Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 909–912

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND ANTITUMOR ACTIVITY OF LEINAMYCIN DERIVATIVES : MODIFICATIONS OF C-8 HYDROXY AND C-9 KETO GROUPS

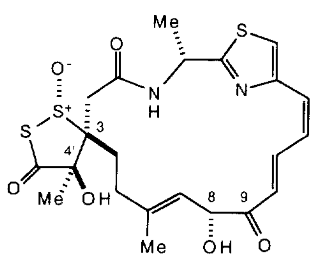
Yutaka Kanda,[a]* Tadashi Ashizawa,[b] Yutaka Saitoh,[a] Hiromitsu Saito,[a] Katsushige Gomi,[b] and Masami Okabe[b]

[a]*Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. 3-6-6 Asahimachi, Machida, Tokyo 194, Japan and* [b]*Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd. 1188 Shimotogari, Nagaizumi, Sunto, Shizuoka 411, Japan*

Received 21 January 1998; accepted 4 March 1998

Abstract: A series of leinamycin derivatives were synthesized and evaluated for antitumor activity. Modifications at C-8 and C-9 positions revealed a broad structure-activity relationship in vitro and some derivatives showed potent antiproliferative activity against HeLa S_3 cells.

Leinamycin (**1**), a novel antitumor antibiotic with unusual spiro 1-oxo-1,2-dithiolan-3-one moiety, was isolated from a culture broth of *Streptomyces* sp. and was shown to possess potent antitumor activities against murine experimental tumors.[1] Leinamycin causes single strand scission of plasmid DNA in the presence of thiol cofacors.[2] Isolation of a guanine-leinamycin adduct revealed the unprecedented chemical reactions which would be responsible for the thiol-mediated alkylative DNA cleavage by leinamycin.[2,3] As a part of our program aimed at discovering clinically useful leinamycin analogs, chemical modification of natural leinamycin have been investigated.[4] In this communication, synthesis, in vitro antiproliferative activity, and in vivo antitumor activity of C-8 and C-9 substituted leinamycin derivatives are described.



Leinamycin (1)

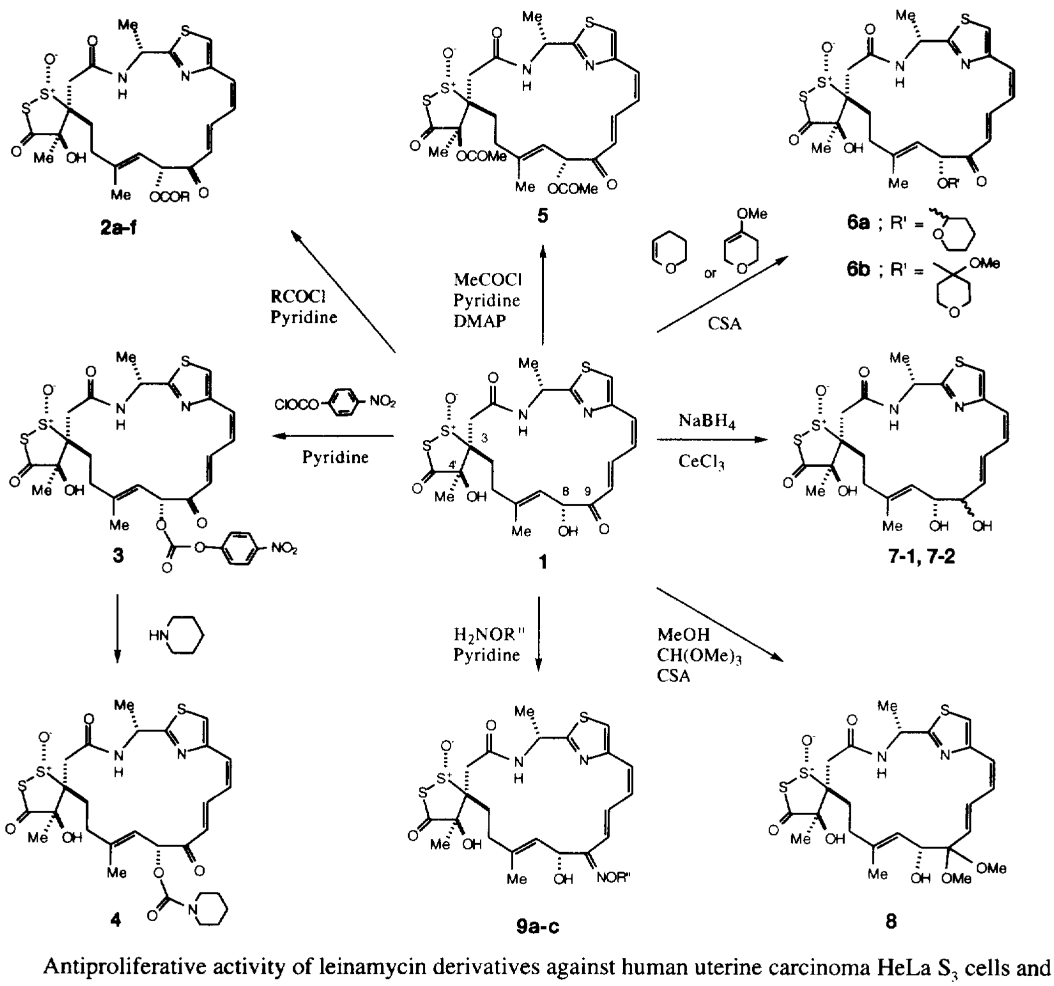
Since the dithiolanone moiety of leinamycin plays a crucial role for the DNA cleaving activity,[2] the modifications without affecting this labile moiety were explored (Scheme 1). Monoacetate **2a** was obtained by the treatment of leinamycin with acetic anhydride and pyridine in the presence of *N,N*-dimethylaminopyridine (DMAP).[5] Various C-8 acyloxy derivatives **2b-e** were synthesized using acid chlorides and pyridine in fairly good yields. Although carbonates **2f** and **3** were also prepared from leinamycin and the corresponding chloroformate, reactions of leinamycin with carbamoyl chlorides or isothianates did not afford the corresponding carbamates. Carbamate **4** could be obtained from 4-nitrophenyl carbonate **3** and piperidine in 26% yield. Diacetate **5** was obtained when excess acetyl chloride

0960-894X/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(98)00133-4

was used. Tetrahydropiranyl (THP) ether **6a** and methoxytetrahydropiranyl (MTHP) ether **6b** could be prepared in good yields from leinamycin and the corresponding dihydropirans in the presence of camphorsulfonic acid (CSA).

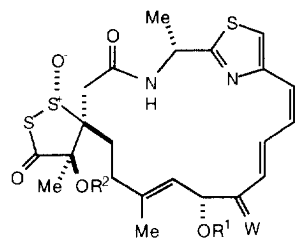
Selective reduction of the ketone at C-9 was achieved using $NaBH_4$ and $CeCl_3$ in MeOH at 0 °C to afford C-9 hydroxy derivative **7-1** and its epimer **7-2**. Dimethylacetal **8** could be prepared from leinamycin and a catalytic amount of CSA in MeOH and $CH(OMe)_3$. Oxime derivatives **9a-c** were prepared by treating leinamycin with hydroxylamine or hydroxylamine ethers.[4b,6]

Scheme 1. Chemical modifications of leinamycin



Antiproliferative activity of leinamycin derivatives against human uterine carcinoma HeLa S_3 cells and in vivo antitumor activity against murine leukemia P388 are shown in Table 1. Since diacetate **5** showed much weaker antiproliferative activity than monoacetate **2a**, a free hydroxy group at C-4' might be important for the activity. Acylation of C-8 hydroxy group resulted in potent derivatives such as **2a** and **2e** in HeLa S_3 assay. THP ether **6a** and the ethyl carbonate **2f** showed more potent antiproliferative activity than that of leinamycin. Conversion of the C-9 ketone to acetal or hydroxy group resulted in much less potent derivatives **7** and **8**. On the other hand, oxime derivatives **9b-1** and **9c-2** maintained the antiproliferative activity. Interestingly, benzyloxime **9c-1** (*Z* isomer) showed 11 times less potent activity than **9c-2** (*E* isomer). These results suggest that the conjugation from the thiazole ring to sp^2 carbon at C-9 and substituents at C-8 and C-9 would be critical for the antiproliferative activity.

Table 1. Leinamycin derivatives



compd	R^1	R^2	W	yield [a] (%)	HeLa S_3 [b] IC_{50} (μM)	P388 (ip-ip)[c] ILS_{max} (%)[d]	P388 (ip-ip)[c] OD (mg/kg)[e]
2a	COMe	H	O	82	0.010	34	1.0
2b	CO(*c*-hex)	H	O	81	0.058	49	4.0
2c	$CO(CH_2)_{14}Me$	H	O	72	7.7	37	16
2d	COPh	H	O	84	0.13	36	2.0
2e	CO(2-quinoxalinyl)	H	O	75	0.0068	44	2.0
2f	CO_2Et	H	O	85	0.0014	31	2.0
3	$CO_2C_6H_4(4\text{-}NO_2)$	H	O	96	0.022	57	2.0
4	$CO\text{-}N(CH_2)_5\text{-}$	H	O	26[l]	0.18	nt[n]	-
5	COMe	COMe	O	74	0.78	15	2.0
6a	THP[j]	H	O	58[m]	0.0013	28	0.13
6b	MTHP[k]	H	O	94	0.018	35	2.0
7-1[f]	H	H	H, OH	28	6.2	nt	-
7-2[g]	H	H	H, OH	22	4.9	nt	-
8	H	H	OMe, OMe	72	>10	nt	-
9a	H	H	NOH	52	0.91	nt	-
9b-1[h]	H	H	NOMe	24	0.067	nt	-
9b-2[i]	H	H	NOMe	61	0.11	21	2.0
9c-1[h]	H	H	$NOCH_2Ph$	47	0.11	29	8.0
9c-2[i]	H	H	$NOCH_2Ph$	43	0.0095	31	2.0
1	H	H	O	-	0.011	57	0.38

a) Yields from leinamycin unless otherwise noted. 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 lymphocytic leukemia P388 in mice. $CD2F_1$ mice (five mice/group) were implanted intraperitoneally (ip) with 10^6 cells, and compounds were administered ip on day 1. d) Maximal increase in life span, calculated (*T*/*C*-1) x 100, where *T* and *C* are mean survival days of treated and control mice, respectively. e) Optimal dose. f) Less polar isomer. g) More polar isomer. h) Less polar isomer (*Z* oxime configuration). i) More polar isomer (*E* oxime configuration). j) 2-Tetrahydropiranyl. k) 1-Methoxytetrahydropiranyl. l) Yield from compound **3**. m) Yield for a 5:4 mixture of diastereomers. n) Not tested.

In vivo antitumor activity of leinamycin derivatives against P388 leukemia seemed to be consistent with their in vitro antiproliferative activity in terms of optimal dose. Thus, the derivatives with low IC_{50} value showed potent in vivo antitumor activity in terms of dose. Some derivatives showed comparable ILS value to that of leinamycin (**2b**, **2e**, **3**, and **6b**).

In summary, chemical modifications of C-8 hydroxy and C-9 keto groups of leinamycin were carried out and the antiproliferative activity against HeLa S_3 cells revealed a broad structure-activity relationship in vitro. Modification of C-8 hydroxy group resulted in potent derivatives **2f** and **6a**. The importance of substituents at C-8 and C-9 for the antitumor activity might be associated with the interaction between leinamycin derivatives and DNA.

Acknowledgment. We thank Dr. M. Yoshida and her staff for NMR and MS spectra. We are grateful to N. Chiba, K. Masunaga, Y. Tsuji, and K. Sugiyama for their excellent technical assistance.

References and Notes

1. (a) Hara, M.; Takahashi, I.; Yoshida, M.; Asano, K.; Kawamoto, I.; Morimoto, M, Nakano, H. *J. Antibiotics* **1989**, *42*, 333. (b) Hara, M.; Asano, K.; Kawamoto, I.; Takiguchi, T.; Katsumata, S.; Takahashi, K.; Nakano, H. *J. Antibiotics* **1989**, *42*, 333. (c) Hirayama, N. Matsuzawa, E. S. *Chem. Lett.* **1993**, 1957.
2. (a) Hara, M.; Saitoh, Y.; Nakano, H. *Biochemistry* **1990**, *29*, 5676. (b) Asai, A.; Hara, M.; Kakita, S.; Kanda, Y.; Yoshida, M.; Saito, H.; Saitoh, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6802.
3. Oxidative DNA cleavage by leinamycin *in addition to* the DNA alkylation was recently reported. Mitra, K.; Kim, W.; Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1997**, *119*, 11691.
4. For our synthetic approach, see: (a) Kanda, Y; Saito, H.; Fukuyama, T. *Tetrahedron Lett.* **1992**, *33*, 5701. (b) Kanda, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **1993**, *115*, 8451.
5. All compounds were fully characterized by ^1H NMR, IR, and HRMS. Spectroscopic data for key compounds is as follows:
   Compound **2a**: ^1H NMR ($CDCl_3$, 400MHz) δ ppm ; 8.80 (dd, *J* = 16.5, 11.6 Hz, 1H), 7.28 (s, 1H), 6.67 (br d, *J* = 6.6 Hz, 1H), 6.65 (d, *J* = 11.6 Hz, 1H), 6.36 (dd, *J* = 11.6, 11.6 Hz, 1H), 6.03 (d, *J* = 16.5 Hz, 1H), 5.79 (br s, 2H), 5.36 (dq, *J* = 6.6, 6.6 Hz, 1H), 4.45 (br s, 1H), 3.06 (br s, 2H), 2.35 (dt, *J* = 12.8, 3.9 Hz, 1H), 2.06 (dt, *J* = 12.8, 5.2 Hz, 1H), 2.00 (s, 3H), 1.88 (dt, *J* = 12.8, 5.2 Hz, 1H), 1.78 (s, 3H), 1.77 (d, *J* = 6.6 Hz, 3H), 1.75 (m, 1H), 1.72 (s, 3H). HRFABMS *m/z* calcd for $C_{24}H_{29}N_2O_7S_3$ $(M+H)^+$ 553.1137, found 553.1160.
   Compound **2f**: ^1H NMR ($CDCl_3$, 400MHz) δ ppm ; 8.76 (dd, *J* = 16.6, 11.4 Hz, 1H), 7.27 (s, 1H), 6.66 (br d, *J* = 6.5 Hz, 1H), 6.66 (d, *J* = 11.4 Hz, 1H), 6.37 (dd,*J* = 11.4, 11.4 Hz, 1H), 6.07 (d, *J* = 16.6 Hz, 1H), 5.78 (br d, *J* = 10.0 Hz, 1H), 5.73 (d, *J* = 10.0 Hz, 1H), 5.36 (dq,*J* = 6.5, 6.5 Hz, 1H), 4.41 (br s, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.04 (s, 2H), 2.34 (dt, *J* = 12.7, 4.0 Hz, 1H), 2.06 (ddd,*J* = 13.0, 12.7, 5.3 Hz, 1H), 1.89 (ddd, *J* =12.7, 12.4, 5.3 Hz, 1H), 1.77 (s, 3H), 1.76 (ddd, *J* = 13.0, 12.4, 4.0 Hz, 1H), 1.75 (d, *J* = 1.2 Hz, 3H), 1.74 (d, *J* = 6.5 Hz, 3H), 1.23 (t, *J* =7.1 Hz, 3H). HRFABMS *m/z* calcd for $C_{25}H_{31}N_2O_8S_3$ 583.1242 $(M+H)^+$, found 583.1259.
   Compound **6a**: ^1H NMR ($CDCl_3$, 500MHz) δ ppm ; major isomer 9.25 (dd, *J* = 16.5, 11.6 Hz, 1H), 7.25 (s, 1H), 6.88 (br d, *J* = 6.4 Hz, 1H), 6.63 (d, *J* = 11.6 Hz, 1H), 6.36 (dd, *J* = 11.6, 11.6 Hz, 1H), 6.01 (d, *J* = 16.5 Hz, 1H), 5.94 (d,*J* = 9.7 Hz, 1H), 5.30 (dq, *J* = 6.7, 6.4 Hz, 1H), 5.08 (dd, *J* = 9.7,1.2 Hz, 1H), 5.00 (br s, 1H), 4.58 (t, *J* = 4.6 Hz, 1H), 3.78-3.74 (m, 1H), 3.50-3.45 (m, 1H), 3.25 (d, *J* = 15.0 Hz, 1H), 2.90 (d, *J* = 15.0 Hz, 1H), 2.35-2.28 (m, 1H), 2.12-2.06 (m, 1H), 1.95-1.44 (m, 8H), 1.88 (s, 3H), 1.79 (d, *J* = 6.7 Hz, 3H), 1.72 (d, *J* = 1.2 Hz, 3H) ; minor isomer 9.04 (dd, *J* = 16.5, 11.6 Hz, 1H), 7.25 (s, 1H), 6.86 (br d, *J* = 6.4 Hz, 1H), 6.63 (d, *J* = 11.6 Hz, 1H), 6.39 (dd, *J* = 11.6, 11.6 Hz, 1H), 6.05 (d, *J* = 16.5 Hz, 1H), 5.87 (d, *J* = 9.8 Hz, 1H), 5.27 (dq, *J* = 6.4, 6.4 Hz, 1H), 4.93 (br s, 1H), 4.91 (dd, *J* = 9.8, 1.2 Hz, 1H), 4.63 (br s, 1H), 3.72-3.67 (m, 1H), 3.45-3.39 (m, 1H), 3.22 (d, *J* = 14.6 Hz, 1H), 2.88 (d,*J* = 14.6 Hz, 1H), 2.35-2.28 (m, 1H), 2.14-2.06 (m, 1H), 1.90-1.40 (m, 8H), 1.90 (s, 3H), 1.72 (d, *J* = 6.4 Hz, 3H), 1.72 (s, 3H). HRFABMS *m/z* calcd for $C_{27}H_{35}N_2O_7S_3$ $(M+H)^+$ 595.1606, found 595.1606.
6. Each isomer at the C=N double bond was isolated in case of **9b** and **9c**. The stereochemistry was determined by NMR spectra. For the determination of oxime stereochemistry, see: Gasc, J.-C.; Gouin D'Ambrieres, S.; Lutz, A.; Chantot, J.-F. *J. Antibiotics* **1991**, *44*, 313.