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Synthesis and Antitumor Activity of Novel C-8 Ester Derivatives of Leinamycin

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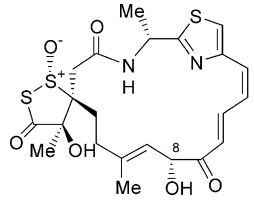
Abstract—A novel series of C-8 ester derivatives of leinamycin are described. Condensation of *N*-substituted amino acids or carboxylic acids containing polyether moiety with leinamycin resulted in the C-8 ester derivatives with good antitumor activity in several experimental models. Among these derivatives, compound **4e**, which has five ethylene glycol ether units in the C-8 acyl group, showed potent antitumor activity against human tumor xenograft. Combination with the modification of the dithiolanone moiety was applied to these C-8 ester derivatives and some of them also showed good antitumor activity.

Leinamycin (**1**), a potent 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 in addition to the DNA alkylation and related mode of action studies were also reported.[5] A theoretical study of chemical reactions related to the biological actvity of leinamycin was recently appeared.[6]



Leinamycin (1)

Although leinamycin shows marginal antitumor activity against murine experimental tumors, leinamycin derivatives with potent antitumor activity against human tumor xenografts have been desired for further development. As a part of our program aimed at discovering clinically useful leinamycin analogues, chemical modification[7,8] of natural leinamycin and total synthesis approach[9] have been investigated. In our earlier studies, we found that chemical modifications of the C-8 hydroxy group and the spiro dithiolanone moiety of leinamycin resulted in the leinamycin derivatives, such as **5** (KF22678), with potent antitumor activity.[8,10] Herein we report novel ester derivatives of the C-8 hydroxy group of leinamycin with potent antitumor activity against various experimental models.

Since some C-8 ester derivatives of leinamycin showed potent antiproliferative activity,[7] we synthesized more ester derivatives using carboxylic acids with functional groups to increase antitumor activity and water solubility. Selective esterification of the C-8 hydroxy group was achieved by the treatment of leinamycin with carboxylic acids and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of 4-dimethylaminopyridine (DMAP) (Scheme 1). Among a number of C-8 ester derivatives we prepared, *N*-substituted glycine esters showed good in vivo activity. So we focused our attention to introduce a series of *N*-substituted glycine and other related amino acid residues to the C-8 hydroxy group of leinamycin. Ester

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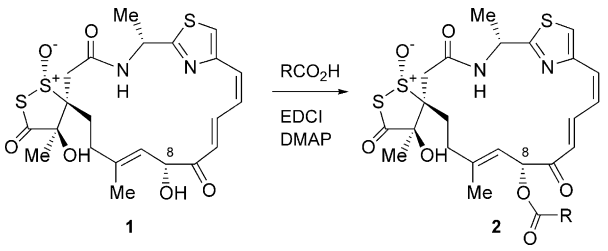


Scheme 1. Synthesis of C-8 ester derivatives of leinamycin.

derivatives **2a–p** were synthesized in reasonable yields from leinamycin (Table 1).[11]

Table 1. Structures, chemical yields, antiproliferative activity, and in vivo antitumor activity of C-8 ester derivatives **2a–p**

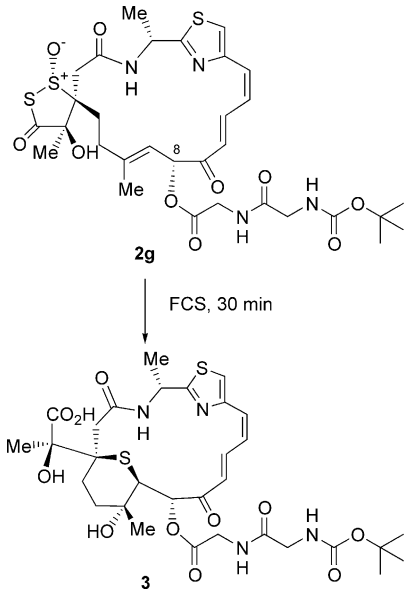
Compd (RCO_2H)[a]	Yield[b] (%)	HeLa S3[c] IC_{50} μmol/L	Sarcoma 180[d] T/C[e] (mg/kg)[f]	P388[g] ILS%[h] (mg/kg)[f]
2a (Formyl Gly)	57	0.011	0.46 (4.0)	56 (8.0)
2b (AcGly)	56	0.013	0.67 (2.0)	66 (4.0)
2c (CbzGly)	80	0.0062	0.89 (2.0)	54 (8.0)
2d (CbzAla)	76	0.045	0.56 (4.0)	52 (4.0)
2e (CbzβAla)	77	0.040	0.79 (4.0)	53 (4.0)
2f (CbzSar)	55	0.052	0.74 (8.0)	48 (4.0)
2g (BocGlyGly)	55	0.020	0.22 (8.0)	46 (8.0)
2h (CbzGlyGly)	47	0.023	0.30 (8.0)	72 (4.0)
2i (BzGlyGly)	55	0.0087	0.42 (8.0)	78 (8.0)
2j (CbzAlaGly)	50	0.034	0.61 (16)	58 (8.0)
2k (CbzSarGly)	54	0.067	0.61 (8.0)	70 (4.0)
2l (BocβAlaGly)	42	0.17	0.45 (8.0)	67 (8.0)
2m (CbzβAlaGly)	41	0.020	0.55 (8.0)	76 (16)
2n (CbzLeuGly)	43	0.016	0.56 (8.0)	67 (8.0)
2o (CbzGlyβAla)	53	0.15	0.69 (16)	71 (16)
2p (CbzβAlaβAla)	53	0.061	0.58 (16)	62 (16)
1	—	0.028	0.44 (2.0)	64 (1.0)

[a]Carboxylic acids used for the esterification of C-8 hydroxy group of leinamycin.
[b]Chemical yields from leinamycin.
[c]In vitro antiproliferative activity against HeLa S3 cells. The cells were precultured for 24 h in 96-well plates and treated with compounds for 72 h. IC_{50} values are determined by the neutral red dye-uptake method.
[d]In vivo antitumor activity against murine sarcoma 180. Sarcoma 180 cells were inoculated into the axillary region of ddY mice on day 0. Compounds were administered iv on day 1.
[e]Treated versus control value of tumor volume.
[f]Optimal dose.
[g]In vivo antitumor activity against lymphocytic leukemia P388 in mice. CD2F1 mice (five mice/group) were implanted intraperitoneally (ip) with 10^6 cells, and compounds were administered ip on day 1.
[h]Maximal increase in life span, calculated (T/C-1)×100, where T and C are mean survival days of treated and control mice, respectively.

Antitumor activities of these C-8 ester derivatives were evaluated in murine sarcoma 180 and P388 leukemia models as well as antiproliferative activity against HeLa S3 cells (Table 1).[12]

Various *N*-substituted glycine and alanine esters were prepared and most of them showed antitumor activity against sarcoma 180 and P388 leukemia. For example, single intravenous (iv) administration of *N*-substituted glycylglycine ester **2g** and **2h** showed significant antitumor activity against sarcoma 180 (T/C 0.22 and 0.30, respectively). Several dipeptide esters, such as **2h**, **2i**, **2k**, **2m**, and **2o**, showed better ILS values (ILS > 70%) than leinamycin in P388 leukemia model. Some compounds, such as **2c** and **2i**, showed potent antiproliferative activity against HeLa S3 cells (IC_{50} < 0.01 μmol/L).

Considering that esters might be converted to hydroxy group in vivo, stability of these ester derivatives of leinamycin in fetal calf serum (FCS) was investigated by HPLC analysis.[8] Half-lives of leinamycin and the amino acid ester derivatives, such as **2g**, in FCS were less than 10 min at 37 °C. The main metabolite of **2g** in FCS after 30 min was determined as compounds **3**, which is a C-8 ester derivative of the inactive degradation product of leinamycin[4] (Scheme 2). The results suggested that the hydrolysis of C-8 ester might be much slower than DNA cleavage by **2g**. Although the activation mechanisms for DNA cleavage in vivo system as well as pharmacokinetic analyses should be studied in detail, the C-8 ester groups would play some important roles for the DNA cleavage and in vivo antitumor activity.

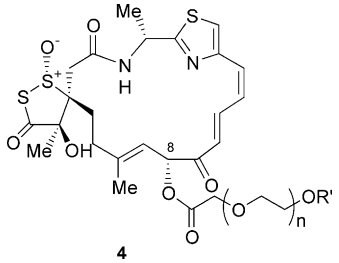


Scheme 2. Degradation of ester **2g** in FCS.

Introduction of the carboxylic acids containing polyether moiety, which could be effective for better water solubility, to C-8 hydroxy group was also investigated (Table 2). Treatment of leinamycin with various acetic acids substituted with some ethylene glycol ether units and EDCI in the presence of DMAP resulted in the compounds with excellent antitumor activity against sarcoma 180. Compounds **4g** and **4h**, which possess terminal hydroxy group in the C-8 acyl group, were also synthesized by the treatment of leinamycin with the carboxylic acids, in which the terminal hydroxy group was protected as TBS ether, followed by deprotection of TBS group under acidic conditions (HCl aq/THF, 20 °C). Substituted acetic acids with ethylene glycol ether units were easily prepared from sodium alkoxide of corresponding alcohols and bromoacetic acid. It should be noted that the number of ethylene glycol ether units (n) could be important for both in vitro antiproliferative activity against HeLa S3 cells and in vivo antitumor activity against sarcoma 180. Although narrow therapeutic range was observed in case of **4a** ($n = 1$), esters **4b** ($n = 2$) and **4e** ($n = 5$) showed good antitumor activity against sarcoma 180 (T/C 0.30). Since ester **4c** ($n = 3$) and **4f** ($n = 6$) showed weak antiproliferative activity

against HeLa S3 cells, the number of ethylene glycol ether units might influence the interaction of these derivatives with DNA. However, molecular modeling and biochemical studies would be needed for understanding the role of C-8 acyloxy groups on DNA interaction. It should be noted that compound **4h** with terminal hydroxy group in the C-8 acyl group and compound **4i**, an ester of **4g**, showed excellent antitumor activity against sarcoma 180 (T/C 0.18 and 0.12, respectively).

Table 2. Structures, chemical yields, antiproliferative activity, and in vivo antitumor activity of C-8 ester derivatives **4a**–**i**

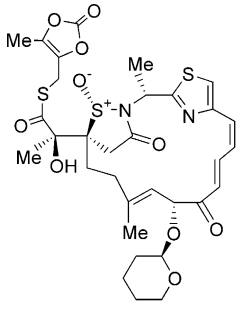


4

Compd	*n*	R′	Yield[a] (%)	HeLa S3[b] IC_{50}, μmol/L	Sarcoma 180[c] T/C (mg/kg)
4a	1	Me	72	0.026	0.72 (1.0)
4b	2	Me	61	0.066	0.30 (4.0)
4c	3	Me	69	0.61	0.82 (4.0)
4d	4	Me	49	0.24	0.51 (8.0)
4e	5	Me	52	0.011	0.30 (8.0)
4f	6	Me	8	0.21	nt
4g	2	H	58, 49[d]	0.025	0.36 (4.0)
4h	4	H	45, 28[e]	nt	0.18 (8.0)
4i	2	$COCH_2O(CH_2)_2OMe$	82[f]	nt	0.12 (8.0)
1	—	—	—	0.028	0.44 (2.0)

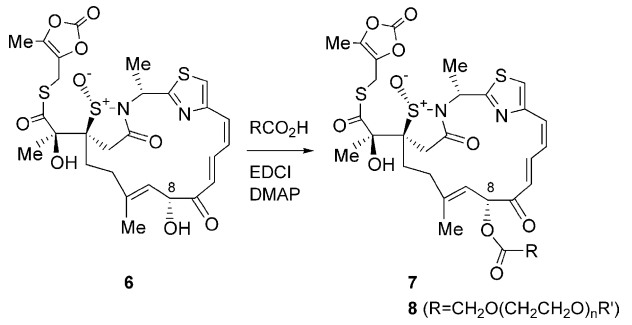
[a]Chemical yields from leinamycin unless otherwise noted.
[b]In vitro antiproliferative activity against HeLa S3 cells. See Table 1.
[c]In vivo antitumor activity against sarcoma 180 in mice. See Table 1.
[d]Yield of deprotection of TBS ether of **4g**.
[e]Yield of deprotection of TBS ether of **4h**.
[f]Yield from **4g**. (nt = not tested).

We previously reported that some thioester derivatives in combination with modification of C-8 hydroxy group resulted in compound **5** (KF22678), which showed a broad antitumor activity against human tumor xenografts such as lung, colon, ovary, and prostate.[10]



5 (KF22678)

Application of the structure–activity relationships of C-8 esters to the thioester derivatives of leinamycin, in which the dithiolanone moiety was converted to thioester with 3-isothiazolidinone 1-oxide, was carried out. These thioester derivatives of leinamycin could be considered as more stable prodrugs of corresponding dithiolanone compounds. Thioester **6**, which was synthesized by the reported method[8] from leinamycin,



6 **7** **8** (R=$CH_2O(CH_2CH_2O)_nR'$)

Scheme 3. Synthesis of ester derivatives **7** and **8**.

was selected as the starting material for the esterification of C-8 hydroxy group. Several C-8 esters **7** and **8** were synthesized by esterification of **6** by the method described for the synthesis of **2** (Scheme 3).

The acyl groups to be introduced were selected from the acids that showed good activity in case of compounds **2** and **4**. Table 3 shows the structures, chemical yields, and antitumor activity of compounds **7** and **8**. In case of *N*-substituted glycine esters **7**, most compounds showed comparable or better antitumor activity than corresponding dithiolanone compounds **3**. For example, *N*-carbobenzoxyglycine ester **7a** showed better ILS value (ILS 98%) than corresponding dithiolanone compound **2c** (ILS 52%). *N*-Substituted β-alanylglycine esters **7e** and **7f** showed excellent antitumor activity against sarcoma 180 (T/C 0.15 and 0.23). Unexpected results for esters **8**, containing polyether moiety in the C-8 acyl group, were obtained. Although compound **8a**, which has two ethylene glycol ether units, showed good antitumor activity against sarcoma 180 (T/C 0.37), the antitumor activity was less potent in comparison with the corresponding dithiolanone compound **4b** (T/C 0.30).

Table 3. Antiproliferative activity and in vivo antitumor activity of C-8 ester derivatives **7** and **8**

Compd (RCO_2H)[a]	Yield (%)[b]	HeLa S3[c] IC_{50}, μmol/L	Sarcoma 180[d] T/C (mg/kg)	P388[e] ILS% (mg/kg)
7a (CbzGly)	54	0.0040	0.36 (16)	98 (8.0)
7b (BocGlyGly)	22	0.014	0.39 (8.0)	39 (4.0)
7c (CbzGlyGly)	29	0.015	0.61 (4.0)	64 (8.0)
7d (BzGlyGly)	62	0.032	0.46 (8.0)	90 (16)
7e (CbxβAlaGly)	40	0.011	0.15 (16)	91 (16)
7f (BocβAlaGly)	21	0.012	0.23 (16)	70 (8.0)
8a (*n* = 2, R′ = Me)	41	0.0055	0.39 (8.0)	54 (2.0)
8b (*n* = 5, R′ = Me)	51	0.043	0.58 (8.0)	nt
6	—	0.0046	0.67 (2.0)	65 (2.0)

[a]Carboxylic acids that used for the modification of C-8 hydroxy group (for compound **7**).
[b]Chemical yields for the esterification from compound **6** unless otherwise noted.
[c]In vitro antiproliferative activity against HeLa S3 cells. See Table 1.
[d]In vivo antitumor activity against sarcoma 180 in mice. See Table 1.
[e]In vivo antitumor activity against lymphocytic leukemia P388 in mice. See Table 1. (nt = not tested).

Among all the ester derivatives described here, some compounds were selected for further evaluation in human tumor xenografts, Lu-65 (non-small cell lung calcinoma), A2780 (ovary carcinoma), and HCT116 (colon carcinoma). The results are summarized in Table 4.

Compounds **2h**, **4e**, **7b**, and **8a** showed potent antitumor activity against Lu-65 (T/C < 0.4). Especially, compound **4e** showed significant antitumor activity against Lu-65 (T/C 0.20). Compound **2a** showed good antitumor activity against A2780 (T/C 0.29). In HCT116 xenograft, compound **2h** showed marginal antitumor activity (T/C 0.48).

Table 4. Antitumor activity of ester derivatives of leinamycin against human tumors inoculated in nude mice

Compd	Dose[a] (mg/kg)	Lu-65 [b] T/C[c]	A2780[b] T/C[c]	HCT116[b] T/C[c]
2a	5.3	0.43	0.29	0.62
2g	8.0	0.43	0.73	0.70
2h	12	0.30	0.63	0.48
4e	8.0	0.20	nt	nt
7b	12	0.33	0.51	nt
7e	18	0.48	0.46	0.64
8a	4.0	0.35	nt	nt
5 (KF22678)	8.0	0.33	0.43	0.48

[a]Optimal dose.
[b]Tumor cells (8-mm^3 fragment) were inoculated sc into male BALB/c-nu/nu mice. When tumors had grown to the size between 50 and 300 mm^3 (day 0), compounds were administered iv.
[c]Treated versus control value of tumor volume. (nt = not tested).

In conclusion, novel C-8 ester derivatives of leinamycin showed significant antitumor activity against several experimental models. Some of these ester derivatives, such as **4e**, are being evaluated for further development as potential antitumor agents.

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