SYNTHESIS AND ANTIVIRAL EVALUATION OF 3-HYDROXY-METHYLPYRIMIDO[1,6-*c*][1,3]OXAZEPIN DERIVATIVES

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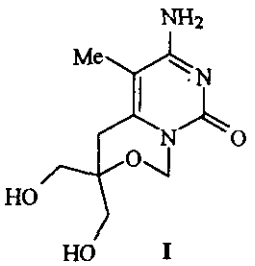
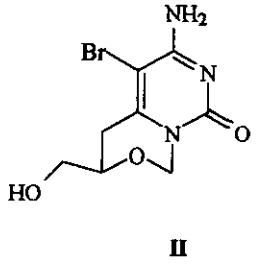
Abstract- A number of pyrimido[1,6-*c*][1,3]oxazepin derivatives, the *anti* glycosidic constrained acyclic analogs of uridine and cytidine have been prepared. Compounds synthesized were evaluated for activity against herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). All of these compounds were inactive.

From the initial discovery of a cyclonucleoside, 2',3'-O-isopropylidene-O^2,5-cyclocytidine by Todd and co-wokers[1] in 1951, a variety of derivatives of cyclonucleosides bridged by oxygen, sulfur, nitrogen and carbon have been prepared for several reasons. These include that cyclonucleosides are useful intermediates for nucleoside conversion,[2] effective antileukemic agents,[3] and suitable probes[4-7] to study the relationship between substrate-inhibitor conformation and the specificities of the enzymes of nucleic acid biosynthesis. In the course of our research concerning new antitumor and antiviral agents,[8-14] we have synthesized a new class of cyclonucleoside, namely anti-conformationally restricted pyrimidine acyclic nucleosides. We found 6-amino-3,3-bis(hydroxymethyl)-5-methyl-1*H*,3*H*,4*H*-pyrimido[1,6-*c*][1,3]-oxzazine-8-one (**I**) had slight activity against HIV[8] and 3-hydroxymetyl derivatives (**II**) of **I** showed slight activity against Breast Cancer cell line.[14] In order to explore potent antitumor and antiviral agents, we



I

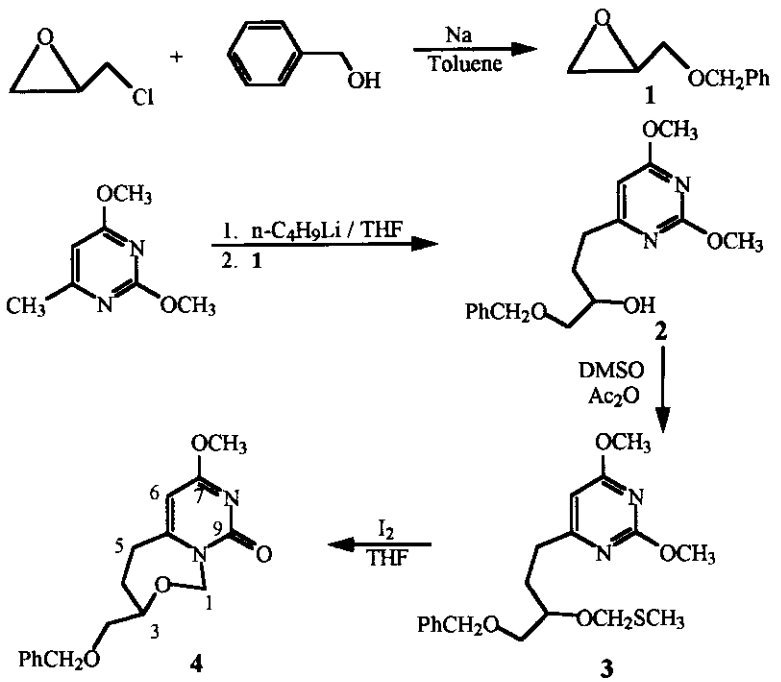


II

began to expand the six member oxazine ring to seven member oxazepin ring. We thought this ring

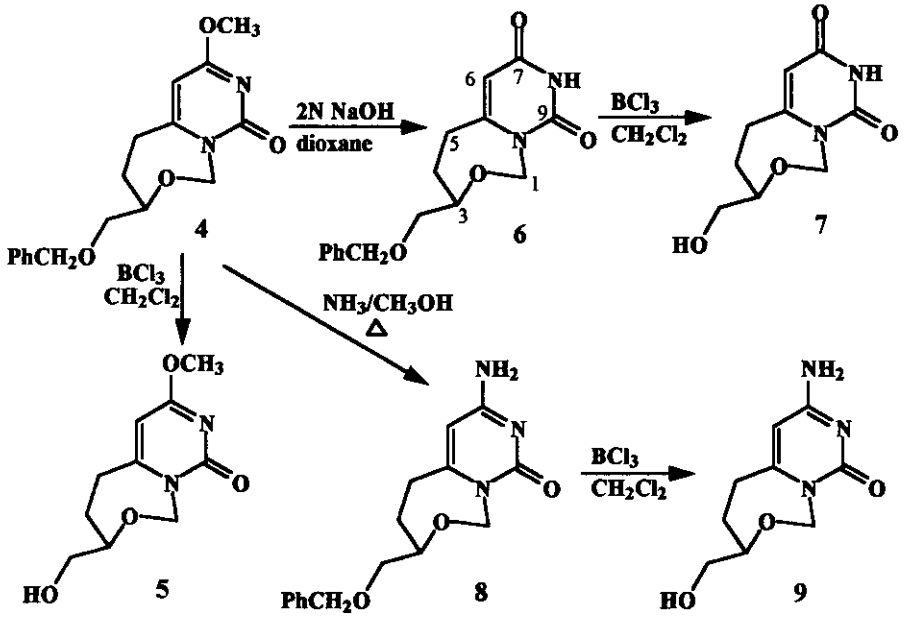
modification might vary the 3-hydroxymethyl group on the oxazepin ring to a different spatial position from the 3-hydroxymethyl group on the oxazine ring. In this paper we wish to report on the synthesis and antiviral activity of these ring-modified analogue.

RESULTS AND DISCUSSION

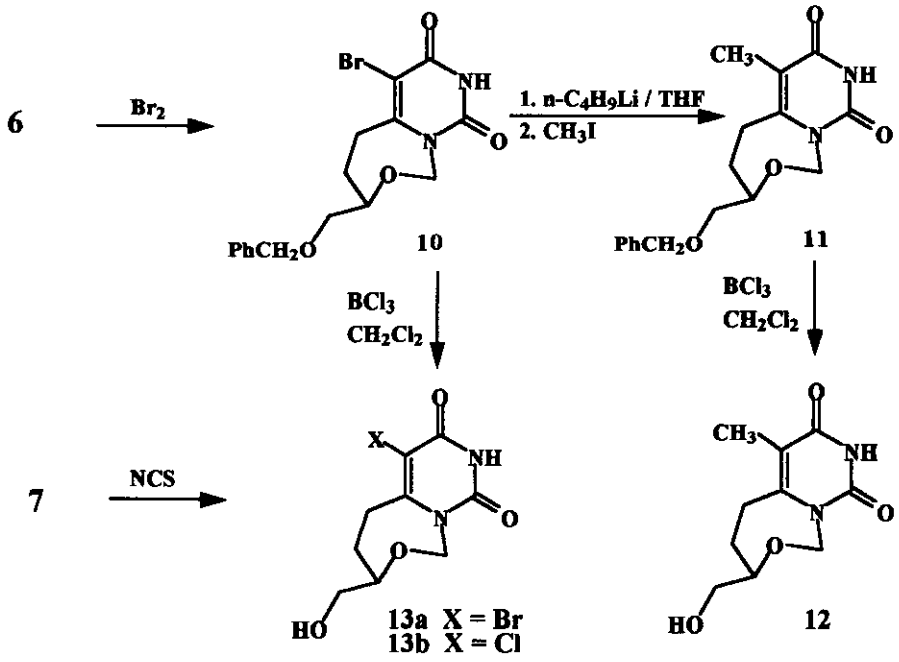


Scheme 1

The 2,4-dimethoxy-6-methylpyrimidine was synthesized from 6-methyluracil by chlorination with phosphorus oxychloride and subsequent substitution of the 2- and 4-chloro group with sodium methoxide in methanol. The benzyl glycidyl ether (**1**) was prepared by a coupling of benzyl alcohol with epichlorohydrin in a solution of sodium in dry toluene. Treatment of 2,4-dimethoxy-6-methylpyrimidine with n-butyllithium in dry tetrahydrofuran at -70 °C gave the lithiated derivative, which was reacted with **1** at -70 °C to afford **2** in 65% yield (Scheme 1). The introduction of a C-1 carbon of the acyclic moiety was performed by a conversion of the hydroxy group of **2** to the corresponding methylthiomethyl ether derivative (**3**). This was carried out by treating **2** with a mixture of acetic anhydride and anhydrous dimethyl sulfoxide at rt for 72 h. Cyclization of **3** was initiated with iodine in dry tetrhydrofuran at rt to give the cyclized product (**4**). Treatment of **4** with 2 N sodium hydroxide in dioxane at reflux overnight gave **6** in a yield of 80% (Scheme 2). The cytosine derivative (**8**) was obtained by a conversion of the 6-methoxy group to an amino group under high pressure at 100 °C. Deblocking the benzyl group of **4**, **6**, and



Scheme 2



Scheme 3

8 with boron trichloride in methylene chloride at -55 °C furnished **5**, **7** and **9**, respectively. The thymine derivative (**11**) was synthesized by a two-step conversion starting with a displacement of the 6-proton of **6** with bromo, using bromine water at rt, followed by a conversion of 6-bromo of **10** to 6-methyl, using

n-butyllithium, followed with methyl iodide (Scheme 3). Deblocking the benzyl group of **10** and **11** with boron trichloride in methylene chloride at -55 °C furnished **12** and **13a,** respectively. Chlorination of 7 with *N*-chlorosuccinimide in the presence of acetic acid afforded 6-chloro derivative **(13b)**.

Target compounds **(5-12)**, and **(13a)** and **(13b)** were evaluated for activity against herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). All of these compounds were inactive at the highest concentration tested (100 μM).

EXPERIMENTAL SECTION

General. Melting points were taken on a BUCHI 530 apparatus and are uncorrected. The silica gel used for chromatography was silica gel 60 70-230 mesh (E. Merck, Darmstadt, Germany), TLC was performed *on prescored* DC-Alufolien Kieselgel $60F_{254}$ (E. Merck, Darmstadt, Germany). Compounds were visualized by illuminating under UV light (254 nm). Evaporations were carried out at < 50 °C using a rotary evaporator at reduced pressure (water aspirator). Solvent ratios are reported as v/v. ^1H and ^{13}C NMR spectra were obtained at Varian 300 NMR spectrometer. Where necessary, deuterium exchange experiments were used to obtained proton shift assignments. IR spectra were recorded on a Perkin-Elmer 938G spectrophotometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of P_2O_5 for at least 12 h unless otherwise specified. Elemental analyses were obtained from Perkin-Elmer *2400 Elemental Analyzer*.

Benzyl glycidyl ether (1) Sodium (6.36 g, 0.28 mol) was added to a mixture of benzyl alcohol (30 g, 0.28 mol) and dry toluene (180 mL). The mixture was warmed until the sodium metal was completely dissolved and then was cooled to rt. Epichlorohydrin (126 g, 1.36 mol) was added to the cool mixture. The mixture was stirred at rt for 90 min and then concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with n-hexane / ethyl acetate (10/1). The desired fractions were evaporated under reduced pressure to give **1** (25.9 g, 67%). mp 60-61 °C (methanol). Rf: 0.55 (n-hexane / ethyl acetate = 5/1). ^1H NMR (DMSO-d_6):δ 7.30 (*m*, 5H, Ph), 4.52 (s, 2H, CH_2Ph), 3.57, 3.30 (m, 1H each, OCH_2), 3.15 (m, 1H, *CH*), 2.73, 2.55 (m, 1H each, CH_2). ^{13}C NMR (DMSO-d_6): δ 138.5, 128.6, 125.8, 72.5, 71.1, 50.6, 40.1.

6-(4-Benzyloxy-3-hydroxybutyl)-2,4-dimethoxypyrimidine (2) Under nitrogen atmosphere n-butyl-lithium (1.6 M, 44 mL, 71 mmol) was added dropwise to a solution of 2,4-dimethoxy-6-methylpyrimidine (10 g, 65 mmol) in dry tetrahydrofuran (150 mL) at -70 °C. The mixture was raised and stirred at -55 °C for 30 min. Compound (1) (12.8 g, 78 mmol) was added and the stirring was continued for 2 h. The

solution was neutralized with acetic acid to pH 7, and the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic layer was separated, dried over sodium sulfate, and then the solvent was removed. The residue was chromatographed on silica gel eluting with methylene chloride / methanol (9/1). The desired fractions were evaporated under reduced pressure to give oily **2** (16 g, 65%). Rf: 0.2 (n-hexane/ ethyl acetate = 2/1). ^{1}H NMR (DMSO-d_6):δ 7.26 (m, 5H, Ph), 6.37 (s, 1H, H-5), 4.74 (d, J = 5.7 Hz, 1H, OH, D_2O exchangeable), 4.47 (s, 2H, CH_2Ph), 3.84 (s, 6H, OCH_3), 3.63 (m, 1H, CH), 3.32 (m, 2H, CH_2OBn), 2.62 (m, 2H, CH_2-6), 1.64 (m, 2H, $C\underline{H_2}$-CH_2-6). MS (*m/z*): 319 (M^+). Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N, 8.80. Found: C, 64.02; H, 7.15; N,8.74.

6-(4-Benzyloxy-3-methylthiomethoxybutyl)-2,4-dimethoxypyrimidine (3) Acetic anhydride (63 mL) was added to a mixture of **2** (16 g, 50 mmol) in dry dimethyl sulfoxide (63 mL). The solution was stirred at rt for 72 h. The solution was extracted with methylene chloride and the extract was washed with brine and water. The extracted residue was dried over anhydrous sodium sulfate, and concentrated. The residue was applied to a silica gel column which was eluted with n-hexane / ethyl acetate (5/1) The desired fractions were collected and concentrated under reduced pressure to give oily **3** (11.1 g, 58%). Rf: 0.34 (n-hexane / ethyl acetate = 3/1). ^{1}H NMR (DMSO-d_6):δ 7.26 (m, 5H, Ph), 6.40 (s, 1H, H-5), 4.75 (s, 2H. OCH_2S), 4.47 (s, 2H, CH_2Ph), 3.84 (s, 6H, OCH_3), 3.71 (m, 1H, CH), 3.49 (d, J = 4.6 Hz, 2H, CH_2OBn), 2.62 (m, 2H, CH_2-6), 2.09 (s, 3H, SCH_3), 1.62 (m, 2H, $C\underline{H_2}$-CH_2-6). MS (*m/z*): 379 (M^+). Anal. Calcd for $C_{19}H_{26}N_2O_4S$: C, 60.30; H, 6.92; N, 7.40. Found: C, 60.35; H, 7.06; N, 7.22.

3-Benzyloxymethyl-7-methoxy-1*H*, 3*H*, 4*H*, 5*H*-pyrimido[1,6-*c*][1,3]oxazepin-9-one (4) Iodine (5 g, 39 mmol) was added into a mixture of **3** (8.5 g, 13.2 mmol) in dry tetrahydrofuran (58 mL). The solution was stirred under nitrogen at rt for 64 h. A 5% aq sodium sulfite solution was added until the brown color of the mixture disappeared and the resulting solution was extracted with methylene chloride. The combined extracts were washed with brine and water and then dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the solvent was evaporated. The residue was chromatographed on silica gel and eluted with methylene chloride / ethyl acetate (4 / 1). The fractions were collected and concentrated to give oily **4** (2.2 g, 53%). Rf: 0.25 (methylene chloride / ethyl acetate = 3/1). ^{1}H NMR (DMSO-d_6):δ 7.31 (m, 5H, Ph), 6.2, 5.1 (d, J = 9.7 Hz, 1H each, H_2-1), 5.98 (s, 1H, H-6), 4.48 (s, 2H, CH_2Ph), 3.90(m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.39 (d, J = 4.6 Hz, 2H, CH_2OBn), 3.20, 2.80 (m, 1H each, H_2-5), 2.10, 1.40 (m, 1H, H_2-4). MS (*m/z*): 317 (M^+). Anal. Calcd for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.25; H, 6.55; N, 8.84.

3-Hydroxymethyl-7-methoxy-1*H*, 3*H*, 4*H*, 5*H*-pyrimido[1,6-*c*][1,3]oxazepin-9-one (5) A mixture of

4 (740 mg, 2.3 mmol) in methylene chloride (12 mL) was cooled to -78 °C. Boron trichloride (1 M in methylene chloride, 9.3 mL, 9.3 mmol) was added *via* syringe and under nitrogen gas. The mixture was stirred at -78 °C for 3 h, then temperature was raised to -40 °C. A solution of methanol / methylene chloride (1/1, 15 mL) was added, and the cooling bath was removed. The solution was neutralized with sodium bicarbonate. After filtration, the solvent was removed under reduced pressure. The residue was chromatographed on silica gel and eluted with methylene chloride / methanol (20/1). The fractions were collected and concentrated to give **5** (380 mg, 72%). mp 162-163 °C (methanol). Rf: 0.17 (methylene chloride / methanol = 20/1). ^{1}H NMR (DMSO-d_6):δ 6.10, 5.10 (d, J = 9.6 Hz, 1H each, H_2-1), 5.99 (s, 1H, H-6), 4.79 (s, 1H, OH), 3.79 (s, 3H, OCH_3), 3.60(m, 1H, CH), 3.30 (m, 2H, CH_2O), 3.07, 2.85 (m, 1H each, H_2-5), 2.00, 1.40 (m, 1H each, H_2-4). MS (*m/z*): 226 (M^+). Anal. Calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.04; H, 6.15; N, 11.98.

3-Benzyloxymethyl-1*H*, 3*H*, 4*H*, 5*H*, 8*H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (6) A solution of **4** (2 g, 6.3 mmol) in 2 N sodium hydroxide / dioxane (1/1, 150 mL) was vigorously stirred under reflux for 14 h. The organic layer was neutralized by acetic acid to pH 7. The mixture was concentrated. The residue was chromatographed on silica gel and eluted with methylene chloride / ethyl acetate (9/1). The fractions were collected and concentrated to give **6** (1.54 g, 80%). mp 160-161 °C (methanol). Rf: 0.13 (methylene chloride / ethyl acetate = 3/1). ^{1}H NMR (DMSO-d_6):δ 11.24 (s. 1H, NH), 7.27 (m, 5H, Ph), 5.88, 4.96 (d, J = 9.7 Hz, 1H each, H_2-1), 5.58 (s, 1H, H-6), 4.47 (s, 2H, CH_2Ph), 3.90 (m, 1H, CH), 3.40 (s, 2H, CH_2OBn), 3.27, 2.75 (m, 1H each, H_2-5), 2.00, 1.40 (m, 1H each, H_2-4). MS (*m/z*): 302 (M^+). Anal. Calcd for $C_{16}H_{18}N_2O_4$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.96; H, 6.00; N, 8.94.

3-Hydroxymethyl-1*H*, 3*H*, 4*H*, 5*H*, 8*H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (7) A mixture of **6** (3 g, 10 mmol) in methylene chloride (50 mL) was cooled to -78 °C. Boron trichloride (1 M in methylene chloride, 40 mL, 40 mmol) was added *via* syringe and under nitrogen gas. The mixture was stirred at -78 °C for 3 h, then temperature was raised to rt. A solution of methanol / methylene chloride (1/1, 50 mL) was added. The solution was neutralized with sodium bicarbonate. After filtration, the solvent was evaporated. The residue was crystallized with methanol to give **7** (1.51 g, 72%). mp 206-207 °C (methanol). Rf: 0.28 (methylene chloride / ethyl acetate/ methanol = 3/2/0.1). ^{1}H NMR (DMSO-d_6):δ 11.25 (s. 1H, NH), 5.87, 4.96 (d, J = 9.7 Hz, 1H each, H_2-1), 5.58 (s, 1H, H-6), 4.79 (s, 1H, OH), 3.65 (m, 1H, CH), 3.30 (s, 2H, CH_2O), 2.98, 2.75 (m, 1H each, H_2-5), 2.00, 1.40 (m, 1H each, H_2-4). ^{13}C NMR (DMSO-d_6): δ 163.1, 158.3, 151.6, 100.9, 83.2, 73.3, 64.1, 30.9, 30.7. MS (*m/z*): 212 (M^+). Anal. Calcd for $C_9H_{18}N_2O_4$: C, 50.94; H,5.70; N, 13.20. Found: C, 50.80; H, 5.71; N, 12.95.

3-Benzyloxymethyl-7-amino-1*H*, 3*H*, 4*H*, 5*H*-pyrimido[1,6-*c*][1,3]oxazepin-9-one (8) A mixture of

4 (1.6 g, 5 mmol) in saturated methanolic ammonia (100 mL) was heated at 120 °C for 24 h in a sealed tube. The solution was cooled to 0 °C and the precipitate was collected by filtration and crystallized with methanol to obtain **8** (1.02 g, 67%). mp 262-263 °C (methanol). Rf: 0.54 (methylene chloride / methanol = 9/1). ^{1}H NMR (DMSO-d_6):δ 7.29 (m, 5H, Ph) 7.10, 7.00 (s. 1H each, NH_2), 6.10, 4.96 (d, J = 9.7 Hz, 1H each, H_2-1), 5.59 (s, 1H, H-6), 4.47 (s, 2H, CH_2Ph), 3.90 (m, 1H, CH), 3.38 (d, J = 4.7 Hz, 2H, CH_2O-Bn), 2.90, 2.60 (m, 1H each, H_2-5), 1.80, 1.40 (m, 1H each, H_2-4). MS (*m/z*): 301 (M^+). Anal. Calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 63.91; H, 6.13; N, 13.80.

3-Hydroxymethyl-7-amino-1*H*, 3*H*, 4*H*, 5*H*-pyrimido[1,6-*c*][1,3]oxazepin-9-one (9) A mixture of **8** (0.9 g, 3 mmol) in methylene chloride (21 mL) was cooled to -78 °C. Boron trichloride (1 M in methylene chloride, 17 mL, 17 mmol) was added *via* syringe and under nitrogen gas. The mixture was stirred at -78 °C for 3 h, then temperature was raised to rt. A solution of methanol / methylene chloride (1/1, 5 mL) was added. The solution was neutralized with sodium bicarbonate. After filtration, the solvent was evaporated. The residue was chromatographed on silica gel and eluted with methylene chloride / methanol (10/1). The fractions were collected and concentrated to give **9** (186 mg, 30%). mp 261-262 °C (methanol). Rf: 0.26 (methylene chloride / ethyl acetate = 5/1). IR (KBr): 3416, 3353, 1643 cm^{-1}. ^{1}H NMR (DMSO-d_6):δ 7.14, 7.01 (s. 1H each, NH_2), 6.05, 4.89 (d, J = 9.6 Hz, 1H each, H_2-1), 5.58 (s, 1H, H-6), 4.78 (s, 1H, OH), 3.64 (m, 1H, CH), 3.32 (s, 2H, CH_2O), 2.95, 2.67 (m, 1H each, H_2-5), 2.00, 1.37 (m, 1H each, H_2-4). ^{13}C NMR (DMSO-d_6): δ 165.8, 158.558, 151.5, 93.2, 83.3, 73.8, 64.1, 31.2, 30.6. MS (*m/z*): 211 (M^+). Anal. Calcd for $C_9H_{13}N_2O_3$: C, 51.18; H, 6.20; N, 19.89. Found: C,51.43; H, 5.98; N, 19.59.

3-Benzyloxymethyl-6-bromo-1*H*, 3*H*, 4*H*, 5*H*, 8*H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (10) Bromine water (3%, 50 mL, 18 mmol) was added to a solution of **6** (3 g, 10 mmol) in pyridine (50 mL) and the mixture was stirred at rt for 2 h. The solvent was removed under reduced pressure. The residue was crystallized from methanol to give **10** (2.4 g, 62%). mp 140-141 °C (methanol). Rf: 0.13 (methylene chloride / ethyl acetate = 3/2). ^{1}H NMR (DMSO-d_6):δ 11.80 (s. 1H, NH), 7.31 (m, 5H, Ph), 5.94, 5.08 (d, J = 9.6 Hz, 1H each, H_2-1), 4.47 (s, 2H, CH_2Ph), 3.90 (m, 1H, CH), 3.45, 3.10 (m, 1H each, H_2-5), 3.40 (s, 2H, CH_2OBn), 2.10, 1.50 (m, 1H each, H_2-4). MS (*m/z*): 380 (M^+). Anal. Calcd for $C_{16}H_{17}N_2O_4Br$: C, 50.41; H, 4.49; N, 7.35. Found: C, 50.38; H, 4.57; N, 7.01.

3-Benzyloxymethyl-6-methyl-1*H*, 3*H*, 4*H*, 5*H*, 8*H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (11) Under nitrogen atmosphere n-butyllithium (1.5 M, 7.6 mL, 11.4 mmol) was added dropwise to a solution of **10** (2 g, 5.5 mmol) in dry tetrahydrofuran (26 mL) at -70 °C. The mixture was stirred at -50 °C for 1 min. Methyl iodide (0.5 mL, 5.2 mmol) was added and the stirring was continued for 1 min. The cold bath was removed and the mixture was further stirred for 30 min. The solution was neutralized with acetic acid to

pH 7, and the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic layer was separated, dried over sodium sulfate, and then the solvent was removed. The residue was chromatographed on silica gel eluting with methylene chloride / ethyl acetate (4/1). The desired fractions were evaporated under reduced pressure to give **11** (535 mg, 32%). mp 162-163 °C (methanol). Rf: 0.38 (methylene chloride / ethyl acetate = 3/2). ^{1}H NMR (DMSO-d_6):δ 11.31 (s, 1H, NH), 7.28 (m, 5H, Ph), 5.89, 5.00 (d, J = 9.6 Hz, 1H each, H_2-1), 4.48 (s, 2H, OCH_2Ph), 3.90 (m, 1H, CH), 3.40 (d, J = 4.6 Hz, 2H, CH_2OBn), 3.10, 2.80 (m, 1H each, CH_2-5), 2.00 (m, 1H, CH_2-4), 1.83 (s, 3H, CH_3), 1.40 (m, 1H, CH_2-4). MS (*m/z*): 316 (M^+). Anal. Calcd for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.62; H, 6.33; N,8.66.

3-Hydroxymethyl-6-methyl-1*H*, 3*H*, 4*H*, 5*H*, 8*H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (12) Compound (**12**) was prepared in a manner similar to the preparation of **7**. Reagent: **11** (660 mg, 2.1 mmol) in methylene chloride (50 mL), boron trichloride (1 M, 8.3 mL, 8.3 mmol). Yield (304 mg, 65%). mp 236-237 °C (methanol). Rf: 0.20 (methylene chloride / methanol = 20/1). IR (KBr): 3461, 1691 cm^{-1}. ^{1}H NMR (DMSO-d_6):δ 11.28 (s, 1H, NH), 5.91, 4.98 (d, J = 9.6 Hz, 1H each, H_2-1), 4.77 (t, J = 5.4 Hz, 1H, OH), 3.70 (m, 1H, *CH*), 3.33 (m, 2H, CH_2O), 3.10, 2.90 (m, 1H each, CH_2-5), 1.85 (s, 3H, CH_3), 2.00, 1.40 (m, *1H each*, CH_2-4). ^{13}C NMR: 163.9, 153.0, 150.84, 106.5, 82.8, 73.3, 64.9, 30.1, 26.7, 10.7. MS (*m/z*): 226 (M^+). Anal. Calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.16; H, 6.51; N,12.58.

3- Hydroxymethyl-6-bromo-1*H*, 3*H*, 4*H*, 5*H*, 8*H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (13a) Compound (**13a**) was prepared in manner similar to the preparation of **7**. Reagents: **10** (200 mg, 0.5 mmol) in methylene chloride (20 mL), boron trichloridr (1 M, 2.1 mL, 2.1 mmol). Yield (86 mg, 56%). mp 196-197 °C (methanol). Rf: 0.38 (methylene chloride / ethyl acetate / methanol = 3/2/0.1). IR (KBr): 3484, 1682 cm^{-1}. ^{1}H NMR (DMSO-d_6):δ 11.81 (s, 1H, NH), 5.94, 5.06 (d, J = 9.6 Hz, 1H each, H_2-1), 4.79 (t, J = 5.4 Hz, 1H, OH), 3.72 (m, 1H, CH), 3.40 (m, 1H, H_2-5), 3.34 (m, 2H, CH_2O), 3.11 (m, 1H, H_2-5), 2.00, 1.39 (m, 1H each, H_2-4). ^{13}C NMR (DMSO-d_6): δ 159.4, 156.1, 150.3, 97.5, 82.8, 74.4, 63.8, 30.9, 29.3. MS (*m/z*): 290 (M^+). Anal. Calcd for $C_9H_{11}N_2O_4Br$: C, 37.13; H, 3.81; N, 9.62. Found: C, 37.03; H, 3.85; N, 9.31.

3-Hydroxymethyl-6-chloro-*1H*, *3H*, *4H*, *5H*, *8H*-pyrimido[1,6-*c*][1,3]oxazepin-7, 9-dione (13b) A mixture of **7** (800 mg, 2.5 mmol) ans *N*-chlorosuccinimide (500 mg, 3.8 mmol) in glacial acetic acid (20 mL) was heated at 60 °C with stirring for 4.5 h. The solution was evaporated *in vacuo* to dryness. The residue was coevaporated with methanol (3 x 10 mL) three times. The residue was crystallized from methanol to give **13b** (570 mg, 91%). mp 207-208 °C (methanol). Rf: 0.26 (methylene chloride / methanol = 95/5). IR (KBr): 3489, 1690 cm^{-1}. ^{1}H NMR (DMSO-d_6):δ 11.81 (s. 1H, NH), 5.92, 5.04 (d, J = 9.6 Hz,

1H each, H_2-1), 4.86 (t, J = 5.4 Hz, 1H, OH), 3.72 (m, 1H, *CH*), 3.35 (m, 2H, CH_2O), 3.00, 2.27 (m, 1H each, H_2-5), 2.05, 1.40 (m, 1H each, H_2-4). ^{13}C NMR (DMSO-d_6): δ 159.0, 154.5, 150.0, 106.5, 82.9, 74.3, 63.7, 29.3, 27.8. MS (*m/z*): 246 (M^+). Anal. Calcd for $C_9H_{11}N_2O_4Cl$: C, 43.83; H, 4.50; N, 11.36. Found: C, 43.81; H, 4.55; N, 11.63.

In Vitro Antiviral Assays for Herpesviruses

(a) Cells and Viruses. Diploid human foreskin fibroblasts (HFF cells) were grown in minimal essential medium (MEM) with Earle's salts [MEM(H)] supplemented with 10% fetal bovine serum. BSC-1 (African green monkey kidney) cells were grown in MEM(E) supplemented with 10% calf serum. Cells were passaged according to conventional procedures as detailed previously.[15] A plaque-purified isolate, Po, of the Towne strain of HCMV was used and was a gift of Dr. M. F. Stinski, University of Iowa. The KOS strain of HSV-1 was provided by Dr. S. K. Weller of University of Connecticut. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere. [15] The HTML-IIIB strain of HIV-1 was propagated in the human T-lymphocyte cell line, H9 as detailed elsewhere.[16] The virus inoculum consisted of supernatant fluids from H9-IIIB producer cultures.

(b) Antiviral Assays for Herpesviruses. HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above[15] for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. HSV-1 was grown in BSC-1 cells and was assayed using an enzyme immunoassay described by Prichard and Shipman.[17]

(c) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. During-induced cytopathology was estimated at 30-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration.[15] Cytotoxicity in KB cells was determined colorimetrically using a staining assay as described previously. [18]

(d) Data Analysis. Dose-response relationships were constructed by linearly regressing the present inhibition of parameters derived in the preceding sections against log of drug concentration. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for KB cytotoxicity) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

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