Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 9, pp. 1021-1024, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00162-X

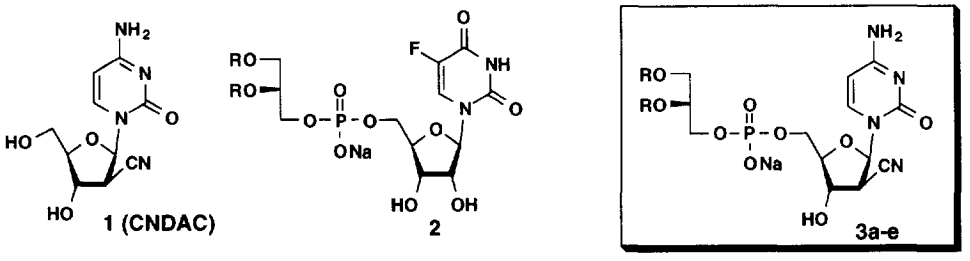
NUCLEOSIDES AND NUCLEOTIDES. 150. ENZYMATIC SYNTHESIS OF 5'-PHOSPHATIDYL DERIVATIVES OF 1-(2-*C*-CYANO-2-DEOXY-β-D-*ARABINO*-PENTOFURANOSYL)CYTOSINE (CNDAC) AND THEIR NOTABLE ANTITUMOR EFFECTS IN MICE [1]

Satoshi Shuto,[a] Hirokazu Awano,[a] Naomi Shimazaki,[b] Kenji Hanaoka,[b] and Akira Matsuda[a,*]

[a]*Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan*
[b]*Biological Research Laboratories, Sankyo Co., LTD. Japan*

Abstract: 1-(2-*C*-Cyano-2-deoxy-β-D-*arabino*-pentofuranosyl)cytosine (CNDAC, **1**) is a potent antitumor nucleoside developed by us. A series of 5'-phosphatidyl derivatives of CNDAC bearing various fatty acyl residues, namely dimyristoyl derivative **3a**, dipalmitoyl derivative **3b**, distearoyl **3c**, dioleoyl derivative **3d**, and dilinoleoyl derivative **3e**, was synthesized by phospholipase D-catalyzed transphosphatidylation. All of these 5'-phosphatidyl-CNDACs (**3a-e**) administered ip almost perfectly inhibited the growth of sc-implanted M5076 sarcoma in mice, and the effects were clearly superior to that of CNDAC.

To develop efficient antitumor drugs, it is very important to find agents having a new antitumor mechanism of action, namely having characteristic pharmacodynamic properties. We have designed and synthesized 1-(2-*C*-cyano-2-deoxy-β-D-*arabino*-pentofuranosyl)cytosine (CNDAC, **1**) as an antitumor nucleoside due to mechanism-based DNA-strand-breaking activity.[2] In fact CNDAC showed potent growth inhibitory activity against various human tumor cells both *in vitro* and *in vivo*.[2] We also demonstrated that the 5'-triphosphate of CNDAC was incorporated into DNA by DNA polymerases and the resulting oligo-DNA including CNDAC was strand-broken spontaneously, as we hypothesized.[2e]



R = Fatty acyl

On the other hand, it is also significant to modify antitumor agents with a new mechanism of action to use them effectively in cancer chemotherapy. From this point of view, much attention has been focused on the conversion of antitumor nucleosides to their phospholipid derivatives.[3] In recent years, we have been engaged in the studies on 5'-phosphatidylation of antitumor nucleosides to improve their antitumor

potency.[4] We have used 5-fluorouridine (FUR) as a model antitumor nucleoside and demonstrated that 5'-phosphatidyl-FUR (**2**) had more significant antitumor activity against various mouse tumors than FUR, and had excellent pharmacokinetic properties due to being recognized as a phospholipid by the body.[4a,b,d-f] In this communication, we describe synthesis of 5'-phosphatidyl derivatives of CNDAC bearing various fatty acid residues, and their antitumor effects on M5076 sarcoma in mice.

We have reported that CNDAC was very unstable due to unusual acidity of the 2'-proton; the 2'-position was readily epimerized and/or the glycoside linkage was cleaved even in neutral conditions.[2d] Accordingly, its derivatization by the usual nucleoside chemistry would be troublesome. On the other hand, we have developed an enzymatic method for the preparation of 5'-phosphatidylnucleosides from a nucleoside and a 3-*sn*-phosphatidylcholine (PC, **4**) by a one-step reaction, in which phospholipase D-catalyzed transphosphatidylation, namely, the regiospecific transfer reaction of the phosphatidyl residue from PC to the 5'-hydroxyl of a nucleoside, was used.[4a,b] Therefore, we planned to synthesize 5'-phosphatidyl derivatives of CNDAC by this enzymatic method.

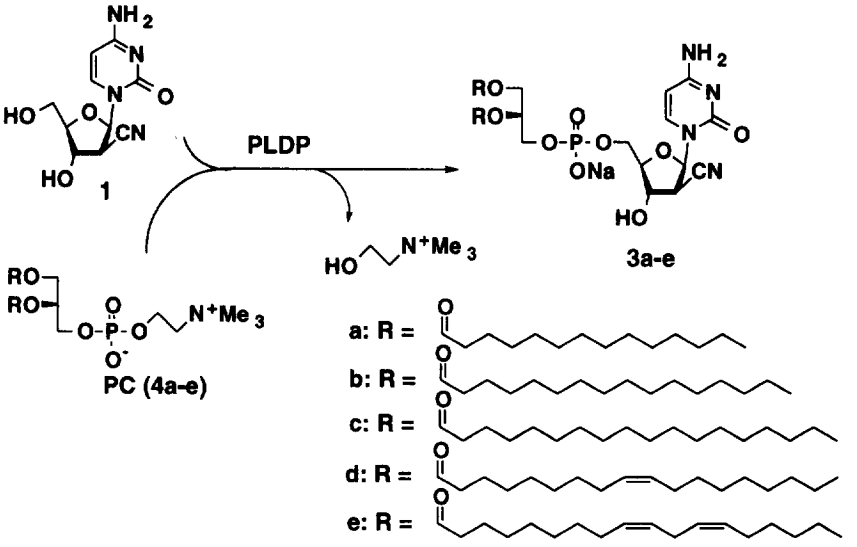


Table I. Yields and Physical Data for 5'-(3-*sn*-Phosphatidyl)-CNDACs.

Compound	Yield[a]	UV $_{max}$ (nm)[b]	FAB-MS (*m/z*)	Formula[c]
3a	52	271	850 (MH^+)	$C_{41}H_{70}N_4O_{11}PNa \cdot 3/4MeOH$
3b	42	271	906 (MH^+)	$C_{45}H_{78}N_4O_{11}PNa$
3c	52	271	962 (MH^+)	$C_{49}H_{86}N_4O_{11}PNa$
3d	53	271	958 (MH^+)	$C_{49}H_{82}N_4O_{11}PNa \cdot 1/2H_2O$
3e	54	271	954 (MH^+)	$C_{49}H_{78}N_4O_{11}PNa \cdot H_2O$

[a]Yields were based on CNDAC used. [b]Measured in MeOH. [c]Compounds were analyzed for C, H, and N and were within ±0.4% of the theoretical value.

In the presence of 1.2 eq. of dimyristoyl PC **4a**, CNDAC was treated with phospholipase D from *Streptomyces* sp. AA 586 (PLDP) in a two-phase system of chloroform and acetate buffer (pH 4.5) at 40 °C. After the usual work-up and treatment with a cation-exchange resin, the desired phosphatidyl-CNDAC derivative **3a** was isolated as sodium salt in a pure form[5] in which epimerization at the 2'-position was not observed. In a similar way, saturated-acyl-type derivatives, **3b** and **3c**, were readily prepared. It is recognized that synthesis of phospholipid derivatives bearing unsaturated acyl residues is usually troublesome due to their instability.[6] However, the dioleoyl and dilinoleoyl phosphatidyl derivatives of CNDAC, **3d** and **3e**, also were successfully synthesized by this enzymatic method.

Table II. Antitumor activities of ip-administered 5'-phosphatidyl-CNDACs (**3a-e**) against M5076 sarcoma implanted sc in mice[a]

Compound	Dose (mg/kg/day)	TGI[b] (%)	ILS[c] (%)	Wt. Change[d] (g)
3a	100	80	4	0.8
	200	97	22	0.3
	300	-	0	toxic (1)[e]
3b	100	93	21	0.9
	200	98	38	0
	300	100	40	toxic (1)[e]
3c	100	88	20	1.2
	200	97	49	-0.2
	300	98	60	-0.3
3d	25	69	-3	1.6
	50	92	7	0.8
	100	98	13	0.6
	200	-	-12	toxic (2)[e]
	300	-	-58	toxic (3)[e]
3e	100	93	15	-0.3
	200	-	10	toxic (1)[e]
	300	-	15	toxic (1)[e]
CNDAC (**1**)	100	38	-11	1.1
	200	47	-7	1.2
	300	67	14	1.0

[a]M5076 cells ($1x10^6$) were implanted sc into female BDF1 mice and ip-therapy was done on Days 1, 5, 9. [b]Tumor volume and tumor growth inhibition (TGI) on Day 21 were calculated as follows: Tumor volume = (length) x $(width)^2/2$; TGI (%) = [1-(average tumor volume in treated group)/(average tumor volume in control group)] x 100. [c]Increase in life-span (ILS) was calculated as follows: ILS = [(median survival day of treated group)/(median survival day of control group) - 1] x 100. [d]Body weight change from Day 1 to 12. [e]Number of toxic deaths out of 6 mice.

Although CNDAC effectively inhibits the growth of various experimental tumors,[3a-c] its antitumor activity against mouse M5076 sarcoma was not strong enough. Therefore, we evaluated the antitumor effects of the newly synthesized compounds with sc-implanted M5076 sarcoma in mice, and compared them with the effects of CNDAC. The compounds were administered ip on Days 1, 5, and 9, and the results are summarized in Table II. The saturated diacyl derivatives, **3a**, **3b**, and **3c**, with the same saturated fatty acyl residues (myristoyl, palmitoyl, and stearoyl, respectively) at the *sn*-1- and *sn*-2-positions, inhibited the growth of sarcoma almost perfectly without body weight changes of mice, at the doses 100 and 200 mg/kg/day. This result was clearly superior to that of CNDAC (tumor growth inhibition (TGI); 38% at 100 mg/kg/day and 47% at 200 mg/kg/day, respectively). At the dose 300 mg/kg/day, although **3a** and **3b** were toxic, **3c** strongly inhibited growth of the tumor (98% inhibition) and also increased the life span of mice (ILS 60%) with a slight weight loss in mice. CNDAC had a moderate antitumor effect at this dose (TGI 67%, ILS 14%). Although the unsaturated diacyl derivative **3d** and **3e** were toxic at 200 mg/kg/day and 300 mg/kg/day, when the treatments were done at lower doses, these compounds showed more excellent effects than saturated derivatives. The linoleoyl deri-

vative **3e** at 100 mg/kg/day and the oleoyl derivative **3d** at 100 and 50 mg/kg/day inhibited the growth of sarcoma effectively. We also investigated the antitumor effects on the same tumor system by po administration of **3a**, **3b**, and **3c**; all the tested compounds showed similar strong antitumor effects as those observed by their ip administration (data not shown).

In conclusion, we enzymatically synthesized 5'-phosphatidyl derivatives of CNDAC that had more significant antitumor effects than the parent compound, CNDAC. These results, as well as the antitumor potency of 5-phosphatidyl-FUR reported previously,[4b,e,f] suggested that the derivatization of antitumor nucleosides to the corresponding 5'-phosphatidyl analogues resulted in increased antitumor effects of the compounds.

Acknowledgment

We thank to Drs. S. Imamura and H. Misaki of Asahi Chemical Industry for their gift of PLDP.

References and Notes

1. Part 149: Obara, T.; Shuto, S.; Saito, Y.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E.; Matsuda, A. *J. Med. Chem.* Submitted for publication.
2. a) Matsuda, A.; Nakajima, Y.; Azuma, A.; Tanaka, M.; Sasaki, T. *J. Med. Chem.* **1991**, *34*, 2917-2919. b) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T. *J. Med. Chem.* **1993**, *36*, 4183-4189. c) Tanaka, M.; Matsuda, A.; Terao, T.; Sasaki., T. *Cancer Lett.* **1992**, *64*, 67-74. d) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem.* **1995**, *38*, 3391-3397. e) Matsuda, A.; Azuma, A.; Nucleosides Nucleotides, **1995**, *14*, 461-471.
3. a) Shuto, S.; Ueda, S.; Imamura, S.; Fukukawa, K.; Ueda, T. *Chem. Pharm. Bull.* **1988,** *36,* 5020-5023. b) Shuto, S.; Itoh, H.; Endo, E.; Fukukawa, K.; Tsujino, M.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1987**, *35*, 3523-3526. c) Turcotte, J. G.; Srivastava, S. P.; Meresak, W. A.; Rizkalla, B. A.; Louzon, F.; Wunz, T. P. *Biochim. Biophys. Acta* **1980**, *619*, 604-618. d) Hong, C. I.; An, S.-H.; Buchheit, D. J.; Nechaev, A.; Kirisits, A. J.; West, C. R.; Berdel, W. E. *J. Med. Chem.* **1986**, *29*, 2038-2044. e) Herrmann, R.; Berdel, W. E. *Cancer Res.* **1992**, *52*, 1865-1867. f) Neumann, J. -M.; Herve, M.; Debouzy, J. -C; Iglesias, G. F.; Gouyette, C.; Dupraz, B.; Huynh-Dinh, T. *J. Am. Chem. Soc.*, **1989**, *111*, 4270-4377. g) Hostetler, K. Y.; Parker, S.; Sridhar, C. N.; Martin, M. J.; Li. J. -L.; Stuhmiller, L. M. van Wijk, G. M. T.; van den Bosch, H.; Gardner, M. F.; Aldern, K. A.; Richman, D. D. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 11835-11839.
4. a) Shuto, S.; Ueda, S.; Imamura, S.; Fukukawa, K.; Tsujino, M.; Matsuda, A.; Ueda, T. *Tetrahedron Lett.* **1987**, *28*, 199-202. b) Shuto, S.; Itoh, H.; Ueda, S.; Imamura, S.; Fukukawa, K.; Tsujino, M.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1988**, *36*, 209-217. c) Shuto, S.; Itoh, H.; Obara, T.; Nakagami, K.; Yaso, M.; Yaginuma, S.; Tsujino, M.; Saito, T.; Matsuda, A.; Ueda, T. *Nucleosides Nucleotides* **1992**, *11*, 437-446. d) Sakai, A.; Mori, N.; Shuto, S.; Suzuki, T. *J. Pharm. Sci.* **1993**, *82*, 575-578. e) Doi, K.; Oku, N.; Toyota, T.; Shuto, S.; Sakai, A.; Itoh, H.; Okada, S. *Biol. Pharm. Bull.*, **1994**, *17*, 1414-1416. f) Shuto, S.; Itoh, H.; Sakai, A.; Nakagami, K.; Imamura, S.; Matsuda, A. *BioMed. Chem.* **1995**, *3*, 235-243.
5. A typical procedure: $CHCl_3$ solution (20 mL) of 3-*sn*-phosphatidylcholine (**4**, 0.6 mmol) was added to a solution of PLDP (7 mg, 1220 units) and CNDAC (126 mg, 0.5 mmol) in sodium acetate buffer (200 mM, pH 4.5, 4 mL). The mixture was stirred at 40 °C for 6 h, then a mixture of H_2O (5 mL), MeOH (20 mL), and $CHCl_3$ (20 mL) was added, and the resulting mixture was shaken. The separated organic layer was washed with H_2O and evaporated to dryness. The residue was purified by flash chromatography (silica gel, $CHCl_3$/MeOH, 3:1) to give white powder of phosphatidyl-CNDAC, which was treated with Diaion WK-20 resin (2 x 8 cm, Na^+ form) giving **3** as a sodium salt.
6. a) Hermetter, A.; Paltauf, F.; Hauser, H. *Chem. Phys. Lipids* **1982**, *30*, 35-45. b) Browning, J.; Seelig, J. *Chem. Phys. Lipids* **1979**, *24*, 103-118.

(*Received in Japan* 14 *February* 1996; *accepted* 1 *April* 1996)