of **1** would allow

Scheme 1. Diels−Alder Adduct of Apoptolidin



access to a diverse array of derivatives with conserved original functionality, tunable physical properties and only modest and predictable variations in the core conformation. Apoptolidin was found to react with *N*-*tert*-butyl maleimide under thermal conditions to produce Diels−Alder adduct **15** in 28% yield (unoptimized) with the stereochemistry indicated in Scheme 1. This was the only observed product of the four possible stereoisomers that could form in this reaction.

The absolute stereochemistry of **15** was determined by analysis of ROESY correlations and proton coupling constants. Strong ROESY correlations between the maleimide derived proton MI-2-H and MI-3-H and the vicinal protons from apoptolidin 10-H and 13-H indicate that adduct **15** is the result of an endo-approach of the dienophile. ROESY correlations between 11-H and 8-Me restrict the newly formed olefin to the bottom hemisphere of the macrolide (Figure 4). The proton−proton coupling constant $J_{8-H,9-H}$

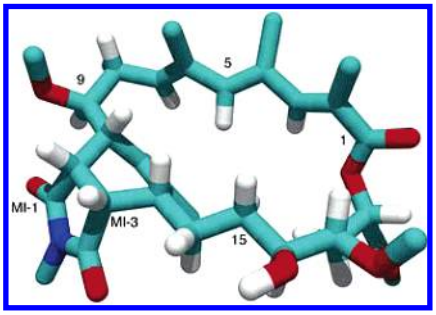


Figure 4. Three-dimensional structure of the macrocyclic core of adduct **15**. Methyl protons, *tert*-butyl group, and C-9 and C-20 substituents are omitted for clarity.

$\approx$ 0 Hz indicates a nearly 90° torsional angle for the C-8/C-9 bond, and the large vicinal coupling between 9-H and 10-H ($J_{9-H,10-H}$ = 11.2 Hz) indicates a dihedral angle of approximately 180° across the C-9/C-10 bond. This combination of torsional and distance constraints restrict the stereochemistry of adduct **15** to that shown in Figure 4. Additional ROESY correlations in the C-15 though C-19 system are consistent with the stereochemistry indicated.

Evaluation of the Diels−Alder cycloadduct **15** in the F_0F_1-ATPase inhibition assay indicated that it was only about 3-fold less potent than **1** (2.3 μM vs 0.7 μM). Collectively, the similar assay results arising from a wide range of varied analogues (e.g., **2**−**9**, **15**) prompted our interest in an alternative assay. Although the F_0F_1-ATPase inhibition assay provides a rapid means of evaluating new compounds in an easily controlled cell-free system, its predictive value in estimating the desired biological effect of apoptolidin derivatives, the selective induction of apoptosis in cancer cells, has not been established.

To address this issue, compounds **1**−**15** and oligomycin (**16**, Figure 5), for which F_0F_1-ATPase inhibition data has

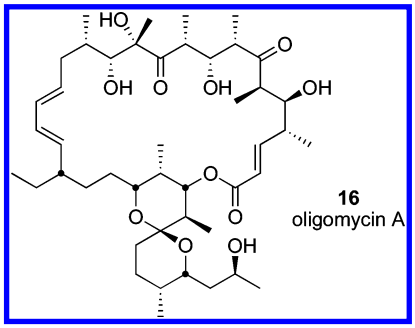


Figure 5. Structure of oligomycin A.

been obtained, were tested for their ability to selectively

inhibit the proliferation of Ad12-3Y1 cells (obtained by transfection of 3Y1 cells with E1A and E1B oncogenes) relative to untransformed 3Y1 cells. This assay system was selected because it is identical to the activity-guided screen that was used to identify apoptolidin as a medicinally relevant natural product and thereby would allow a correlation of previous and current assay results.[1] The results of this cell-based assay (Ad12-3Y1 vs 3Y1) and of the cell-free assay for mitochondrial F_0F_1-ATPase inhibition are summarized in Table 1.

Apoptolidin is active against Adl-3Y1 cells with a GI_{50} of 6.5 nM and exhibits no activity against 3Y1 cells at high concentrations ($>1\ \mu$M). It is considerably less potent ($IC_{50} = 0.7\ \mu$M) in the cell-free F_0F_1-ATPase inhibition assay. The discrepancy in potencies between the two assays, which differ by 2 orders of magnitude, might be explained in several ways, including cellular uptake, chemical modification under the assay conditions, and cumulative effects over the three-day time-course of the cell-based assay.

Acyl derivatives **2**−**7**, which differ little in potency in the F_0F_1-ATPase inhibition assay and are stable under these assay conditions,[5] are again—like apoptolidin—much more potent in the cell proliferation assay. In addition, with one exception (**5**), the derivatives differ slightly in potency from apoptolidin. This could reflect an insensitivity to structure variations in this assay or possible conversion of these esters to **1** under the longer assay time-course. The C-16 acetate (**5**), whose potency (56 nM) is significantly less than apoptolidin, suggests that either the C-16 hydroxyl is important for activity or the action of apoptolidin is sensitive to increased steric demand at C-16. These data also imply that the C-16 acetate might be a poor lipase substrate. The 20-OMe (**8**) and 21-OMe (**9**) derivatives are about 3−4-fold less potent than **1** in the F_0F_1-ATPase assay, a trend that is mirrored in the Ad12-3Y1 cell proliferation assay where they are also marginally less potent than **1**. In contrast, isoapoptolidin (**10**), despite its low potency (17 μM) in the enzymatic assay, performs as well as **1** against transformed cells (9 nM vs 6.5 nM). The comparable activities of **1** and **10** is consistent with previous studies from our laboratory on the facile equilibration between **1** and **10** under the assay conditions.[6a]

Activity data for macrolide fragments **11**−**13** reveal additional differences between the two assay systems. Compound **11** was found to inhibit mitochondrial F_0F_1-ATPase with an IC_{50} of 13 μM—a less than 20-fold decrease from **1**. In contrast, its potency against E1A-transformed cells drops almost 1000-fold relative to **1**. A similar trend can be identified for compounds **12** and **13**. The Diels−Alder adduct **15** retains almost full potency against F_0F_1-ATPase, but loses close to 500-fold potency against transformed cells, suggesting that the loss of activity of **11**−**13** in the cell-based system is a consequence of a change in binding affinity to a biological target rather than a loss of cellular uptake relative to **1**.

These results might alternatively be explained by the details of F_0F_1-ATPase assay. This cell-free assay is performed using mitochondria isolated from yeast. Yeast F_0F_1-ATPase differs not insignificantly from its counterpart in rat, in particular in the oligomycin sensitivity conferring protein subunit (OSCP). Furthermore, mitochondrial F_0F_1-ATPase is believed to undergo significant conformational changes in the ϵ-subunit in response to the proton gradient across the inner mitochondrial membrane.[9] As a result, catalysis in the forward direction, ATP synthesis, is not equivalent to the reverse direction, ATP hydrolysis. It is therefore possible to selectively inhibit ATP hydrolysis or ATP synthesis independently. Although **1** and **16** both have IC_{50} concentrations close to 1 μM for inhibition of ATP hydrolysis by this enzyme, previously reported values for the inhibition of ATP synthesis by **16** in respirating mitochondria are in the low nanomolar range.[10] It is also possible, however, that a more relevant secondary biological target of apoptolidin exists that gives rise to its observed cellular effects.

In conclusion, the biological activities of apoptolidin and a wide range of its derivatives are compared for the first time in two assay systems. Inhibition of ATP hydrolysis mediated by mitochondrial F_0F_1-ATPase isolated from yeast is not sufficient to predict the potency and in some cases relative activity order of new compounds in a cell-based assay. The further development of apoptolidin as a therapeutic lead for the treatment of cancer will be aided by a precise understanding of its biological mechanism of action and structural basis for its activity. This work has led to the identification of several compounds that possess selectivity and potency comparable to, and some better than, apoptolidin, as determined in an assay involving transformed cell lines. Further work on these and new analogues is in progress.

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Supporting Information Available: Experimental procedures and characterization data for compound **15**. This information is available free of charge via the Internet at http://pubs.acs.org.

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(9) Tsunoda, S. P.; Rodgers, A. J. W.; Aggeler, R.; Wilce, M. C. J.; Yoshida, M.; Capaldi, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6560.

(10) (a) Lardy, H. A.; Witonsky, P.; Johnson, D. *Biochemistry* **1965**, *4*, 552. (b) Nieminen, A. L.; Saylor, A. K.; Herman, B.; Lemasters, J. J. *Am. J. Physiol.* **1994**, *267*, C67.