Journal of Medicinal Chemistry

Brief Article

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Fluorocyclopentenyl-cytosine with Broad Spectrum and Potent Antitumor Activity†

Won Jun Choi,[‡,§] Hwa-Jin Chung,[∥] Girish Chandra,[‡] Varughese Alexander,[‡] Long Xuan Zhao,[‡,⊥] Hyuk Woo Lee,[‡] Akshata Nayak,[‡] Mahesh S. Majik,[‡] Hea Ok Kim,[‡] Jin-Hee Kim,[‡] Young B. Lee,[#] Chang H. Ahn,[#] Sang Kook Lee,*[,∥] and Lak Shin Jeong*[,‡]

[‡]Department of Bioinspired Science and College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

[§]College of Pharmacy, Dongguk University, Kyungki-do 410-774, Korea

[∥]College of Pharmacy, Seoul National University, Seoul 151-742, Korea

[⊥]College of Chemistry and Chemical Engineering, Liaoning Normal University, Dalian 116-029, China

[#]Rexan Pharmaceuticals, Inc., Bethesda, Maryland 20850, United States

Supporting Information

ABSTRACT: On the basis of the potent biological activity of cyclopentenyl-pyrimidines, fluorocyclopentenyl-pyrimidines were designed and synthesized from D-ribose. Among these, the cytosine derivative **5a** showed highly potent antigrowth effects in a broad range of tumor cell lines and very potent antitumor activity in a nude mouse tumor xenograft model implanted with A549 human lung cancer cells. However, its 2′-deoxycytidine derivative **5b** did not show any antigrowth effects, indicating that 2′-hydroxyl group is essential for the biological activity.

INTRODUCTION

Neplanocin A (**1**, Figure 1),[1,2] a naturally occurring nucleoside, shows potent antiviral and antitumor activities by inhibiting *S*-adenosylhomocysteine (SAH) hydrolase, which catalyzes the interconversion of *S*-adenosylhomocysteine into adenosine and L-homocysteine.[3] Compound **1** causes the depletion of the cofactor NAD^+ by inhibiting SAH hydrolase. This inhibition is reversed by the addition of excess cofactor NAD^+.[4] Its 6′-fluoro analogue **2** was also reported to be a potent inhibitor of SAH hydrolase and to exhibit potent antiviral activity against vesicular stomatitis virus (VSV).[5] Compound **2** was shown to be a novel dual mechanism-based inhibitor of SAH hydrolase.[5]

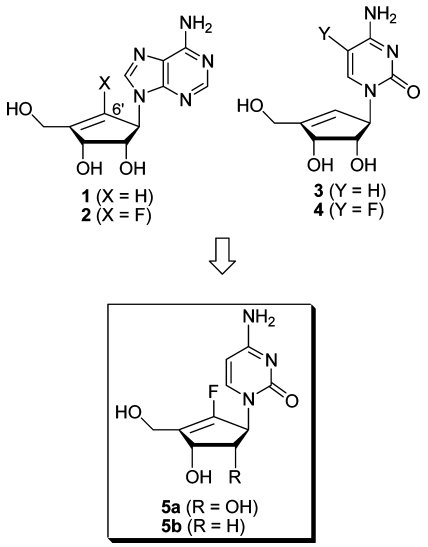


Figure 1. The rationale for the design of fluorocyclopentenyl-cytosine derivatives **5a** and **5b**.

On the other hand, the cyclopentenyl-cytosine analogue **3** showed potent antitumor and antiviral activities by reducing cytidine-5′-triphosphate (CTP) pools.[6] Another 5-fluorocytosine analogue **4** also exhibited potent antiviral activity against the West Nile virus although the compound is rather cytotoxic.[7] Thus, on the basis of the potent biological activity of **3** and **4**, there is interest in designing and synthesizing the fluorocyclopentenyl-cytosine derivative **5a** (R = OH) and measuring its biological activity. Additionally, the synthesis of its 2′-deoxy analogue **5b** (R = H) will help determine if the 2′-hydroxyl group is critical for the biological activity. Moreover, it is also interesting to study the biological target involved in the antitumor activity of **5a**. Herein, we report the synthesis of the fluorocyclopentenyl-cytosine derivatives **5a** and **5b**, the potent antitumor activity of **5a**, both in in vitro and in vivo studies, and the mechanism of action of **5a**.

RESULTS AND DISCUSSION

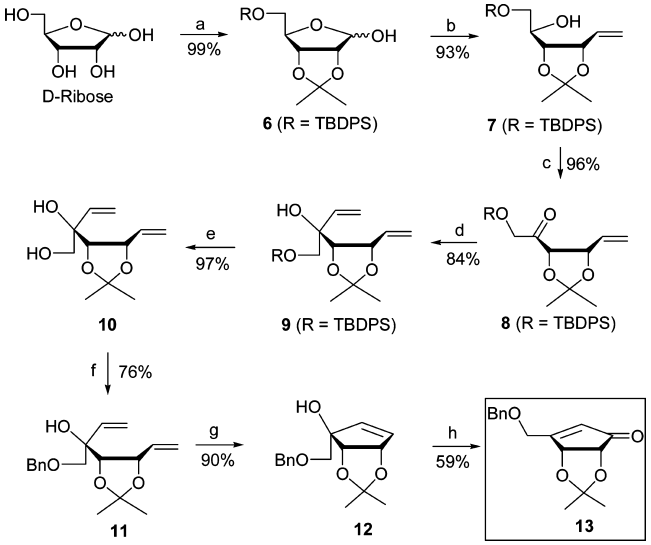
For the synthesis of the desired nucleosides, the key intermediate **13**[8,9] was first synthesized with a benzyl protecting group, as shown in Scheme 1, because other protecting groups such as trityl, TBS, and TBDPS were not successful in the electrophilic vinyl fluorination.

We previously reported that the Grignard reaction to the ketone **8** in the case of the benzyl protecting group produced desired β-hydroxydiene **11** as the minor isomer and α-

Received: March 22, 2012
Published: April 23, 2012

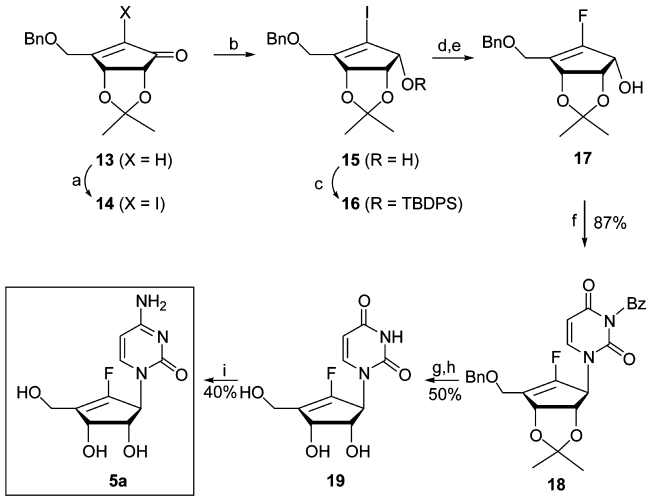
   dx.doi.org/10.1021/jm3004009 | J. Med. Chem. 2012, 55, 4521−4525

Scheme 1. Synthesis of the Key Intermediate 13 with Benzyl Protecting Group[a]



[a]Reagents and conditions: (a) *c*-H_2SO_4, acetone, then TBDPSCl, imidazole; (b) Ph_3PCH_3Br, KO*t*-Bu, THF; (c) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 1 h, then Et_3N, rt; (d) CH_2═CHMgBr, THF, −78 °C; (e) *n*-Bu_4NF, THF; (f) (i) $Bu_2Sn(O)$, toluene, 15 h, (ii) TBAI, BnBr, 50 °C, 16 h; (g) Grubbs catalyst, toluene, 80 °C; (h) PDC, molecular sieves, DMF, rt, 18 h.

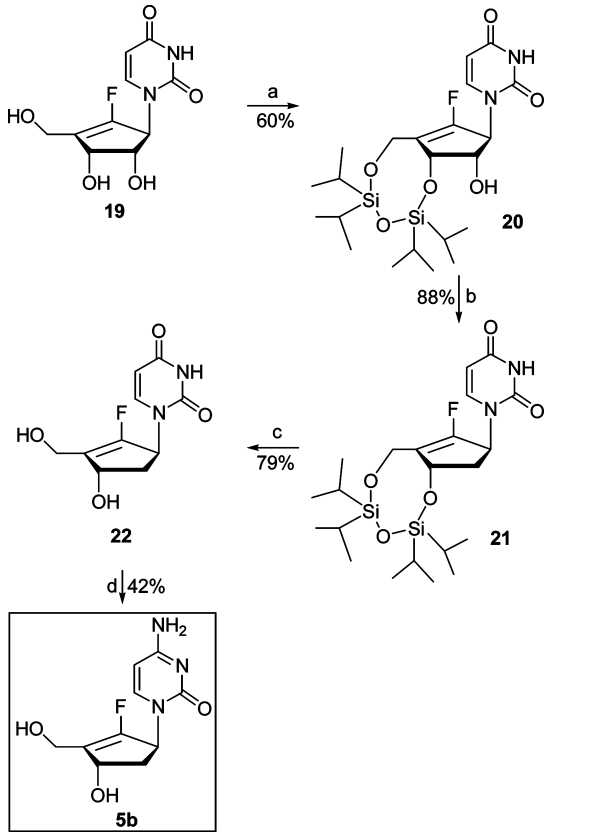
Scheme 2. Synthesis of the 2′-Hydroxyl Derivatives 5a and 19[a]



[a]Reagents and conditions: (a) I_2, pyridine, THF; (b) $NaBH_4$, $CeCl_3$-$7H_2O$, MeOH; (c) TBDPSCl, imidazole, DMF; (d) NFSI, *n*-BuLi, THF, −78 °C; (e) *n*-Bu_4NF, THF; (f) N^3-benzoyluracil, DEAD, Ph_3P, THF; (g) NH_3/MeOH; (h) BBr_3, CH_2Cl_2, −78 °C ; (i) (i) Ac_2O, pyridine, (ii) $POCl_3$, Et_3N, 1,2,4-triazole, (iii) NH_4OH, 1,4-dioxane; (iv) NH_3/MeOH.

hydroxydiene as the major isomer, but the minor isomer **11** could only undergo oxidative rearrangement to give **13**.[8] Thus, we first synthesized *β*-hydroxydiene **9** with the TBDPS protecting group stereoselectively in five steps, according to a previously published procedure,[8] and then converted the TBDPS group into the benzyl group. Treatment of D-ribose with acetone and *c*-H_2SO_4, followed by TBDPS protection of the resulting 2,3-acetonide, gave the lactol **6**. A Wittig reaction

Scheme 3. Synthesis of the 2′-Deoxy Derivatives 5b and 22[a]



[a]Reagents and conditions: (a) $TIDPSCl_2$, pyridine, rt; (b) PhOC(S)-Cl, pyridine, then *n*-Bu_3SnH, AIBN, toluene, 120 °C; (c) TBAF, THF, rt; (d) (i) Ac_2O, pyridine, (ii. $POCl_3$, Et_3N, 1,2,4-triazole, (iii) NH_4OH, 1,4-dioxane, (iv) NH_3/MeOH.

of **6** followed by Swern oxidation produced ketone **8**. A Grignard reaction of **8** with vinylmagnesium bromide yielded the diene **9** as a single stereoisomer. Removal of the TBDPS group in **9**, followed by selective benzylation of the resulting diol **10**[10] using organotin chemistry, produced the benzylate **11**. A ring-closing metathesis (RCM) reaction of **11** using the second-generation Grubbs catalyst gave the *β*-hydroxycyclopentene **12**. Oxidative rearrangement of **12** with PDC in DMF yielded the benzylated cyclopentenone **13**[6] with concomitant minor formation of its benzoylated derivative, resulting from the benzylic oxidation (see page S4 in Supporting Information).

The key intermediate **13** was first converted to known glycosyl donor **17**[5] and then to the final cytosine derivative **5a** (Scheme 2). Conventional iodination of **13** with iodine and pyridine in CCl_4 was slow, but when the solvent was changed to THF, iodocyclopentenone **14** was obtained at a very good yield. Reduction of **14** with sodium borohydride, followed by TBDPS protection of the resulting **15**, produced TBDPS ether **16**. Electrophilic vinyl fluorination was successfully achieved by adding *n*-BuLi to a solution of **16** and *N*-fluorobenzenesulfonimide (NFSI) at −78 °C, giving **17** after desilylation.[5] Condensation of **17** with N^3-benzoyluracil under the standard Mitsunobu conditions yielded the uracil derivative **18**. Treatment of **18** with methanolic ammonia, followed by treatment with boron tribromide, produced the uracil derivative **19**. The uracil derivative **19** was converted to the cytosine derivative **5a** in three steps. Treatment of **19** with acetic anhydride gave the triacetate, which was converted to the triazole derivative by

dx.doi.org/10.1021/jm3004009 | J. Med. Chem. 2012, 55, 4521−4525

Table 1. Broad Spectrum and Potent Antitumor Activity (IC_{50}, μM) of Compound 5a in a Variety of Human Tumor Cell Lines

	tumor cell lines								
	OVCAR-3 (ovary)	MCF-7 (breast, hormone dependent)		MDA-MB-231 (breast)	HeLa (cervix)	PC-3 (prostate)	LNCap (prostate)	HepG2 (liver)	A549 (lung)	UMRC2 (kidney)
IC_{50} (μM)	0.80	0.34		0.18	1.35	0.63	2.67	0.79	0.50	0.83
	tumor cell lines									
	NCIH226 (lung)	HT-29 (colon)	HCT116 (colon)	SK-MEL-28 (melanoma)	PANC-1 (pancreas)	U251 (brain)	MKN45 (stomach)	K562 (leukemia)		
IC_{50} (μM)	0.25	0.28	0.19	1.38	0.62	0.83	0.34	0.82		

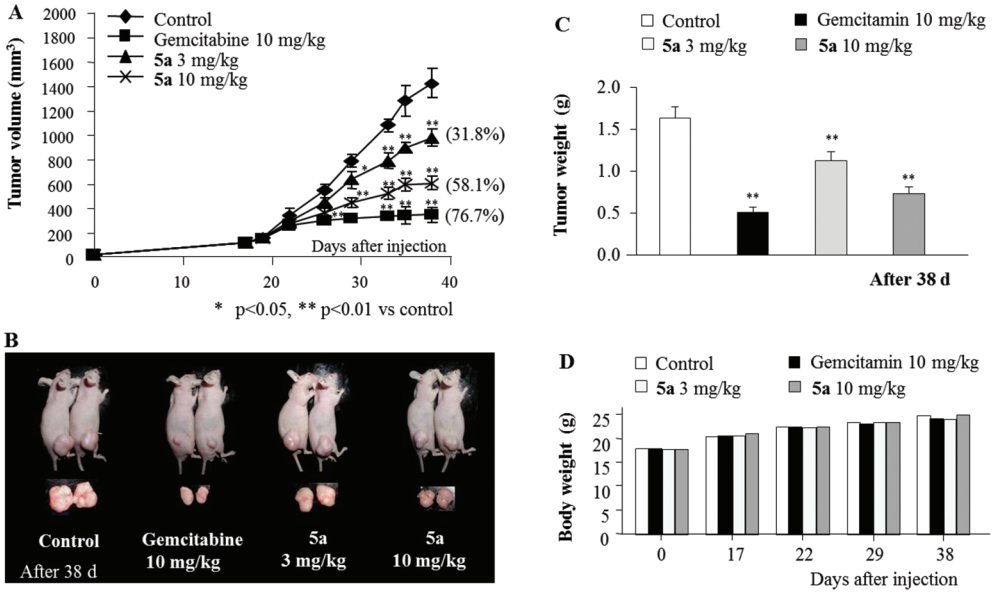


Figure 2. Inhibition of tumor growth by **5a** using an A549 xenograft model. (A,B) A549 cells (3×10^6 cells) were injected subcutaneously into the right flank of nude mice. When tumor volumes reached ca. 100 mm^3, treatment was initiated. **5a** (3 or 10 mg/kg) was administered intraperitoneally three times a week for three weeks. Tumor volumes were measured with a caliper every 2−3 days. (C) Weight of the removed xenograft tumors on day 38 was measured. *p < 0.05, **p < 0.01 indicates statistically significant differences from the control group. (D) Body weight changes of the mice were monitored during the experiments.

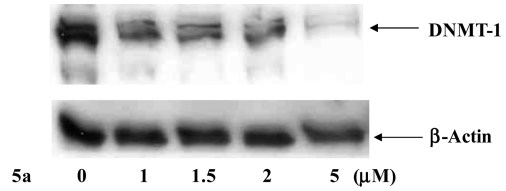


Figure 3. Effect of **5a** on the expression of DNMT-1 protein in MDA-MB-231 cancer cells. Cells were treated without or with **5a** at 1, 1.5, 2, or 5 μM for 24 h. The expression of DNMT-1 protein was determined by Western blot analysis. Actin was determined as a loading control. Representative data are shown from two independent cell culture experiment.

treating with $POCl_3$ and 1,2,4-triazole in the presence of triethylamine. The triazole derivative was converted to the cytosine derivative by treating with ammonium hydroxide in 1,4-dioxane. Removal of the acetate group with methanolic ammonia yielded the final cytosine derivative **5a**.

To determine if a ribosyl template is essential for the biological activity, the 2′-deoxy derivative **5b** was synthesized as shown in Scheme 3. Compounds **5a** was treated with $TIPDSCl_2$ to give the 3′,5′-TIPDS-protected nucleoside **20**. Treatment of **20** with phenyl chlorothionoformate in pyridine gave the thiocarbonate, which was treated with n-Bu_3SnH and AIBN in refluxing toluene to give the 2′-deoxy derivative **21**. Removal of the TIPDS group of **21** with tetra-n-butylammonium fluoride produced the final 2′-deoxyuridine derivative **22**. The 2′-deoxyuridine derivative **22** was converted to the 2′-deoxycytidine derivative **5b** according to the same procedure used in Scheme 2. All synthesized final nucleosides **5a**, **5b**, **19**, and **22** were tested for in vitro antigrowth effects in a variety of human tumor cell lines using the sulforhodamine B (SRB) method. The cytotoxic effects were measured at a concentration of 1 μM of the final nucleosides. As shown in Table 1, only the cytosine derivative **5a** exhibited highly potent antitumor activity against a broad range of tumor cell lines at the above-mentioned concentration. Interestingly, compound **5a** showed much weaker antileukemic activity than **3**,[6] a CTP-synthetase inhibitor. This finding indicates that different mechanism of action may be involved in the antitumor activity of **5a**. However, the corresponding 2′-deoxy analogue **5b** did not show any cytotoxic effect, indicating that the 2′- hydroxyl group is essential for the biological activity of compound **5a**.

Lung cancer is the leading cause of cancer-related death in the world.[11] Along with surgery and radiotherapy, chemotherapy is one of the most common treatments for lung cancer therapy. Although the overall survival has been improved significantly in advanced nonsmall cell lung cancer (NSCLC) using platinum-based combinations with chemotherapeutic agents such as vinorelbine, gemcitabine, and the taxanes during

dx.doi.org/10.1021/jm3004009 | J. Med. Chem. 2012, 55, 4521−4525

the past decade, the one-year survival rates are typically 35% and the two-year survival rates approach only 15−20% in patients with advanced NSCLC.[12−14] Therefore, the development of novel approaches to prevent and treat lung cancer is an important goal.

Thus, we evaluated the antitumor activity of **5a** in a nude mouse tumor xenograft model implanted with A549 human lung cancer cells to determine if in vitro antigrowth effects were correlated with in vivo antitumor effects. A549 cells (3×10^6 cells/mouse) were injected subcutaneously into the right flank region of male nu/nu mice. When the tumor reached to approximately 100 mm^3, compound **5a** was administered three times a week for three weeks by intraperitoneal injection (3 or 10 mg/kg). The tumor volume in the control group was approximately 1400 mm^3 on day 38 after the cells were inoculated. Treatment of **5a** significantly inhibited the tumor growth, and the tumor volume (Figures 2A,B) and tumor weight (Figure 2C) were decreased in a dose-dependent manner. The percent inhibition of the tumor volume compared with the vehicle-administered control group were 31.8% and 58.1% at 3 and 10 mg/kg doses of **5a**, respectively. Under the same experimental conditions, gemcitabine (10 mg/kg), a drug clinically used to treat lung cancer, showed 76.7% inhibition. No overt toxicity or body weight change was apparent in the **5a**-treated group compared to the control group (Figure 2D). These results suggest that compound **5a** might be a promising new chemotherapeutic candidate for the treatment of lung cancer.

To determine the mechanism of action involved in potent antitumor activity of **5a**, we examined the effect of **5a** against DNA methyltransferase (DNMT-1) because of the structural resemblance between **5a** and 5-azacytidine,[15] which is a potent inhibitor of DNMT-1. When MDA-MB-231 (breast cancer) cells were incubated with various concentrations of **5a**, the expression of DNMT-1 protein was inhibited in a dose-dependent manner (Figure 3). This preliminary study indicates that **5a** shows different mechanism of action from **3**,[6] which is a CTP-synthetase inhibitor.

CONCLUSIONS

On the basis of the potent biological activity of pyrimidine nucleosides with a cyclopentene ring, we have synthesized novel pyrimidine nucleosides with a fluorocyclopentene ring via a stereoselective Grignard reaction and electrophilic vinyl fluorination as key steps. Among the compounds tested, cytosine derivative **5a** demonstrated broad spectrum and potent antitumor activity in a broad range of human tumor cell lines as well as in a xenograft nude mouse model. These biological activity data reported here suggest that compound **5a** is a promising, clinically useful anticancer agent that should be further investigated. The detailed mechanism of action of **5a** is currently being studied and will be described in subsequent reports.

ASSOCIATED CONTENT

Supporting Information

Complete experimental procedures and characterization data and ^1H and ^{13}C NMR, ^{19}F copies of **19**, **22**, **5a**, and **5b**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*For L.S.J.: phone, 82-2-3277-3466; fax, 82-2-3277-2851; E-mail, lakjeong@ewha.ac.kr. For S.K.L.: phone, 82-2-880-2475; fax, 82-2-762-8322; E-mail, sklee61@snu.ac.kr.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the grant from the WCU project (R31-2008-000-10010-0), National Research Foundation (NRF), Seoul R&BD Program (ST100039), the GRRC program of Gyeonggi province(GRRC-DONGGUK2011-B02), and the Ewha Global Top5 Grant 2011 of Ewha Womans University.

ADDITIONAL NOTE

†Part of this work was presented in the 4th International Symposium on Nucleic Acids Chemistry, Fukuoka, Japan, September 20−22, 2005.

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