Synthesis and Antiviral Activity of Novel Anti-VZV 5-Substituted Uracil Nucleosides with a Cyclopropane Sugar Moiety

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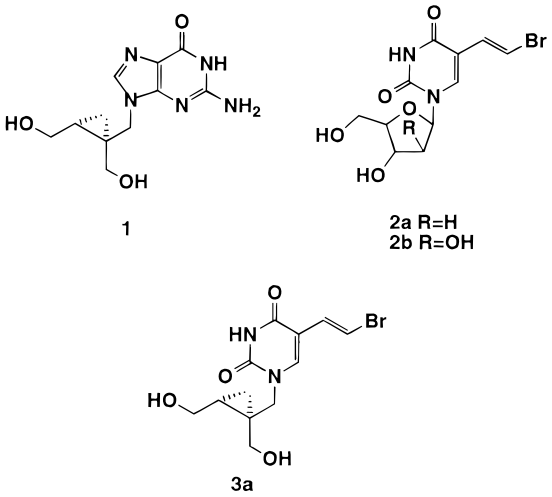
A series of 5-substituted uracil nucleoside derivatives with a 1(1′*S*,2′*R*)-[1′,2′-bis(hydroxymethyl)-cyclopropyl]methyl group as an acyclosugar moiety were synthesized and evaluated for their anti-herpetic activities. Among the compounds synthesized, (*E*)-5-halovinyluracil derivatives showed superior anti-varicella zoster virus (VZV) activity over acyclovir (ACV) but were less potent than ACV against herpes symplex virus type-1 (HSV-1). IC_{50} values for the VZV Kawaguchi strain were 0.027 for Br, 0.070 for Cl, and 0.054 μg/mL for I derivatives and 3.4 μg/mL for ACV. The most potent compound, (1′*S*,2′*R*)-5-[(*E*)-2-bromoethenyl]-1-[[1′,2′-bis-(hydroxymethyl)cycloprop-1′-yl]methyl]-2,4-(1*H*,3*H*)-pyrimidinedione **(3a)**, was 40−60-fold more potent than ACV against clinical isolates of VZV. It showed good oral bioavailability in rats (68.5%) and, unlike (*E*)-5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil (BVaraU), did not result in the release of (*E*)-5-(2-bromovinyl)uracil (BVU), a potent dihydropyrimidine dehydrogenase inhibitor, in plasma after oral administration.

Introduction

Since the discovery of acyclovir (ACV) as a potent antiherpetic agent,[1] acyclic nucleosides have attracted the interest of both medicinal chemists and virologists. The search for a new agent having superior activity to ACV has resulted in the discovery of ganciclovir (GCV),[2] which shows broader antiviral activity, and penciclovir (PCV)[3] as new therapeutic agents. As represented by these two drugs, one of the approaches to improve antiherpetic activity is to design a compound with two hydroxyl groups mimicking the 3′- and 5′-hydroxyl groups of the 2′-deoxyribose moiety of nucleosides. In the search for new acyclonucleosides based on this hypothesis, we previously found that (1′*S*,2′*R*)-9-[[1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl]guanine (A-5021, **1**) (Chart 1) showed extremely potent antiherpetic activity.[4] Another important chemical class of antiherpetic nucleosides is a series of 5-substituted uracil nucleosides—such as (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU)[5] (**2a**)—that show specific anti-varicella zoster virus (VZV) activity. Among these uracil nucleosides (*E*)-5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil (BVaraU)[6] (**2b**) shows extremely potent anti-VZV activity; however, potentiation of the toxicity of 5-fluorouracil (5-FU) by its metabolite, (*E*)-5-(2-bromovinyl)uracil (BVU),[7] has limited its use as a therapeutic agent. Several uracil nucleosides with 5-substituents other than the 5-halovinyl group have been developed to overcome this problem.[8] Among this class of compounds, however, only those with cyclic sugar moieties have thus far been demonstrated to show specific anti-VZV activity. Though the sugar moiety of **1** has a cyclopropane ring, its overall feature is acyclic because of its flexibility. Thus, it would be of interest to determine whether 5-substituted uracil nucleosides with a sugar moiety of **1** show VZV-specific antiviral activity. In this paper, we report the synthesis and antiviral activity of these compounds. Some of the compounds showed potent anti-VZV activity, and preliminary results on the pharmacokinetics of the most active compound, (1′*S*,2′*R*)-5-[(*E*)-2-bromoethenyl]-1-[[1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl]-2,4-(1*H*,3*H*)-pyrimidinedione **(3a)**, are also presented.

Chart 1



Chemistry

In Scheme 1, the syntheses of 5-substituted uracil derivatives with a 1(1′*S*,2′*R*)-[1′,2′-bis(hydroxymethyl)-cyclopropyl]methyl group are represented by the preparation of **3a**. To prepare a series of analogues, the sugar moiety was attached to 5-substituted uracil bases[10,11] by alkylation reaction. The chiral trisubstituted cyclopropane **4** was prepared from chiral epichlorohydrin and diethyl malonate in >97% ee as described previously.[4]

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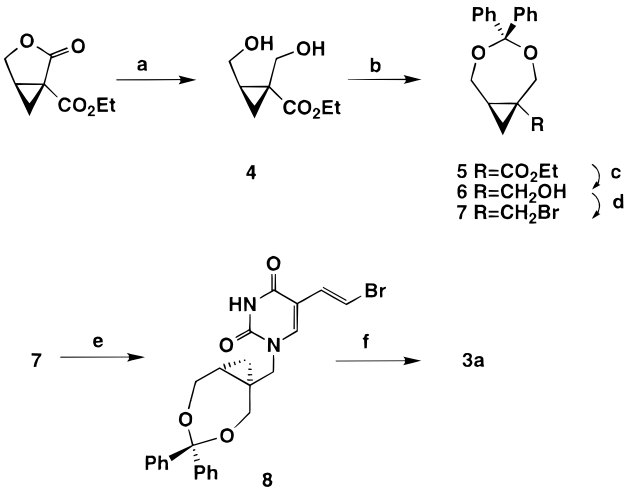
Scheme 1[a]



[a] (a) $NaBH_4$; (b) Ph_2CN_2, DDQ; (c) $LiBH_4$; (d) CBr_4, PPh_3, Et_3N; (e) BVU, K_2CO_3; (f) HCl / MeOH-H_2O.

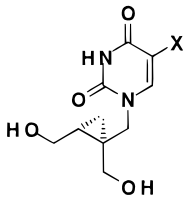
Because of high reactivity of halomethylcyclopropanes such as **7**, the *cis*-hydroxymethyl groups were protected with diphenylketal **5**, which is more stable under acidic conditions than the previously reported dimethyketal derivative.[4] **6** was converted to bromide **7** by triphenylphosphine/CBr_4 in the presence of triethylamine to avoid decomposition. The alkylation of unprotected 5-substituted uracils occurred mainly at the 1-position, but limited amounts of 3-alkylated and 1,3-dialkylated products were also obtained. These byproducts were separated by C_{18} reversed-phase chromatography after removal of the ketal protective group with 1 M HCl treatment. The yield of the alkylation products varies from 16% to 72% depending on the type of the uracil base. The other enantiomer of **3a** was prepared by the same procedure using the enantiomer of **4**.

Biological Studies

The antiherpetic activities of this series of compounds were measured by quantitative CPE reduction assay[4] against herpes simplex virus type 1 (HSV-1) Tomioka strain and plaque reduction assay against Kawaguchi strain.[12] The results are summarized in Table 1. Among the compounds tested, 5-halovinyl derivatives (**3a**, **15**, **16**) showed the most potent activity against VZV. **3a** was the most potent compound and was more than 100 times as potent as ACV**.** It showed no cytotoxic effect up to 500 μg/mL in Vero cells. Unlike ACV and **1**, **3a** showed only marginal activity against HSV-1. The enantiomer (**3b**) of **3a** showed weak activity against VZV, and even this activity may have been due not to **3b** but to **3a** in the preparation. Among the other compounds, only 5-iodo (**13**) and 5-propynyl (**18**) derivatives showed anti-VZV activity, and this activity was weak.

The antiviral activities of **3a** were further evaluated against several strains of herpes viruses, including clinical isolates of VZV, by plaque reduction assay. **3a** was extremely potent against clinical isolates of VZV—more potent than ACV, PCV, or **1**—while BVaraU was about 100 times more potent than **3a**. Like other nucleosides of this chemical class, **3a** showed no activity against HSV-2, and its activity was specific to VZV.

Table 1. Anti-HSV-1 Activity (Tomioka in Vero Cells) by Quantitative CPE Assay and Anti-VZV Activity (Kawaguchi in HEL Cells) by Plaque Reduction Assay of the Compounds



		HSV-1		VZV
compd	X	IC_{50}[a]	CC_{50}[b]	IC_{50}
ACV		0.81	820	3.4
1 (A-5021)		0.020	240	0.67
9	H	>500	>500	>100
10	F	>500	>500	>100
11	Cl	295	>500	>100
12	Br	215	>500	97
13	I	90	>500	9.3
14[c]	Me	>500	>500	>100
15	(*E*)-2-chloroethenyl	4.30	>500	0.070
3a	(*E*)-2-bromoethenyl	14.5	>500	0.027
3b[d]	(*E*)-2-bromoethenyl	>500	>500	7.4
16	(*E*)-2-iodoethenyl	24.6	330	0.054
17	ethynyl	310	>500	>100
18	1-propynyl	325	>500	19.8

[a] Concentrations in μg/mL. [b] Cytotoxicity in Vero cells. [c] Racemate.[4] [d] With (1′*R*,2′*S*)-configuration in the sugar portion.

Despite its potent activity, BVaraU generates BVU, a potent inhibitor of dihydropyrimidine dehydrogenase, after oral administration. Since **3a** has no glycosidic linkage, **3a** is expected to be more metabolically stable than BVaraU. When 0.1 mmol/kg of the compounds was given to rats, **3a** showed oral bioavailability of 68.5%, versus 47.5% for BVaraU, as shown in Table 3. It is noted that, unlike BVaraU, BVU was not detected in the plasma of the rats given **3a** orally.

Discussion

Series of 5-substituted uracil nucleosides are known as antivirals with specific activity against VZV. These nucleosides include 5-bromovinyluracil derivatives such as BVdU, its carbocyclic analogue GR-95168,[13] BVaraU, and a 5-alkynyluracil derivative, Netivudine.[8] 5-Iodo derivatives such as 5-iodo-2′-deoxyuridine (IDU) and its hexitol analogue show some antiherpetic activity,[14] and compounds with even more bulky 5-substituents, such as heteroaromatics, have also been reported.[15] These nucleosides have cyclic sugar moieties similar to 2′-deoxyribose. Even an isoribose derivative of BVdU is active against VZV.[16] In contrast, the ACV and GCV type compounds with 5-bromovinyluracil as a base moiety have been reported to show no antiherpetic activities.[17] A 5-bromovinyluracil derivative with a carbocyclic oxetane moiety has recently been reported as a potent anti-VZV agent.[18] This compound is structurally similar to **3a**; however, because of the structural constraint, it belongs to a class of nucleosides with cyclic sugar moieties. Though **3a** has a cyclopropane ring, this ring is connected via a methylene linkage, so that the pseudo-sugar moiety of **3a** has an overall flexibility like that of the acyclonucleosides. Thus, **3a** is the first example of a 5-substituted uracil acyclonucleoside with potent anti-VZV activity. The reason some 5-substituted uracil derivatives show specific activity against VZV is

Table 2. Antiherpetic Activity of **3a** and Reference Compounds against Various Strains by Plaque Reduction Assay

			IC_{50}[a]				
virus	strain	cells	**3a**	**1**	ACV	PCV	BVaraU
HSV-1	Tomioka	MRC-5	0.77	0.0093	0.29	0.54	0.14
HSV-2	186	MRC-5	>100	0.12	0.25	1.2	ND[b]
VZV	Kawaguchi	HEL	0.027	0.67	3.4	5.6	ND
VZV	clinical isolates[c]	MRC-5	0.035 ± 0.021	0.21 ± 0.096	1.3 ± 0.40	ND	0.00031 ± 0.000072
VZV	clinical isolates[d]	HEL	0.097 ± 0.029	ND	5.4 ± 1.5	10 ± 3.8	ND

[a] Concentrations in μg/mL. [b] Not determined. [c] Average of 6 isolates. [d] Average of 11 isolates.

Table 3. Oral Bioavailability of **3a** and BVaraU in Rats

	route	**3a**	BVaraU
AUC_{0-12h} (μM·h)	iv	86.9	100.5
AUC_{0-12h} (μM·h)	po	59.5	47.7
oral bioavailability (%)[a]		68.5	47.5
BVD[b]		not detected[c]	detected[d]

[a] AUC_{0-12h}(po)/AUC_{0-12h}(iv) × 100. [b] Presence of BVU in plasma after oral dosage. [c] Detection limit > 0.5 μM. [d] 9.38 ± 3.02 μM at 12 h.

not clear. One possible explanation is that the thymidine kinase of VZV may have a large binding pocket to accommodate these derivatives.[19] The sugar moiety of **3a** may take a conformation resembling that of 2′-deoxyribose in terms of the position of the two hydroxyl groups in the binding site of the thymidine kinase, and **3a** may exhibit potent anti-VZV activity through efficient phosphorylation.

Among the series of 5-substituted uracil nucleosides, BVaraU shows the most potent anti-VZV activity. Despite this potent activity, however, its clinical use is limited because of the metabolic formation of BVU. BVU is known to be a potent inhibitor of dihydropyrimidine dehydrogenase, and use of BVaraU in combination with 5-FU delays the degradation of 5-FU and potentiates its toxicity. The release of BVU is catalyzed by pyrimidine phosphorylase of the enterobacteria.[7a] Since **3a** has no glycosidic linkage, it is expected that **3a** is more metabolically stable than BVaraU. Our preliminary experiment revealed the good oral bioavailability and stability of **3a**. There may be other pathways of metabolism, such as oxidative degradation at the liver, and thus additional studies will be needed on the metabolic stability of **3a**. However, **3a** in combination with 5-FU is expected to be a safer drug than BVaraU or BVdU. Further studies on the pharmacology and pharmacokinetics of **3a** are currently in progress.

Experimental Section

General. Reagents used were the highest quality available commercially. Unless otherwise noted, organic extracts were dried over anhydrous $MgSO_4$ or Na_2SO_4 and temperature refers to the temperature of the bath. ^1H and ^{13}C NMR spectra were recorded with a Varian XL-300 300-MHz or a JEOL JNM-GX-400 400-MHz spectrometer, using tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL JMS-DX300 spectrophotometer, and accurate masses were measured on a JEOL JMS-HX110 spectrometer. Optical rotations were recorded on a JASCO DIP-370 polarimeter. Thin-layer chromatography was carried out on silica gel 60F254 precoated plates (Merck art. no. 5715) and silica gel column chromatography was conducted on silica gel 60 (70−230 mesh; Merck art. no. 7734). Preparative reversed-phase column chromatography was conducted on a Merck LiChroprep RP-18 column (40−63 μm). Elemental combustion analyses, where indicated only by the elements, were within ±0.4% of the theoretical values.

MRC-5 cells were purchased from Dainippon Pharmaceuticals (Osaka, Japan). HEL cells and clinical isolates of VZV were provided by Dr. Koichi Yamanishi of Osaka University Medical School. ACV, PCV, and BVaraU were synthesized in the Central Research Laboratories of Ajinomoto Co.

Ethyl (1*R*,7*R*)-3,5-Dioxa-4,4-diphenylbicyclo[5.1.0]octane-1-carboxylate (5). Ethyl (1*R*,2*R*)-1,2-bis(hydroxymethyl)-1-cyclopropanecarboxylate[4] (**4**) (5.23 g, 30 mmol, >97% ee) and diphenyldiazomethane (5.83 g, 30 mmol) in 130 mL of 1,2-dichloroethane were slowly added to a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (6.81 g, 30 mmol) in 250 mL of 1,2-dichloroethane at room temperature. The mixture was stirred for 1 h and the reaction mixture was concentrated in vacuo. The concentrate was dissolved in toluene and the solution was washed with saturated $NaHCO_3$. The organic layer was concentrated in vacuo and the residue was chromatographed on silica gel eluting with CH_2Cl_2 to yield **5** as a yellow oil (8.72 g, 85%): ^1H NMR ($CDCl_3$) δ 1.22 (t, J = 7.0 Hz, 3H), 1.33 (dd, J = 3.6, 7.1 Hz, 1H), 1.43 (dd, J = 3.6, 9.3 Hz, 1H), 1.80 (m, 1H), 3.68 (d, J = 13.3 Hz, 1H), 3.76 (dd, J = 3.1, 12.9 Hz, 1H), 4.1 (m, 3H), 4.65 (d, J = 13.0 Hz, 1H), 7.20−7.60 (m, 10H); FD MASS m/z 338 (M^+).

(1*S*,7*R*)-3,5-Dioxa-4,4-diphenylbicyclo[5.1.0]octane-1-methanol (6). To a solution of **5** (7.01 g, 20.7 mmol) in 10 mL of dry THF was added 12 mL of a 2 M solution of $LiBH_4$ in THF, and the mixture was heated at 72 °C for 16 h. Saturated NH_4Cl was added to the mixture at 0 °C and the product was extracted with EtOAc. The organic layer was washed with H_2O and concentrated in vacuo. The residue was chromatographed on silica gel eluting with CH_2Cl_2−MeOH (19:1) to yield **6** as a white solid (5.54 g, 90%): mp 93 °C; ^1H NMR ($CDCl_3$) δ 0.67 (dd, J = 4.4, 8.9 Hz, 1H), 0.96 (dd, J = 4.4, 5.8 Hz, 1H), 1.08 (m, 1H), 3.42 (dd, J = 11.0, 27.9 Hz, 2H), 3.65 (dd, J = 3.9, 12.9 Hz, 1H), 3.76 (d, J = 12.7 Hz, 2H), 4.1 (m, 3H), 7.20−7.60 (m, 10H); FD MASS m/z 296 (M^+).

(1*R*,7*R*)-1-Bromomethyl-3,5-dioxa-4,4-diphenylbicyclo-[5.1.0]octane (7). To a solution of **6** (5.00 g, 16.9 mmol) in 100 mL of CH_2Cl_2 were added Et_3N (1.2 mL, 8.4 mmol), triphenylphosphine (7.97 g, 30.4 mmol), and CBr_4 (10.1 g, 30.4 mmol). After the mixture stirred for 20 min at room temperature, saturated $NaHCO_3$ was added and the product was extracted with hexane. The organic layer was washed with H_2O and the solvent was removed in vacuo. The residue was chromatographed on silica gel eluting with a gradient of 0−25% EtOAc in hexane to yield **7** as a yellow oil (5.45 g, 90%): ^1H NMR ($CDCl_3$) δ 0.83 (dd, J = 4.2, 8.5 Hz, 1H), 1.16−1.28 (m, 2H), 3.13 (d, J = 10.2 Hz, 1H), 3.60 (dd, J = 3.5, 12.9 Hz, 2H), 3.80 (d, J = 12.9 Hz, 2H), 4.07 (dd, J = 4.8, 13.0 Hz, 2H), 7.20−7.60 (m, 10H); FD MASS m/z 358 (M^+).

General Method for Preparation of 3a, 3b, and 9−18. To a solution of **8** (358 mg, 1.0 mmol) in dry DMF (20 mL) were added the corresponding 5-substituted uracil[10,11] (1.2 mmol), K_2CO_3 (138 mg, 1.0 mmol), and 18-crown-6 (264 mg, 1.0 mmol). After stirring for 2 h at 60 °C, the mixture was cooled to room temperature and insoluble substances were removed by filtration. The filtrate was concentrated in vacuo and the residue was dissolved in 10 mL of MeOH and 2.5 mL of HCl. After the mixture stirred for 30 min at room temperature, MeOH was removed in vacuo. The concentrate was brought to pH = 4 by adding K_2CO_3 and was chromatographed on reversed-phase C_{18} silica gel eluting with 0−30% MeOH/

H_2O. Evaporation of the desired fractions gave **3a**, **3b**, **9**−**13**, and **15**−**18**. **14** was prepared as a racemate as described previously.[4]

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-[(*E*)-2-bromoethenyl]-2,4(1*H*,3*H*)-pyrimidinedione (3a): colorless needles from water; yield 20%; mp 99−102 °C; $[\alpha]^{26}_D = +14.7$ ($c = 0.689\%$, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.42 (t, $J = 5.4$ Hz, 1H), 0.80 (dd, $J = 4.8$, 8.7 Hz, 1H), 1.20−1.30 (m, 1H), 3.24−3.37 (m, 2H), 3.50 (dd, $J = 6.0$, 12.0 Hz, 1H), 3.61 (dt, $J = 12.0$, 6.0 Hz, 1H), 3.61 (d, $J = 14.1$ Hz, 1H), 3.77 (d, $J = 14.1$ Hz, 1H), 4.50−4.59 (m, 2H), 6.81 (d, $J = 13.5$ Hz, 1H), 7.23 (d, $J = 13.5$ Hz, 1H), 7.91 (s, 1H); ^{13}C NMR (CD_3OD) δ 14.9, 25.7, 27.5, 54.3, 62.8, 63.2, 108.6, 111.5, 130.2, 145.6, 152.5, 164.1; HRMS calcd for $C_{12}H_{16}BrN_2O_4$ (MH^+) 331.0293, found 331.0298. Anal. ($C_{12}H_{15}BrN_2O_4$) C, H, N.

1-[(1′*R*,2′*S*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-[(*E*)-2-bromoethenyl]-2,4(1*H*,3*H*)-pyrimidinedione (3b): enatiomer of **8** was used instead of **8** in this preparation; colorless needles from water; yield 14%; ^{1}H NMR was identical to that of **3a**; mp 97−100 °C; $[\alpha]^{26}_D = -16.7$ ($c = 0.563\%$, MeOH); HRMS calcd for $C_{12}H_{16}BrN_2O_4$ (MH^+) 331.0293, found 331.0295. Anal. ($C_{12}H_{15}BrN_2O_4$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-2,4(1*H*,3*H*)-pyrimidinedione (9): colorless solid; yield 22%; mp 129−132 °C; $[\alpha]^{25}_D = +21.1$ ($c = 0.885\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (t, $J = 5.4$ Hz, 1H), 1.00 (dd, $J = 5.4$, 9.0 Hz, 1H), 1.35−1.46 (m, 1H), 3.46 (dd, $J = 9.3$, 12.0 Hz, 1H), 3.49 (d, $J = 12.3$ Hz, 1H), 3.77 (d, $J = 12.3$ Hz, 1H), 3.83−3.92 (m, 3H), 5.68 (d, $J = 7.8$ Hz, 1H), 7.69 (d, $J = 7.8$ Hz, 1H); ^{13}C NMR (CD_3OD) δ 15.0, 25.5, 27.7, 54.1, 62.8, 63.1, 101.8, 147.8, 153.4, 166.8; HRMS calcd for $C_{10}H_{15}N_2O_4$ (MH^+) 227.1032, found 227.1008. Anal. ($C_{10}H_{14}N_2O_4$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-fluoro-2,4(1*H*,3*H*)-pyrimidinedione (10): colorless solid; yield 16%; mp 138−141 °C; $[\alpha]^{25}_D = +22.1$ ($c = 1.48\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.56 (t, $J = 5.3$ Hz, 1H), 0.99 (dd, $J = 5.3$, 8.7 Hz, 1H), 1.37−1.47 (m, 1H), 3.46 (dd, $J = 9.3$, 11.8 Hz, 1H), 3.52 (d, $J = 12.0$ Hz, 1H), 3.78 (d, $J = 12.0$ Hz, 1H), 3.80 (d, $J = 15.0$ Hz, 1H), 3.84 (d, $J = 15.0$ Hz, 1H), 3.87 (dd, $J = 6.2$, 11.8 Hz, 1H), 7.89 (d, $J = 14.4$ Hz, 1H); ^{13}C NMR (CD_3OD) δ 14.9, 25.5, 27.5, 54.5, 62.8, 63.2, 131.6 (d, $J = 32.8$ Hz), 141.4 (d, $J = 230.4$ Hz), 152.1, 159.9 (d, $J = 25.5$ Hz); HRMS calcd for $C_{10}H_{14}FN_2O_4$ (MH^+) 245.0938, found 245.0941. Anal. ($C_{10}H_{13}FN_2O_4$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-chloro-2,4(1*H*,3*H*)-pyrimidinedione (11): colorless solid; yield 31%; mp 122−124 °C; $[\alpha]^{25}_D = +18.2$ ($c = 0.955\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.56 (t, $J = 5.5$ Hz, 1H), 1.00 (dd, $J = 5.5$, 8.7 Hz, 1H), 1.38−1.48 (m, 1H), 3.46 (dd, $J = 9.3$, 11.4 Hz, 1H), 3.52 (d, $J = 12.2$ Hz, 1H), 3.78 (d, $J = 12.2$ Hz, 1H), 3.85 (d, $J = 14.6$ Hz, 1H), 3.87 (dd, $J = 6.9$, 11.4 Hz, 1H), 3.87 (d, $J = 14.6$ Hz, 1H), 8.02 (s, 1H); ^{13}C NMR (CD_3OD) δ 15.0, 25.6, 27.5, 54.6, 62.8, 63.2, 108.4, 144.6, 152.6, 162.0; HRMS calcd for $C_{10}H_{14}ClN_2O_4$ (MH^+) 261.0642, found 261.0638. Anal. ($C_{10}H_{13}ClN_2O_4 \cdot 0.2H_2O$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-bromo-2,4(1*H*,3*H*)-pyrimidinedione (12): colorless solid; yield 72%; mp 141−143 °C; $[\alpha]^{25}_D = +11.3$ ($c = 3.13\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.56 (t, $J = 5.3$ Hz, 1H), 1.00 (dd, $J = 5.3$, 9.0 Hz, 1H), 1.38−1.48 (m, 1H), 3.45 (dd, $J = 9.2$, 11.8 Hz, 1H), 3.52 (d, $J = 12.2$ Hz, 1H), 3.77 (d, $J = 12.2$ Hz, 1H), 3.86 (s, 2H), 3.87 (dd, $J = 6.3$, 11.8 Hz, 1H), 8.11 (s, 1H); ^{13}C NMR (CD_3OD) δ 15.0, 25.6, 27.5, 54.6, 62.8, 63.2, 96.1, 147.1, 152.8, 162.1; HRMS calcd for $C_{10}H_{14}BrN_2O_4$ (MH^+) 307.0117, found 307.0110. Anal. ($C_{10}H_{13}BrN_2O_4$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione (13): colorless solid; yield 39%; mp 148−150 °C; $[\alpha]^{25}_D = +7.6$ ($c = 0.841\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (t, $J = 5.4$ Hz, 1H), 1.00 (dd, $J = 5.4$, 8.7 Hz, 1H), 1.36−1.47 (m, 1H), 3.45 (dd, $J = 9.0$, 11.7 Hz, 1H), 3.51 (d, $J = 12.0$ Hz, 1H), 3.76 (d, $J = 12.0$ Hz, 1H), 3.86 (dd, $J = 6.3$, 11.7 Hz, 1H), 3.86 (2H, s), 8.17 (s, 1H); ^{13}C NMR (CD_3OD) δ 15.0, 25.6, 27.6, 54.4, 62.8, 63.2, 147.1, 152.2, 153.3, 163.4; HRMS calcd for $C_{10}H_{14}IN_2O_4$ (MH^+) 352.9998, found 353.0007. Anal. ($C_{10}H_{13}IN_2O_4 \cdot 0.2H_2O$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-[(*E*)-2-chloroethenyl]-2,4(1*H*,3*H*)-pyrimidinedione (15): colorless needles from water; yield 51%; mp 74−77 °C; $[\alpha]^{25}_D = +21.7$ ($c = 2.56\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.56 (t, $J = 5.4$ Hz, 1H), 1.01 (dd, $J = 5.4$, 8.7 Hz, 1H), 1.38−1.49 (m, 1H), 3.45 (dd, $J = 9.3$, 12.2 Hz, 1H), 3.51 (d, $J = 12.3$ Hz, 1H), 3.76 (d, $J = 12.3$ Hz, 1H), 3.81 (d, $J = 14.4$ Hz), 3.88 (dd, $J = 5.9$, 12.2 Hz, 1H), 3.92 (d, $J = 14.4$ Hz, 1H), 6.56 (d, $J = 13.4$ Hz, 1H), 7.26 (d, $J = 13.4$ Hz, 1H), 7.78 (s, 1H); ^{13}C NMR (CD_3OD) δ 15.7, 26.4, 28.3, 55.1, 63.6, 64.0, 111.2, 121.5, 127.3, 146.1, 153.2, 164.9; HRMS calcd for $C_{12}H_{16}ClN_2O_4$ (MH^+) 287.0799, found 287.0812. Anal. ($C_{12}H_{15}ClN_2O_4 \cdot 0.1H_2O$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-[(*E*)-2-iodoethenyl]-2,4(1*H*,3*H*)-pyrimidinedione (16): colorless solid; yield 39%; mp 105−107 °C; $[\alpha]^{23}_D = +12.4$ ($c = 0.105\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.56 (t, $J = 5.4$ Hz, 1H), 1.00 (dd, $J = 5.4$, 8.7 Hz, 1H), 1.38−1.48 (m, 1H), 3.45 (dd, $J = 9.6$, 12.3 Hz, 1H), 3.5 (d, $J = 12.3$ Hz, 1H), 3.76 (d, $J = 12.3$ Hz, 1H), 3.81 (d, $J = 14.6$ Hz), 3.88 (dd, $J = 5.6$, 12.3 Hz, 1H), 3.92 (d, $J = 14.6$ Hz, 1H), 7.16 (d, $J = 14.7$ Hz, d), 7.34 (d, $J = 14.7$ Hz, 1H), 7.80 (s, 1H); ^{13}C NMR (CD_3OD) δ 14.9, 25.7, 27.5, 54.3, 62.8, 63.2, 78.3, 113.1, 137.7, 145.5, 152.5, 164.2; HRMS calcd for $C_{12}H_{16}IN_2O_4$ (MH^+) 379.0155, found 379.0156. Anal. ($C_{12}H_{15}IN_2O_4 \cdot 0.1H_2O$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-ethynyl-2,4(1*H*,3*H*)-pyrimidinedione (17): colorless solid; yield 23%; mp 155−158 °C; $[\alpha]^{25}_D = +19.9$ ($c = 0.269\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.56 (t, $J = 5.6$ Hz, 1H), 1.01 (dd, $J = 5.6$, 8.7 Hz, 1H), 1.38−1.48 (m, 1H), 3.46 (dd, $J = 9.2$, 12.3 Hz, 1H), 3.51 (d, $J = 12.3$ Hz, 1H), 3.59 (s, 1H), 3.77 (d, $J = 12.3$ Hz, 1H), 3.86 (dd, $J = 5.7$, 12.3 Hz, 1H), 3.87 (s, 2H), 8.05 (s, 1H); ^{13}C NMR (CD_3OD) δ 15.0, 25.6, 27.6, 54.6, 62.8, 63.2, 76.0, 82.8, 98.9, 151.3, 152.7, 165.2; HRMS calcd for $C_{12}H_{15}N_2O_4$ (MH^+) 251.1032, found 251.1027. Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

1-[(1′*S*,2′*R*)-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]-methyl-5-(1-propynyl)-2,4(1*H*,3*H*)-pyrimidinedione (18): colorless solid; yield 61%; mp 156−159 °C; $[\alpha]^{25}_D = +20.7$ ($c = 0.453\%$, MeOH); ^{1}H NMR (CD_3OD) δ 0.55 (t, $J = 5.4$ Hz, 1H), 1.00 (dd, $J = 5.4$, 8.6 Hz, 1H), 1.36−1.46 (m, 1H), 2.03 (s, 3H), 3.46 (dd, $J = 9.3$, 12.0 Hz, 1H), 3.50 (d, $J = 12.3$ Hz, 1H), 3.76 (d, $J = 12.3$ Hz, 1H), 3.80 (d, $J = 14.3$ Hz, 1H), 3.86 (dd, $J = 6.2$, 12.0 Hz, 1H), 3.90 (d, $J = 14.3$ Hz, 1H), 7.87 (s, 1H); ^{13}C NMR (CD_3OD) δ 4.0, 15.0, 25.5, 27.6, 54.4, 62.8, 63.1, 71.5, 90.7, 100.5, 149.4, 152.5, 165.4; HRMS calcd for $C_{13}H_{17}N_2O_4$ (MH^+) 265.1188, found 265.1190. Anal. ($C_{13}H_{16}N_2O_4 \cdot 0.3H_2O$) C, H, N.

Antiviral Activity. Quantitative CPE reduction assay against HSV-1 was performed using the neutral red dye uptake method as described previously.[4] Plaque reduction assay for HSV-1,2 and VZV was carried out as described in the previous report.[12] All antiviral titrations were done in either triplicate or quadruplicate.

Pharmacokinetics in Rats. Drugs were dissolved in PBS by addition of an equimolar amount of NaOH and were given to male SD rats of 5 weeks of age. Blood samples were collected by cardiac puncture at 1, 5, 10, 30, 60, 120, 240, 480, and 720 min after injection and at 15, 30, 60, 120, 240, 480, and 720 min after oral administration from 3 ether-anesthetized rats at each time point. Plasma was recovered by centrifugation and mixed with an equal volume of 20% TCA to remove protein. The drug concentrations in plasma were determined by reversed-phase HPLC analysis using a YMCpack AM-302 column with a linear gradient of 0.1% TFA and CH_3CN, monitoring of UV absorbance at 254 and 295 nm. The elimination portion of the semilogarithmic plasma concentration was analyzed by linear regression. AUC values were determined by the linear trapezoidal method and extrapolated to 12 h.

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