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COMMUNICATIONS

Published on Web 11/26/2003

Fundamental Role of the Fostriecin Unsaturated Lactone and Implications for Selective Protein Phosphatase Inhibition

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Fostriecin (**1**), is a novel phosphate monoester produced by *Streptomyces pulveraceus*[1] that displays antitumor activity (Figure 1).[2] It entered NCI-sponsored clinical trials, but the studies were discontinued before dose-limiting toxicities or therapeutic plasma levels were reached when concerns regarding storage stability surfaced.[3] Although early studies demonstrated that **1** inhibits topoisomerase II ($IC_{50} = 40\ \mu M$),[4] its potency and cell cycle effects were inconsistent with this target of action. Recently, **1** was shown to inhibit the mitotic entry checkpoint through much more potent inhibition of protein phosphatase 2A (PP2A).[2] Moreover, **1** was found to be the most selective PP inhibitor disclosed to date (PP2A/PP4 vs PP1 selectivity $\geq 10^4$).[5−7] Consequently, **1** or a more stable derivative represents an ideal candidate with which to establish the utility of this novel mechanism of action.

At the time that fostriecin's therapeutic effects were emerging, only its two-dimensional structure was known.[1,8] Thus, we established its stereochemistry[9] and thereafter reported its first total synthesis.[10] In a subsequent review of fostriecin,[2] we compared its structure with the pharmacophore[11] for nonselective PP1 inhibition and modeled its binding to PP2A enlisting a homology model[12] derived from PP1 X-ray structures.[13,14] Conserved features of this pharmacophore present in **1** include (1) a phosphate that binds the active site metal ions, (2) a methyl group proximal to this acidic group proposed to mimic the substrate phosphothreonine methyl group, and (3) an extended hydrophobic segment thought to mimic the substrate hydrophobic residues (Figure 1). Fostriecin's most significant feature that does not correspond to the pharmacophore is its unsaturated lactone. Our modeled fostriecin−PP2A complex placed the β12−β13 loop C269 thiol within 2.5 Å of the unsaturated lactone β-carbon (C3) at a trajectory poised for conjugate addition. Thus, the unsaturated lactone emerged as a candidate source of the PP2A/4 inhibition selectivity and potency. Herein, we report a series of fostriecin derivatives designed to define the importance of the unsaturated lactone and confirm that of the phosphate monoester. Their evaluation established a fundamental role for the unsaturated lactone, and the chemical behavior of **1** implicates its participation in a reversible, conjugate addition reaction with an active site nucleophile including C269.

The derivatives **2**−**5**, including the known dephosphofostriecin (**2**)[8,9] and cyclic phosphodiester **4**,[9] were examined to confirm the importance of the phosphate, whereas **6**−**15** were prepared to explore the unsaturated lactone. Of the latter compounds, the saturated lactone **7**, **15** lacking the entire lactone subunit, and diol **14** were viewed as the key analogues. The remainder (**8**−**13**) were intermediates prepared en route to **7** and **14** or generated in efforts to chronicle the chemical behavior of **1** (Scheme 1).

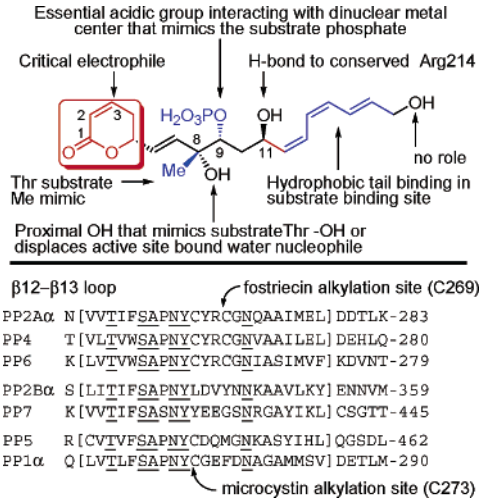

Figure 1. Fostriecin and key elements of its PP activity.

Consistent with the importance of the unsaturated lactone and its potential alkylation of the PP2A C269 thiol, treatment of **1** with EtSH (3 equiv) under mild conditions ($KHCO_3$, THF−H_2O, 0 °C, 5 h, 77%) provided **8** or **10** (K_2CO_3, THF−H_2O, 0 °C, 4 h, 99%), Scheme 1.[15] Analogous conjugate addition reactions were observed with MeOH as the nucleophile.

Thus, the unsaturated lactone of fostriecin increases the PP2A inhibition roughly 200-fold, which potentially may be attributed to C269 alkylation within the variable PP β12−β13 active site loop. Supporting this expectation is the observation that PP2A C269S and C269F mutants are much less sensitive to fostriecin (>10-fold).[16] This behavior of **1** would be analogous to the PP active site alkylation by microcystin-LR revealed in a cocrystal X-ray with PP1.[13] However, the addition of the PP1 C273 thiol to the *N*-methyldehydroalanine residue of microcrystin-LR (which does not alter the PP inhibition potency) constitutes alkylation of

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10.1021/ja038672n CCC: $25.00

Scheme 1

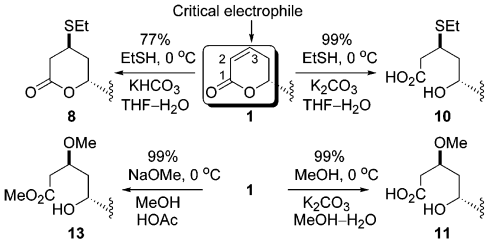
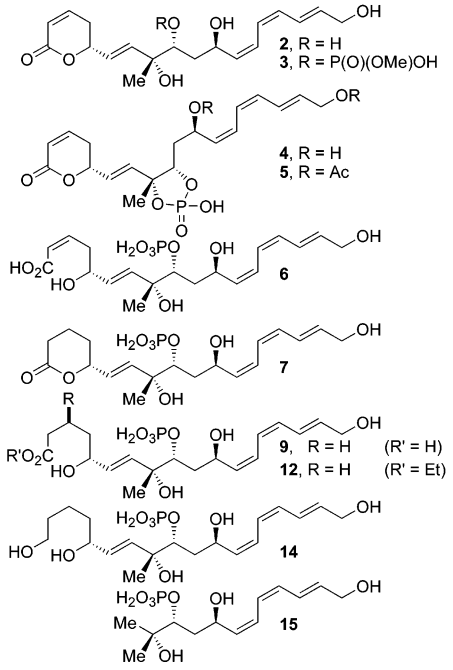


Table 1. Protein Phosphatase[a] and Cytotoxic Activity[b] (IC_{50}, μM)



compd	PP2A	PP1	PP5	L1210	L1210/CI-920
1	0.001 (±0.0007)	50 (±10)[c]	70 (±33)	0.3	35
2	350 (±100)	>100	>100	20	35
3	2.9 (±1.5)	>100[c]	>100	15	>50
4	3.2 (±1.1)	>100	>100	15	35
5	>100	>100	>100	>100	>100
6	73 (±9)	>100	>100	>25	>25
7	0.21 (±0.05)	>100[c]	>100	>50	>50
8	0.5 (±0.4)	>100[c]	>100	3	>25
9	>50	>100	>100	>50	>50
10	8.8 (±2.5)	>100[c]	>100	>25	>25
11	>100	>100	>100	>25	>25
12	1.7 (±0.2)	>100	>100	>25	>25
13	2.0 (±2.8)	>100	>100	>25	>25
14	2.1 (±0.6)	≥100[c,d]	140 (±50)[d]	>100	>100
15	0.19 (±0.02)	≥100[d]	≥100[d]	40	60

[a] Assays were conducted with native PP2A (rabbit muscle),[5b] rhPP1α,[19] and rhPP5 catalytic subunits[19] as detailed.[20] [b] L1210/CI-920 is a L1210 cell line resistant to **1** by virtue of an impaired folate transporter required to import **1**.[2b] [c] Also assayed with native PP1 (rabbit muscle) with identical results.[5b] [d] Enzyme inhibition at 100 μM = 40−50%.

a nearby conserved Cys residue found in all PPs (except PP2B/7), whereas that of **1** occurs at C269 unique to PP2A/PP4/PP6 contributing to both its potency and selectivity (Figure 1).

As anticipated, but not yet disclosed, the phosphate proved to be critical to phosphatase inhibition. Its removal with **2** resulted in a 10^5-fold loss in PP2A inhibition, whereas its conversion to the phosphodiesters **3** and **4** resulted in a 10^3-fold loss in activity. Acetylation of **4** providing **5**[17] resulted in a further ≥50-fold loss in PP2A inhibition, suggesting that the C11 alcohol[18] contributes significantly to fostriecin's potency. The studies also reveal that two easily generated metabolites, dephosphofostriecin (**2**) and the hydrolyzed lactone **6**, experience a 10^5-fold loss in activity, suggesting sites of modification that might enhance the in vivo efficacy. Interestingly and despite its 200-fold loss in potency, **15** still represents a remarkably potent and selective PP2A inhibitor. This suggests that there are additional features of **1** beyond the unsaturated lactone that contribute to its PP inhibition selectivity and that **15** may serve as a lead for further optimization. Studies addressing such issues and those that exploit the newly recognized origin of the PP2A potency detailed herein are in progress and will be reported in due course.

Acknowledgment. We gratefully acknowledge the financial support of NIH (CA93456, D.L.B.; CA60750 and HL59154, R.E.H.), the Skaggs Institute for Chemical Biology, and a NASA fellowship (NTGB-52896, M.R.S.). We are indebted to Kimberly L. Moring for her assistance with the inhibition studies and NCI for samples of fostriecin.

Supporting Information Available: Experimental details for the preparation and characterization of **3** and **7**−**15** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA038672N