Communications to the Editor

Synthesis and Antitumor Activity of Novel Thioester Derivatives of Leinamycin

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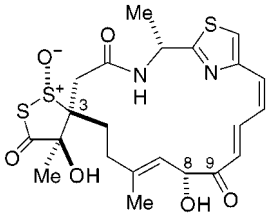
Leinamycin (**1**), a novel antitumor antibiotic, was isolated from a culture broth of *Streptomyces* sp.[1] The unique structural features of leinamycin include the 1-oxo-1,2-dithiolan-3-one moiety which is fused in a spiro fashion to an 18-membered lactam with an extensively conjugated thiazole ring.[2] No other natural products with such an unusual dithiolanone moiety have been reported to date. Leinamycin causes single-strand scission of plasmid DNA in vitro in the presence of thiol cofactors.[3] Isolation of a guanine−leinamycin adduct revealed the unprecedented chemical reactions which would be responsible for the thiol-mediated DNA cleavage by leinamycin.[4] Oxidative DNA cleavage by leinamycin in addition to DNA alkylation was also reported.[5] As a part of our program aimed at discovering clinically useful leinamycin analogues, chemical modification[6] of natural leinamycin and a total synthesis approach[7] have been investigated. Herein we report the discovery of novel thioester derivatives of leinamycin with enhanced stability and potent antitumor activity.



1
Leinamycin

We recently reported C-8- and C-9-modified leinamycin derivatives with potent in vitro antiproliferative activity.[6] However, we suspected that the in vivo antitumor activity of these agents was compromised by their instability. The half-life of leinamycin in aqueous solution at pH 7 at 37 °C is about 6 h and hydrolysis of the reactive dithiolanone moiety could be one of the reasons of instability. However, this dithiolanone moiety of leinamycin has been shown to be essential for its DNA-cleaving activity and antiproliferative activity.[3]

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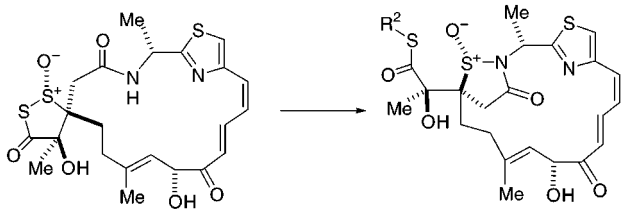
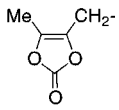
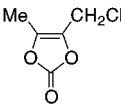
Table 1. Synthesis of Thioester Derivatives of Leinamycin



1 **2**
Leinamycin

compd	R^2	Reagents	Yield (%)
2a	Me	MeI/K_2CO_3	50
2b	EtO_2CCH_2	$EtO_2CCH_2Br/i\text{-}PrNEt/n\text{-}Bu_4NI$	77
2c	$t\text{-}BuCO_2CH_2$	$t\text{-}BuCO_2CH_2Cl/K_2CO_3/KI$	59
2d		 $/K_2CO_3/KI$	73

Accordingly, we sought stable yet biologically active derivatives of leinamycin for their further development as chemotherapeutic agents.

In the course of our chemical modification studies of leinamycin, we found that leinamycin reacted with iodomethane in the presence of potassium carbonate to form *S*-methyl thioester **2a**, containing the 3-isothiazolidinone 1-oxide moiety in place of the 1-oxo-1,2-dithiolan-3-one moiety, in 50% yield. Other thioesters **2b**−**d** were also prepared in a similar manner (Table 1).[8] The structures were determined by extensive 2D NMR studies and confirmed by an X-ray crystallographic analysis[9] of compound **2d**.

The thioester derivatives were more stable than leinamycin in pH 7 aqueous solution (Table 2). Although **2a**,**b** showed weak antiproliferative activity against HeLa S_3 cells, introduction of a pivaloyloxymethyl group or a (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group,[10] both of which have been used for prodrug moieties of carboxylic acids, resulted in cell-active derivatives **2c**,**d**. In combination with the modification at the C-8 hydroxy group, 8-*O*-modified derivatives **3** were synthesized from compound **2d** by the reported method.[6] Among these, the 2-tetrahydropyranyl (THP) ether **3c** (*R*-configuration in the THP group) and methoxymethyl ether **3e** showed strong antiproliferative activity against HeLa S_3 cells. Compounds **3a**−**c** showed significant in vivo antitumor activity against mouse sarcoma 180 (Table 2).[11] The structure of **3c** was determined by an X-ray crystallographic analysis.[9] The configuration of asymmetric carbon in the THP group was shown to be important for the biological activity, with the *R*-isomer **3c** being more potent than the *S*-isomer **3d** in vitro and in vivo.

Since **3c** and other thioester derivatives did not show any DNA-cleaving activity in vitro,[12] we suspect that

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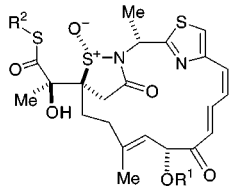
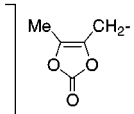
Table 2. Stability and Antiproliferative Activity of Leinamycin Derivatives



2, 3

compd	R^1	R^2	Stability[a] $t_{1/2}$ (h)	HeLa S_3[b] IC_{50} (nM)	sarcoma 180[c] T/C[d]	OD[e] (mg/kg)
2a	H	Me	18	830	0.94	16
2b	H	EtO_2CCH_2	>24	1300	0.98	16
2c	H	t-$BuCO_2CH_2$	>24	2.6	0.83	0.50
2d	H		16	4.6	0.67	2.0
3a	MeCO		24	3.9	0.38	2.0
3b	MTHP[f]		18	1.4	0.25	8.0
3c	THP (R)		30	0.67	0.26	8.0
3d	THP (S)		30	2.4	0.63	8.0
3e	$MeOCH_2$		24	0.17	0.58	2.0
1			6	11	0.49	2.0

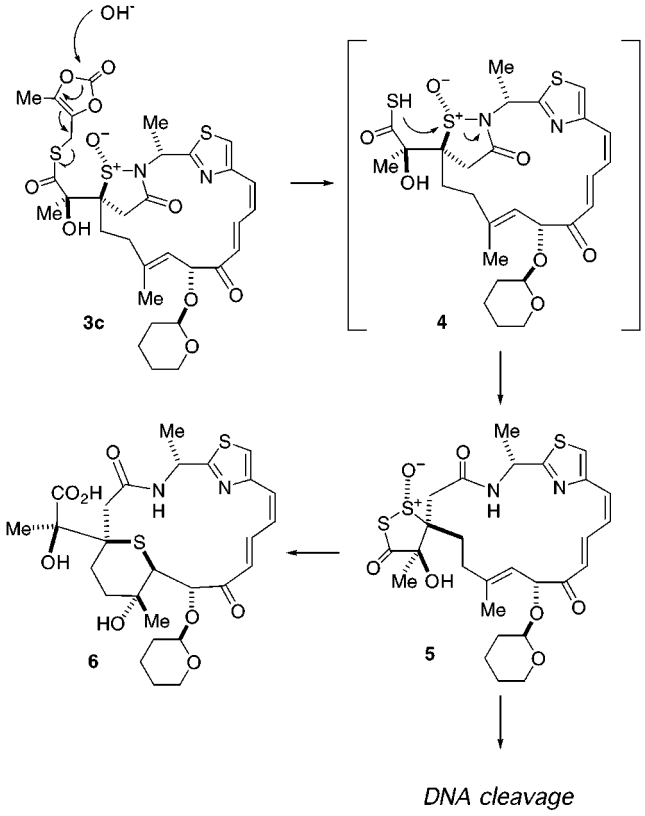
[a] Half-life of compounds determined by HPLC (330 nm); 20 μg/mL in pH 7 phosphate buffer/acetonitrile (4:1) at 37 °C. [b] In vitro antiproliferative activity against HeLa S_3 cells. The cells were precultured for 24 h in 96-well plates and treated with compounds for 72 h. On day 4, the antiproliferative activity was determined by the neutral red dye-uptake method. [c] In vivo antitumor activity against sarcoma 180. Sarcoma 180 cells were inoculated into the axillary region of ddY mice on day 0. Compounds were administered iv on day 1. [d] Treated versus control value of tumor volume. [e] Optimal dose. [f] 4-Methoxytetrahydropyran-4-yl.

the thioester derivatives with the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group can serve as prodrugs that are converted into active form in biological media. A possible activation pathway of **3c** is illustrated in Scheme 1. The (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group is either chemically or enzymatically hydrolyzed to form thio acid **4**, which then rearranges to dithiolanone compound **5**. Since **5** was not sufficiently stable for identification in fetal calf serum (FCS),[13] we have decided to identify the degradation products in FCS. When we treated **5** with FCS for 40 min, compound **6**[14] was identified in 66% yield by HPLC analysis as a major metabolite of **5**. Similarly, treatment of **3c** with FCS for 60 min, 21% of **6** and 53% of unreacted **3c** were identified by HPLC analysis. The results suggested that **3c** could act as a prodrug of dithiolanone **5**. The improved stability of **3c** results in enhanced antitumor activity relative to the parent compound **5**.

In summary, we have discovered the novel and stable thioester derivatives of leinamycin, which showed significant in vitro antiproliferative activity and in vivo antitumor activity. These derivatives have a unique 3-isothiazolidinone 1-oxide moiety and could be prodrugs that provide dithiolanone compounds in biological media. Compound **3c** was found to show potent antitumor activity against human tumor xenograft, such as lung, liver, ovary, prostate, and colon carcinomas.[15] Thus compound **3c** (KF22678) was selected for further evaluations as a promising antitumor agent.

Acknowledgment. We thank Professor Tohru Fukuyama of University of Tokyo and Professor Noriaki Hirayama of Tokai University for helpful discussions.

Scheme 1. Possible Activation Pathway



Supporting Information Available: Full experimental procedures and spectroscopic data for all new compounds and X-ray crystal structures for **2d** and **3c**. This information is available free of charge via the Internet at http://pubs.acs.org.

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(1) (a) Hara, M.; Takahashi, I.; Yoshida, M.; Asano, K.; Kawamoto, I.; Morimoto, M.; Nakano, H. DC 107, a Novel Antitumor Antibiotic Produced by a *Streptomyces* sp. *J. Antibiot.* **1989**, *42*, 333−335. (b) Hara, M.; Asano, K.; Kawamoto, I.; Takiguchi, T.; Katsumata, S.; Takahashi, K.; Nakano, H. Leinamycin, a New Antitumor Antibiotic from *Streptomyces*; Producing Organism, Fermentaion and Isolation. *J. Antibiot.* **1989**, *42*, 1768−1774.

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(6) Kanda, Y.; Ashizawa, T.; Saitoh, Y.; Saito, H.; Gomi, K.; Okabe, M. Synthesis and Antitumor Activity of Leinamycin Derivatives: Modifications of C-8 Hydroxy and C-9 Keto Groups. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 909−912.

(7) Kanda, Y.; Fukuyama, T. Total Synthesis of (+)-Leinamycin. *J. Am. Chem. Soc.* **1993**, *115*, 8451−8452.

(8) Full experimental procedures and spectroscopic data for all new compounds described in this paper are provided in Supporting Information.

(9) ORTEP drawings of **2d** and **3c** are provided in Supporting Information. A full discussion of these X-ray structures will be published elsewhere.

(10) Sakamoto, F.; Ikeda, S.; Tsukamoto, G. Studies on Prodrugs. II. Preparation and Characterization of (5-Substituted 2-oxo-1,3-dioxolen-4-yl)methyl Esters of Ampicillin. *Chem. Pharm. Bull.* **1984**, *32*, 2241−2248.
(11) All biological studies were performed according to reported procedures; see: Morimoto, M.; Ashizawa, T.; Ohno, H.; Azuma, M.; Kobayashi, E.; Okabe, M.; Gomi, K.; Kono, M.; Saitoh, Y.; Kanda, Y.; Arai, H.; Sato, A.; Kasai, M.; Tsuruo, T. Antitumor Activity of 7-*N*-[2-[[2-(γ-L-Glutamylamino)ethyl]dithio]ethyl] mitomycin C. *Cancer Res.* **1991**, *51*, 110−115.
(12) We thank Dr. A. Asai of our laboratories for the DNA-cleaving assay.
(13) Compound **5** was synthesized by the reported method (see ref 6), and the diastereomers were separated by preparative TLC. The half-life of **5** in FCS at 37 °C was 5 min, while the half-life of **3c** in FCS at 37 °C was 55 min.
(14) Compound **6** was synthesized for HPLC standard by the treatment of **5** with 2-mercaptoethanol in aqueous solution; see ref 4 for the formation of **6**.
(15) Ashizawa, T.; Kawashima, K.; Kanda, Y.; Gomi, K.; Okabe, M.; Ueda, K.; Tamaoki, T. Manuscript in preparation.

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