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Improved synthesis of 6-*epi*-dictyostatin and antitumor efficacy in mice bearing MDA-MB231 human breast cancer xenografts

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Abstract

Structure-activity studies centered on the naturally occurring antitumor agent dictyostatin have recently identified several highly active epimers and analogs. From these compounds, 6-*epi*-dictyostatin was selected for scaleup preparation and evaluation in animals. Here we describe a new total synthesis that produced more than 30 mg of 6-*epi*-dictyostatin. The compound was found to have potent antitumor activity in SCID mice bearing MDA-MB231 human breast cancer xenografts.

Since the approval of paclitaxel in the early 1990's and docetaxel in the early 2000's, interest in compounds that bind to and stabilize microtubules has continued to grow.¹ Recently, a semisynthetic derivative of epothilone B, ixabepilone, was approved by the FDA for use either alone or in combination to combat certain types of breast tumors.² Other potent microtubule stabilizing agents, including members of the discodermolide/dictyostatin class, have also generated significant interest as drug candidates.³

Dictyostatin is a rare macrocyclic lactone isolated from marine sponges^{4,5} that has recently become more available through total synthesis.⁶⁻¹⁰ Members of the dictyostatin family strongly inhibit cancer cell growth, have high affinities for the taxoid binding site, and competitively inhibit the binding of paclitaxel and epothilone B to microtubules.^{4,9,11-13} Among several potent analogs, including 7-*epi*-dictyostatin, 16-normethyldictyostatin and 15 (Z),16-normethyldictyostatin, 6-*epi*-dictyostatin **1** was the most potent inhibitor of binding of epothilone B to microtubules. It was also the most potent inhibitor of HeLa cell growth, and its activity was not affected by mutations that induce paclitaxel resistance in the taxoid binding site.^{9,13}

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dictyostatin, R = Me
 7-*epi*-dictyostatin, R = Me, epimer at C7
 16-normethydictyostatin, R = H
 15(*Z*),16-normethyldictyostatin,
 R = H, C15–C16 *Z*-alkene

6-*epi*-dictyostatin **1**

Accordingly, 6-*epi*-dictyostatin **1** was selected for a scaleup synthesis and *in vivo* evaluation of its antitumor properties. Here we report a new synthetic approach to the dictyostatin family that produced more than 30 mg of 6-*epi*-dictyostatin **1**. In the first animal tests of any dictyostatin, epimer **1** showed high antitumor activity in SCID^a mice bearing xenografts of the MDA-MB231 human estrogen receptor negative breast cancer cell line. 6-*epi*-Dictyostatin **1** was significantly better than paclitaxel in inhibiting tumor growth.

We adopted the retrosynthesis plan for 6-*epi*-dictyostatin shown in Figure 1. Compared to the original fluororous mixture synthesis of **1**,¹² the new route is more convergent because it features fully elaborated bottom **5**, middle **6** and top **3** fragments. It also introduces a new strategy for uniting the bottom and middle fragments by a silicon-tethered ring-closing metathesis (RCM) reaction¹⁴ of **4** to form the critical C10–C11 *Z*-alkene.

^aabbreviations:

CBS	Corey-Bakshi-Shibata
DDQ	dichlorodicyanoquinone GH-II, second-generation Grubbs-Hoveyda catalyst
HWE	Horner-Wadsworth-Emmons reaction
PMB	<i>p</i> -methoxybenzyl
RCM	ring-closing metathesis
SCID	severe combined immunodeficient
T/C	tumor volume of test agent-treated group divided by tumor volume of untreated control group
T/V	tumor volume of test agent-treated group divided by tumor volume of vehicle-treated group

The C1-C10 fragment **5** was synthesized through five steps, starting from readily available intermediate **7**,¹⁵ as shown below. Deprotection of TBS ether **7** with HF·pyr and Dess-Martin oxidation¹⁶ of the resulting alcohol provided the aldehyde **8** in 91% yield over two steps. Addition of vinyl magnesium bromide, Dess-Martin oxidation, and Corey-Bakshi-Shibata (CBS)¹⁷ reduction gave an inseparable mixture of two C9-epimers **5** (α) and **9** (β) in a 5:1 ratio favoring the needed alcohol **5** (74% yield, three steps).

The sequence of fragment couplings and macrolactonization is summarized in Scheme 1. The C11-C17 alcohol **6** was treated with BuLi/dimethyldichlorosilane, followed by addition of imidazole and the C1-C10 alcohol epimer mixture of **5** and **9**. This provided another inseparable C9 mixture of the silylketals **4** and **10** in 85% yield, again in about 5:1 ratio. The RCM reaction of this silylketal mixture was mediated by a Grubbs-Hoveyda II catalyst to provide a third inseparable C9 mixture of the 8-membered disiloxanes in 57-69% yield. This mixture was deprotected with dichloroacetic acid and the crude product was purified by silica gel chromatography. This time, the C9 epimers separated, and the target diol **11** (C9S) was isolated in 45% yield alongside 7% of the C9R-epimer **12**. Protection of the diol **11** as a TBS ether followed by treatment with DDQ gave the alcohol **13** in 62% yield over two steps. The alcohol **13** was oxidized by using the Dess-Martin periodinane to the aldehyde **2**, which was coupled with the phosphonate **3** to provide the enone **14** possessing the full C1-C26 carbon skeleton of 6-*epi*-dictyostatin (89% yield over two steps).

The C17-C18 alkene of the enone **14** was reduced using a Stryker reagent ($[\text{Ph}_3\text{PCuH}]_6$)¹⁸ to give a saturated ketone, then the C21 PMB group was removed with DDQ to provide the β -hydroxyketone **15** in 68% over two steps. The 1,3-*syn*-reduction of the hydroxyketone **15** with NaBH_4 and Et_2BOMe ¹⁹ and then selective protection of the C19 hydroxy group of the resulting diol with TBSOTf furnished the alcohol **16** (74% yield over two steps).^{8,20}

In the usual end game, the C1 methyl ester was hydrolyzed by using KOH to produce a *seco*-acid, which was used for the next reaction without further purification. The macrolactonization of the *seco*-acid using the Yamaguchi reagent (2,4,6-trichlorobenzoyl chloride)²¹ gave a mixture of the C2 *E/Z* isomers of the TBS-protected macrolactone in a varying ratio, but the use of a Shiina reagent (2,6-methylnitrobenzoyl anhydride)²² in toluene suppressed the isomerization of the C2Z-alkene (1:13, *E/Z*). Finally, global deprotection with HCl provided 33 mg of 6-*epi*-dictyostatin in 45% yield over three steps.²³

6-*epi*-Dictyostatin **1** and paclitaxel were each administered intravenously in three doses of 20 mg/kg/dose spaced 7 days apart to ten C.B-17 SCID female mice bearing established MDA-MB231 human breast cancer xenografts. Mean tumor volumes (Figure 2) and body weight loss (Figure 3) were periodically measured. One of the mice receiving 6-*epi*-dictyostatin **1** was accidentally injured then euthanized between days 14 and 17, while the other nine mice in that group continued to be followed.

Mean tumor volumes in the mice treated with 6-*epi*-dictyostatin **1** were significantly smaller than those in the control and vehicle-treated groups beginning on day 7 of treatment. And beginning on day 10, they were also smaller than those in the paclitaxel-treated group (Figure 2). Tumor regression was observed in six of the nine 6-*epi*-dictyostatin **1**-treated mice on day 14 of study, and these tumors continued to regress until day 28 of study. In the remaining three 6-*epi*-dictyostatin **1**-treated mice, tumor volumes did not increase until day 28, when tumor regrowth was observed. Tumors continued to grow in all the paclitaxel-treated mice, albeit at a slower rate than that observed for the tumors in the control and vehicle-treated groups. Paclitaxel-treated mice were euthanized between days 24 and 28 (Figure 2).

Body weight loss (Figure 3) was less than 10% in the 6-*epi*-dictyostatin **1**-treated mice and their body weights were significantly lower than those in the other treatment groups. This difference in body weights may in part be due to the lack of tumor growth in the 6-*epi*-dictyostatin **1**-treated mice.

Tumor doubling times, median optimal %T/C and median optimal %T/V for the various treatment groups are presented in Table 1. Tumors in the 6-*epi*-dictyostatin **1**-treated mice did not double in volume at 28 days. The mean tumor doubling times for the paclitaxel-treated mice were significantly longer than the tumors in the control and vehicle-treated groups. Both the median optimal %T/C (day 14) and median optimal %T/V (day 17) were approximately 30% for the paclitaxel-treated mice, and 13% for the 6-*epi*-dictyostatin **1**-treated mice, a significant difference.

In conclusion, we successfully executed an improved synthesis of 6-*epi*-dictyostatin **1** that yielded quantities sufficient for animal antitumor studies. 6-*epi*-Dictyostatin **1** was more effective than paclitaxel in mouse xenograft studies, and excellent efficacy was observed at a dose that did not cause significant weight loss in the animals. Studies are ongoing to determine tissue distribution, metabolism of **1**, and pharmacodynamic effects in tumor and normal tissue. The present results suggest that dictyostatins such as **1** hold promise as new microtubule-stabilizing chemotherapeutic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Retrosynthetic analysis of 6-*epi*-dictyostatin

Figure 2. Antitumor activity in MDA-MB231 human breast cancer xenograft-bearing C.B-17 SCID female mice ($n = 10$ animals per group) treated with 20 mg/kg iv q7dx3 6-*epi*-dictyostatin **1** (○) or paclitaxel (Δ), vehicle (▲), or no treatment (▽). Arrows indicate the days of dosing.

Figure 3.

Body weight changes in MDA-MB231 human breast cancer xenograft-bearing C.B.-17 SCID female mice ($n = 10$ animals per group) over the course of treatment with 20 mg/kg iv q7dx3 6-*epi*-dictyostatin **1** (○) or paclitaxel (△), vehicle (▲), or no treatment (▽). Arrows indicate the days of dosing.

Scheme 1.
Fragment couplings and macrolactonization

Table 1

Tumor doubling times, median optimal %T/C, and median optimal %T/V in C.B-17 SCID mice bearing MDA-MB231 xenografts

Treatment Group N = 10	Days to One Tumor Doubling	Days to Two Tumor Doublings	%T/C day 14	%T/V day 17
Paclitaxel ^a	7.2 ± 2.9 ^c (7.9) ^d	19.6 ± 7.4 ^c (19.0) ^d	33 ^d	31 ^d
6- <i>epi</i> -Dictyostatin 1 ^a	27.4 ± 18.7 ^c (30.9) ^{d,e}	38.1 ± 12.1 ^c (35.0) ^{d,e}	14 ^{d,e}	13 ^{d,e}
Vehicle ^b	4.9 ± 3.2 (4.0)	9.8 ± 3.1 (8.9)	61	100
Control	2.8 ± 1.7 (2.5)	7.7 ± 2.4 (8.6)	100	—

Tumors were implanted in C.B-17 SCID female mice as 25 mg fragments and mice were stratified on day 17 post-implantation, when tumors reached 101–463 mm³. Treatment began at day 0 (day 17 post-implantation). Results of time to one doubling and time to two doublings are presented as the mean ± std (median).

^a20 mg/kg iv q7dx3.

^bCremophor EL-ethanol-saline 1:1:8 (v/v/v) 0.01 ml/g body weight iv q7dx3.

^cValue significantly different from control and vehicle, $p \leq 0.05$ by Dunnett's test.

^dValue significantly different from control and vehicle, $p \leq 0.05$ by Mann-Whitney test.

^eValue significantly different from paclitaxel, $p \leq 0.05$ by Mann-Whitney test.