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NUCLEOSIDES AND NUCLEOTIDES. 152. 1-(3-*C*-ETHYNYL-β-D-*RIBO*-PENTOFURANOSYL)URACIL AS A BROAD SPECTRUM ANTITUMOR NUCLEOSIDE[1]

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Abstract: 1-(3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl)uracil (EUrd) has been designed as a potential multifunctional antitumor nucleoside antimetabolite. EUrd was synthesized by condensation of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-ethynyl-α,β-D-*ribo*-pentofuranose (**6**) and bis(trimethylsilyl)uracil in the presence of trimethylsilyl triflate in CH_3CN in good yield, followed by debenzoylation with NH_3/MeOH. *In vitro* tumor cell growth inhibitory activity of EUrd against 14 human solid tumor cell lines was compared with 5-fluorouridine (FUrd), 2'-deoxy-5-fluorouridine (FdUrd), and 5-fluorouracil (5-FU) as positive controls. EUrd was a quite potent tumor cell growth inhibitor against almost all the tumor cell lines examined in this study except for human pancreas PANC-1 cells, and the potency of EUrd is about 6 to 650 times stronger than that of 5-FU and comparable to that of FUrd and FdUrd. EUrd showed also potent antitumor activity against human tumors as xenografts in nude mice when given in a daily intravenous dose of 2 mg/kg on consecutive days.

A nucleoside antimetabolite is one of the most important agents for cancer chemotherapy. 2'-Deoxycytidine analogues, such as 1-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)cytosine (DMDC),[2] 2'-deoxy-2',2'-difluorocytidine (gemcitabine),[3] and 1-(2-*C*-cyano-2-deoxy-β-D-*arabino*-pentofuranosyl)cytosine (CNDAC),[4] have been developed as potent antitumor agents, which are, unlike 1-β-D-arabinofuranosylcytosine (araC), effective not only on leukemias and lymphomas, but also on a wide variety of solid tumors *in vitro* as well as *in vivo*. These nucleosides are metabolized to their corresponding 5'-diphosphates and/or 5'-triphosphates, which are potent inhibitors of ribonucleoside diphosphate reductase (RDPR) and/or DNA polymerases, respectively. Therefore the inhibition of DNA synthesis of tumor cells would cause their antitumor activity. However, it has been reported that they also slightly but significantly inhibit RNA synthesis. Although the inhibition of RNA synthesis would not be a major mechanism of tumor cell death, it is conceivable that this inhibition may contribute to the antitumor efficacy against slow-growing solid tumors, because a large population of tumor cells is in other than S-phase *in vivo*, in which only RNA synthesis and DNA repair are done. Therefore, to search for more potent inhibitors of tumor cell growth, we designed a nucleoside antimetabolite inhibiting to both DNA and RNA syntheses.

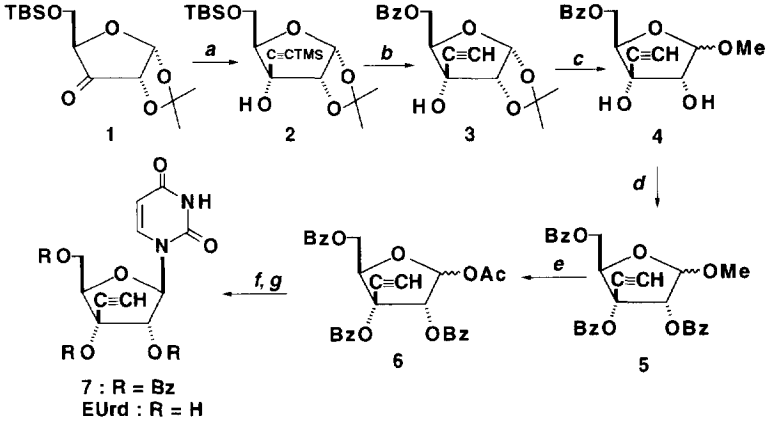
To inhibit DNA synthesis, RDPR is one of the most important target enzymes.[5] RDPR catalyzes the conversion of all the four main ribonucleoside 5'-diphosphates (rNDPs) to the corresponding 2'-deoxyribonucleoside 5'-diphosphates (dNDPs) in a *de novo* pathway. The resulting dNDPs are further phosphorylated to the corresponding 2'-deoxyribonucleoside 5'-triphosphates (dNTPs) by nucleoside diphosphate kinase, which are essential for DNA synthesis. *E. coli* RDPR, which is a prototype of mammalian enzymes, is the best characterized. The R2 subunit contains a tyrosyl radical stabilized by a μ-oxo bridged

binuclear iron (III) center.[5] This radical is transferred to certain amino acid residues in the active site of the R1 subunit in the catalytic site, which initiates the enzyme-catalyzed reduction of the 2'-hydroxyl group in rNDPs, by abstraction of the 3'-hydrogen atom in the ribose moiety of the rNDPs.[6] The amino acid residue corresponding to the radical formation has been postulated to be Cys439 from results of site-directed mutagenesis of the R1 subunit.[7] X-ray crystallographic studies of the R1 subunit also suggested the Cys439 is near the 3'-hydrogen of the rNDP.[8] Therefore, as a functional group that reacts effectively and directly with the thiyl radical, we selected an acetylene group to be introduced at the 3' position, since acetylene derivatives are well-known to react with thiyl radicals to form alkylthio vinyl sulfides.[9]

On the other hand, to inhibit RNA synthesis, a nucleoside should be recognized as a substrate and/or an inhibitor of ribonucleoside and ribonucleotide metabolizing enzymes, such as kinases, CTP synthetase, and RNA polymerase, and therefore should have two hydroxyl groups at the 2' and 3' positions with the *ribo*-configuration. From these considerations, we designed 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)uracil (EUrd) as a potential multifunctional antitumor nucleoside. In this communication, we describe the synthesis and antitumor activity *in vitro* and *in vivo* of EUrd.

Synthesis of EUrd is rather straightforward. Since metal acetylides react from the undesired α-face of 3'-ketonucleosides due to their steric hinderance,[10] we synthesized EUrd from the 3-ulose derivative **1**, which can be easily obtained from D-xylose in four steps in good yield.[11] Addition of LiC≡CTMS to **1** gave the desired β-adduct **2** highly stereoselectively in 99% yield.[12] Desilylation of **2** with tetrabutylammonium fluoride, followed

Scheme 1[a]



[a]Conditions: (a) TMSC≡CH, BuLi, THF, -78 ℃, 99%; (b) i) Bu_4NF, THF, rt, ii) BzCl, pyridine, rt, 93% in two steps; (c) aqueous 20% HCl, MeOH, rt; (d) BzCl, DMAP, pyridine, 100 ℃, 87% in two steps; (e) concentrated H_2SO_4, AcOH, Ac_2O, rt, 93%; (f) bis(TMS)uracil (4 equiv), TMSOTf (4 equiv), 0 ℃ rt, 95%; (g) NH_3/MeOH, rt, 85%.

by benzoylation yielded **3** in 93% yield. Treatment of **3** with HCl in aqueous MeOH, followed by benzoylation of the remaining hydroxyl groups in a mixture of BzCl and DMAP in anhydrous pyridine at 100 ℃ gave **5** in 87% yield. Subsequent acetolysis of **5** furnished 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-3-*C*-ethynyl-α,β-D-*ribo*-

pentofuranose (**6**) in 93% yield as a syrup.

Condensation of bis(trimethylsilyl)uracil (4 equiv) with **6** in CH_3CN in the presence of trimethylsilyl triflate (4 equiv) gave the desired blocked β-nucleoside **7** in 95% as a form. The protected nucleoside **7** was treated with methanolic ammonia at room temperature to furnish EUrd in 85% yield in a crystalline form.[13, 14]

In vitro tumor cell growth inhibitory activity of EUrd against 14 human solid tumor cell lines, including 5 stomach carcinomas, 5 colon carcinomas, 1 lung carcinoma, 1 pancreas carcinoma, 1 bladder transitional-cell carcinoma, and 1 fibrosarcoma, was evaluated. We selected 5-fluorouridine (FUrd), 2'-deoxy-5-fluorouridine (FdUrd) and 5-fluorouracil (5-FU) as positive controls, since 5-FU is widely used for an anti-solid-tumor agent in clinic, and 5-FUrd inhibits mainly RNA synthesis after metabolically converted to its 5'-triphosphate, and FdUrd inhibits DNA synthesis via the inhibition of thymidylate synthase (TS) by its 5'-monophosphate. As can be seen from Table 1, EUrd is a quite potent tumor cell growth inhibitor against almost all the tumor cell lines examined in this study except for pancreas PANC-1 cells. The potency of EUrd is about 6 to 650 times stronger than that of 5-FU and comparable to that of FUrd and FdUrd.

Table 1. Inhibitory Effects of **EUrd**, **FUrd**, **FdUrd**, and **5-FU** on the Growth of Human Tumor Cell lines *in Vitro*[a]

Cell line	Origin	IC_{50} (μM)			
		EUrd	**FUrd**	**FdUrd**	**5-FU**
MKN-28	Stomach	0.080	0.028	0.12	2.5
MKN-45	Stomach	0.024	0.026	3.5	3.0
KKLS	Stomach	0.19	0.016	0.76	6.2
AZ-521	Stomach	0.036	0.050	0.050	3.9
NUGC-3	Stomach	0.034	0.022	0.015	4.4
Colo320DM	Colon	0.060	0.0054	0.65	1.1
SW-48	Colon	0.12	0.016	0.13	5.7
SUN-C2A	Colon	0.018	0.045	0.0033	2.7
DLD-1	Colon	0.066	0.073	0.092	6.0
HCT-15	Colon	0.043	0.047	0.049	5.7
A-549	Lung	0.029	1.9	1.0	1.7
PANC-1	Pancreas	11	11	>40	>78
T24	Bladder	0.034	0.014	0.12	22
HT-1080	Sarcoma	0.024	0.041	0.12	3.6

[a]Tumor cell growth inhibitory activity assay *in vitro* was done following the method of Carmichael *et al.*[15] Each tumor cell (2×10^3 cells/well) was incubated in the presence or absence of compounds for 72 h. MTT-reagent was added to each well and plate was incubated for 4 h more, the resulting MTT-formazan was dissolved in DMSO and the OD (540 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1-OD (540 nm) of sample well/OD (540 nm) of control well] x 100. IC_{50} (μM) was given as the concentration at 50% inhibition of cell growth.

In vivo antileukemic activity of EUrd against mouse leukemia P388 cells (10^5 cells/mouse) using BDF1 mice was also investigated. When EUrd was administered intraperitoneally on days 1 and 5 at a dose of 3

mg/kg/day, it showed a T/C value of 150%. Even at a dose of 1 mg/kg/day under the same schedule of drug administration, the T/C value was calculated to be 144%. We also examined the antitumor effects of EUrd on human tumors (2 stomach, 3 colon, and 2 pancreas cancers) as xenografts in nude mice. The results are summarized in Table 2. In these experiments, the tumor inhibition ratio (%) was used as the parameter of antitumor activity and day 15 was chosen as the day of final evaluation of the effects of treatment on tumor growth. The tumor inhibition ratios were calculated by: Inhibition ratio (%) = (A - B)/A x 100, where A is the average relative tumor weight of the control group and B is that of the treated group. Each group consisted of 8 mice. EUrd had a strong antitumor effect on 6 out of 7 tumor xenografts and a moderate effect on pancreas carcinoma BxPC-3 when given in a daily intravenous dose of 2 mg/kg for 10 consecutive days. There was no detectable toxicity to mice with this dose. Next, the antitumor activity of EUrd was compared with that of 5-FU, since it has been widely used clinically against solid tumors, such as stomach, colon, and breast cancers. Although FUrd and FdUrd are more active than 5-FU *in vitro*, it is known that FUrd has low antitumor activity *in vivo* because of inefficient conversion to 5-fluoro-2'-deoxyuridine 5'-monophosphate, and the clinical use of FdUrd is limited to gastrointestinal adenocarcinoma metastatic to the liver. When 5-FU was intravenously administered 14 consecutive days at a dose of 15 mg/kg, it had antitumor effects only on stomach cancer H-81 but showed moderate antitumor effects on the other human tumors. The doses of EUrd and 5-FU used in this study were each the maximum non-toxic dose. Since a multiplicity of dose schedules were not tested, they may not have been used under optimal circumstances in this study.

Table 2. Antitumor Effects of **EUrd** and **5-FU** on Human Tumor Xenografts in Nude Mice[a]

Tumor	Origin	Tumor inhibition ratio (%)	
		EUrd[b]	**5-FU**[c]
		2 mg/kg x 10, iv	15 mg/kg x 14, iv
AZ-521	Stomach	81	25
H-81	Stomach	92	83
CO-3	Colon	90	49
KM12C	Colon	81	59
DLD-1	Colon	73	50
PAN-12	Pancreas	81	40
BxPC-3	Pancreas	35	50

[a]Tumor mass (2 mm^3) was subcutaneously transplanted into nude mice. [b]EUrd was intravenously administered for 10 consecutive days when the tumor size reached 60-100 mm^3. [c]FU was intravenously administered for 14 consecutive days when the tumor size reached 60-100 mm^3.

Although the optimal therapeutic schedule of EUrd is not yet known, such excellent antituomor activity suggests that EUrd is a promising agent for further evaluation as therapy of human cancer. Whether the mechanism responsible for its excellent antitumor efficacy is related to inhibition of both of DNA and RNA syntheses as described above is now under investigation. If EUrd acts as a multifunctional antitumor nucleoside,

it would be a new approach for anticancer chemotherapy.

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(14) Mp 226-228 ℃ (aqueous MeOH); EI-MS *m/z* 268 (M^+); IR (nujor) 2110 cm^{-1} (C≡C); ^{1}H-NMR (DMSO-d_6) 11.35 (br s, 1 H, NH), 7.99 (d, 1 H, H-6, $J_{6,5}$ = 8.2 Hz), 5.93 (s, 1 H, 3'-OH), 5.86 (d, 1 H, 2'-OH, $J_{2'OH,2'}$ = 6.7 Hz), 5.83 (d, 1 H, H-1', $J_{1',2'}$ = 7.3 Hz), 5.69 (d, 1 H, H-5, $J_{5,6}$ = 8.2 Hz), 5.13 (t, 1 H, 5'-OH, J = 4.5 Hz), 4.18 (dd, 1 H, H-2', $J_{2',1'}$ = 7.3, $J_{2',2'OH}$ = 6.7 Hz), 3.90-3.88 (m, 1 H, H-4'), 3.74-3.60 (m, 2 H, H-5'), 3.55 (s, 1 H, 3'-C≡CH). *Anal.* Calcd for $C_{11}H_{12}N_2O_6$: C, 49.26; H, 4.51; N, 10.44. Found: C, 49.00; H, 4.64; N, 10.52.

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