Highly Potent and Selective Inhibition of Varicella-Zoster Virus by Bicyclic Furopyrimidine Nucleosides Bearing an Aryl Side Chain

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In addition to our recent report on the potent anti-varicella-zoster virus (VZV) activity of some unusual bicyclic furopyrimidine nucleosides bearing long alkyl side chains, we herein report the further significant enhancement of the antiviral potency by inclusion of a phenyl group in the side chain of these compounds. The target structures were prepared by the Pd-catalyzed coupling of a series of para-substituted arylacetylenes with 5-iodo-2′-deoxyuridine, to give intermediate 5-alkynyl nucleosides which were cyclized in the presence of Cu to give the desired bicyclic systems. The compounds display extraordinary potency and selectivity for VZV; the most active are ca. 10 000 times more potent than the reference compound acyclovir and ca. 100 times more potent than the alkyl analogues earlier reported by us. The current compounds show little cytotoxicity, leading to selectivity index values ⩾ 1 000 000. From a range of DNA and RNA viruses tested, only VZV was inhibited by these compounds indicating their extreme selectivity for this target virus. The novelty of the molecules, coupled with their extreme potency and selectivity, their desirable physicochemical properties, and their relative ease of synthesis, makes them of considerable interest for potential drug development for VZV infections.

Introduction

We have recently reported on highly potent and selective inhibitors of varicella-zoster virus (VZV) with unusual bicyclic furopyrimidine structures.[1] These novel molecules displayed potencies against various strains of VZV in cell culture which exceeded those of current anti-VZV agents such as acyclovir by ca. 300-fold.[1] An unusual, but apparently critical, requirement for antiviral activity in this novel series of bicyclic nucleosides is the presence of a long alkyl chain at the 6-position of the furo ring, as typified by structures **1a**−**c** (Chart 1).[1]

Although shorter alkyl chains do lead to antiviral activity comparable to that of acyclovir (R = C_5−C_6 for example), longer chains (R = C_7−C_{10}) were required for optimal potency. In an effort to further explore the structure−activity relationships (SARs) in this unusual series of promising antivirals, we have prepared a small series of ω-substituted compounds[2] and have noted that the ω-chlorononyl compound in particular displays potency and selectivity at least comparable with that of the parent decyl system.[1,2] Substitution at the terminus of the alkyl (nonyl) chain with other halogen atoms (F, Br, I) is also acceptable, while more polar groups such as hydroxyl lead to a significant diminution of potency.[3]

The present compounds display high lipophilicity values, as would be anticipated from their long alkyl side chains. This may have the positive consequence that their membrane permeation may be very efficient and topical formulation and dosing may be notably effective. However, the corollary of this is that the compounds display low water solubility. With this in mind we have recently prepared a series of analogues with (ether) oxygen substitution in the alkyl side chain. Either one or two ether oxygens were incorporated, and the position of these heteroatoms along the chain was varied.[4] The introduction of these oxygen atoms was indeed highly successful in enhancing water solubility, but unfortunately only at the expense of a significant (ca. 1000-fold) reduction in antiviral potency.[4]

Taken together, all of these studies[1−4] indicate the absolute requirement for a nonpolar moiety at the C6 position of the furo ring system for potent anti-VZV activity in these bicyclic nucleosides. To further probe this requirement we now sought to replace a number of the alkyl methylenes by alternative nonalkyl units. In particular, we sought to constrain the highly flexible alkyl systems into more defined conformations by the use of ring moieties. Bearing in mind the above-mentioned SARs we chose to incorporate a series of para-substituted (predominantly alkyl) phenyl groups at C6, and we herein report on the synthesis and characterization of these novel analogues; in particular we note their extreme potency and selectivity as inhibitors of VZV.

Chart 1

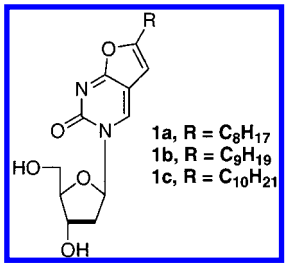

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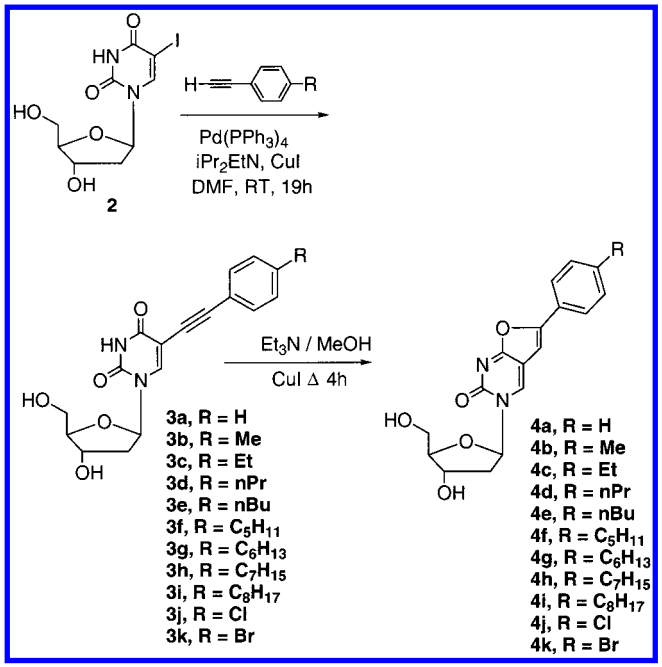
Table 1. Anti-VZV Activity, Cytotoxicity, and ClogP Values for Bicyclic Pyrimidines **4a**−**k** and Reference Compounds **5**, **6**, and **1a**−**c**[a]

			EC_{50} (μM)					
compd	R	ClogP	VZV OKA	VZV YS	VZV TK$^-$ 07	VZV TK$^-$ YS-R	MCC (μM)	CC_{50} (μM)
4a	H	0.35	0.28 ± 0.0	0.16 ± 0.0	>200	162	>200	>200
4b	Me	0.85	0.06 ± 0.0	0.06 ± 0.0	103	>200	>200	>200
4c	Et	1.38	0.09 ± 0.0	0.07 ± 0.0	>50	>50	200	123
4d	Pr	1.91	0.010 ± 0.001	0.008 ± 0.001	>50	>20	⩾50	188
4e	Bu	2.44	0.0022 ± 0.001	0.0005 ± 0.0003	⩾20	>20	⩾20	>200
4f	pentyl	2.97	0.0003 ± 0.0002	0.0001 ± 0.0001	>20	>5	⩾20	>200
4g	hexyl	3.50	0.0005 ± 0.0003	0.0001 ± 0.0001	>5	>5	20	18
4h	heptyl	4.03	0.0054 ± 0.002	0.003 ± 0.002	>5	>5	5	18
4i	octyl	4.56	0.040 ± 0.025	0.027 ± 0.023	>20	>20	⩾20	>200
4j	Cl	1.07	0.1	0.1	>50	>50	200	nd
4k	Br	1.22	0.5	0.5	>50	>50	50	nd
5 (acyclovir)			2.9	1	74	125	>200	>200
6 (BVDU)			0.003	0.003	>150	>150	>150	>400
1a	octyl	2.46	0.008	0.024	>50	>50	>50	⩾50
1b	nonyl	2.99	0.02	0.02	>200	>200	⩾200	>200
1c	decyl	3.52	0.015	0.008	>50	>50	>50	>50

[a] EC_{50} values represent the concentration of compound in μM required to reduce VZV plaque formation after 5 days in HEL cell cultures by 50% compared to untreated controls. Data are given for two thymidine kinase-competent strains of virus (OKA and YS) and for two thymidine kinase-deficient strains (07 and YS-R). CC_{50} represents the 50% cytostatic concentration, defined as the compound concentration in μM required to reduce the cell number by 50% after 5 days in the absence of virus. MCC (minimal cytotoxic concentration) is the compound concentration required to cause a microscopically visible alteration of normal cell morphology. ClogP is the calculated logarithm of the octanol−water partition coefficient *P*.[9]

Scheme 1



Results and Discussion

Chemistry. The synthetic route adopted by us for the current target compounds (Scheme 1) followed closely that we had adopted for the parent alkyl systems,[1] involving the Pd-catalyzed coupling of arylacetylenes with 5-iodo-2′-deoxyuridine **(2)** to give intermediate 5-(2-arylethynyl)-2′-deoxyuridines **3a**−**k**.[5−7] We have previously noted[1] that these intermediates either may be isolated and purified prior to the second synthetic step (cyclization) or may be cyclized in situ. In the first case studied (**3d**) we did isolate and purify the intermediate alkyne (41%) and subjected this to cyclization with copper catalysis (43%). However, in all subsequent cases (**3a**−**c**,**e**−**k**) the alkyne was not isolated and **2** was converted to **4a**−**c**,**e**−**k** in one step. The yields observed ranged from 11−16% (un-optimized), being thus comparable to the overall yield of **4d** (18%) and with a significant saving of time in the purification of intermediates. Target structures **4a**−**k** were characterized by ^{1}H and ^{13}C NMR, high-resolution mass spectrometry, and microanalysis, all data being consistent with their intended structures and confirming high chemical purity.

Antiviral Activity. The target bicyclic systems **4a**−**k** were evaluated for their ability to inhibit the replication of herpes simplex virus (HSV-1, HSV-2), vaccinia virus (VV), cytomegalovirus (CMV), and VZV according to previously described methods.[8] Data are shown in Table 1 for the activity of **4a**−**k** versus two strains of thymidine kinase-competent (TK$^+$) VZV and also two strains of thymidine kinase-deficient (TK$^-$) VZV, with data also included for the reference anti-herpes agents acyclovir (**5**) and BVDU (**6**). We also include corresponding data for the parent alkyl analogues **1a**−**c** of the present systems.[1] Cytotoxicity data are also given for each compound in two assays (Table 1). It is clear from these data that the *p*-alkylaryl systems (**4a**−**i**) we herein report are exquisitely potent and selective inhibitors of VZV in these assays. As previously noted for the parent alkyl analogues,[1] there is a clear dependence on the length of the *p*-alkyl substituent in the homologous series **4a**−**i**. However, whereas optimal alkyl chain lengths were ca. C_8−C_{11} for the parent alkyl series **1a**−**c**, we note that the inclusion of the phenyl ring in the present compounds leads to a shift in optimal *p*-alkyl chain length to ca. C_4−C_7. The *p*-pentylphenyl analogue **4f** displays EC_{50} values against the two thymidine kinase-competent strains of VZV at 0.1−0.3 nM concentrations, thus being ca. 10 000 times more potent than the primary existing medicine for VZV (acyclovir) and 10−30 times more potent than the exceptionally potent BVDU (**6**). Cytotoxicity was in general noted for the current agents only at concentrations ⩾ 20 to 200 μM; combined with the very high potency values these data lead to selectivity index values (SIs) exceeding 1 000 000 for the most active compounds in the series.

Similarly, data for **4j,k** indicate that a *p*-halogen atom is tolerated in the phenyl ring, with similar potencies to that noted for the parent phenyl (**4a**) or toluyl (**4b**) systems. The calculated log *P* values[9] for **4j,k** are roughly in line with this observation (Table 1).

The precise mechanism of action of these compounds remains unclear. However, the clear absence of antiviral activity against thymidine kinase-deficient VZV strains (Table 1) strongly implies an absolute need for VZV thymidine kinase-mediated phosphorylation to the corresponding 5′-monophosphates as a prerequisite for antiviral action. The present data cannot discriminate between action as the monophosphate or further phosphorylation to the di- and triphosphates of these compounds, but selective phosphorylation by VZV thymidine kinase and/or specific inhibition of VZV DNA polymerase by the corresponding nucleoside triphosphates may appear to be the simplest explanation of the very high anti-VZV selectivity of these agents. Indeed, evaluation of **4a**−**j** against a series of other viruses (HSV-1, HSV-2, VV, CMV) indicated a complete absence of antiviral activity, suggesting a mode of action that is entirely specific to one or more VZV-specific enzyme-mediated steps. This mode of action may thus be similar to that suggested for the parent alkyl analogues.

In conclusion we herein report *p*-alkylaryl analogues of bicyclic furopyrimidine nucleosides as exquisitely potent (subnanomolar) and selective inhibitors of VZV. A number of features of these novel compounds indicate that they may have considerable clinical potential for evaluation as improved agents for the treatment of herpes zoster and other VZV infections.

Experimental Section

The bicyclic ring has been numbered in accordance with the recommended IUPAC nomenclature guidelines. Both IUPAC and accepted nomenclature for nucleoside chemistry has been followed for the naming of the compounds described. For thin-layer chromatography, precoated aluminum backed plates (60 F-54, 0.2 mm thickness; supplied by E. Merck AG, Darmstadt, Germany) were used and were developed by the ascending method. After evaporation of solvent, compounds were detected by quenching of the fluorescence, at 254 or 366 nm, upon irradiation with a UV lamp. For column chromatography, glass columns were slurry packed in the appropriate eluent under gravity, with silica gel (C-gel 60A, 40−60 μm; Phase Sep, U.K.). Samples were applied as a concentrated solution, in the same eluent, or preabsorbed onto silica gel. Fractions containing the product were identified by TLC, "pooled" and concentrated in vacuo. Flash column chromatography was performed using an electrical pump. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX300 spectrometer (300 and 75 MHz, respectively) and autocalibrated to the deuterated solvent reference peak. All ^{13}C NMR spectra were proton decoupled. Mass spectra were performed by the service at the University of Birmingham, U.K.. Elemental analyses were performed by the service at the University of Wales, Swansea, U.K. All solvents used were anhydrous and used as supplied from Aldrich. All nucleosides and solid reagents were dried while heating under high vacuum over phosphorus pentoxide. All glassware was oven dried at 130 °C for several hours and allowed to cool in a stream of dry nitrogen.

5-(4-*n*-Propylphenylacetylene)-2′-deoxyuridine (3d). To a stirred solution of 5-iodo-2′-deoxyuridine (**2**) (800 mg, 2.26 mmol) in anhydrous dimethylformamide (8 mL) were added diisopropylethylamine (584 mg, 0.8 mL, 4.52 mmol), 4-*n*-propylphenylacetylene (0.97 g, 6.76 mmol), tetrakis(triphenylphoshine)palladium(0) (261 mg, 0.266 mmol) and copper(I) iodide (86 mg, 0.452 mmol). The mixture was stirred for 18 h, at room temperature, under a nitrogen atmosphere, After this time, the reaction mixture was concentrated in vacuo, and the resulting residue was dissolved in dichloromethane and methanol (1:1) (6 mL), whereupon an excess of Amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness. The crude product was purified by flash column chromatography (eluent: ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give 5-(4-*n*-propylphenylacetylene)-2′-deoxyuridine as the pure product (350 mg, 41%): ^{1}H NMR (DMSO-d_6; 300 MHz) δ 11.56 (1H, broad s, NH), 8.13 (1H, s, H-6), 7.42 (2H, H_a) − 7.25 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 6.18 (1H, dd, ^3J = 6.5 Hz, H-1′), 5.25 (1H, d, ^3J = 4.1 Hz, 3′-OH), 5.16 (1H, t, ^3J = 4.7 Hz, 5′-OH), 4.19 (1H, m, H-3′), 3.73 (1H, m, H-4′), 3.49 (2H, m, H-5′), 2.37 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.21 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.51 (2H, sxt, CH_2, ^3J = 6.9 Hz), 0.85 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.8 (CH_3), 19.5, 21.6, (C_2H_4), 40.7 (C-2′), 60.8 (C-5′), 70.5 (C-3′), 73.7 (α-alkynyl), 85.4, 88.4 (C-1′, C-4′), 93.8 (β-alkynyl), 99.9 (C-5), 124.3 (C−H_b), 129.5 (*ipso*-C), 130.7 (C−H_a), 140.2 (*para*-C), 143.4 (C-6), 150.3 (C-2), 162.6 (C-4).

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-propylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4d). To a solution of 5-(4-*n*-propylphenylacetylene)-2′-deoxyuridine (**3d**) (200 mg, 0.54 mmol) in methanol and triethylamine (7:3) (20 mL) was added copper iodide (20 mg, 0.102 mmol). The mixture was refluxed for 4 h. The solvent was removed in vacuo, and the crude product was purified by flash column chromatography (initial eluent: ethyl acetate, followed by: ethyl acetate/methanol (9:1)). The combined fractions were combined and the solvent was removed in vacuo to give the crude product, which was recrystallized from methanol to give pure 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-propylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4d**) (86 mg, 43%): ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.72 (1H, s, H-4), 7.43 (2H, H_a) − 7.28 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.15 (1H, s, H-5), 6.18 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.31 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.12 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.31 (1H, m, H-3′), 3.89 (1H, m, H-4′), 3.51 (2H, m, H-5′), 2.65 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.31 and 2.12 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.58 (2H, sxt, CH_2, ^3J = 6.9 Hz), 0.85 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.2 (CH_3), 20.1, 22.3, (C_2H_4), 41.5 (C-2′), 62.3 (C-5′), 71.6 (C-3′), 83.2, 88.4 (C-1′, C-4′), 100.4 (C-5), 104.6 (C-4a), 125.3 (C−H_b), 128.4 (*ipso*-C), 131.8 (C−H_a), 141.2 (*para*-C), 138.5 (C-4), 154.6 (C-6), 159.1 (C-2), 172.3 (C-7a); MS (ES$^+$) *m/e* 393 (MNa^+, 100%), 277 (baseNa^+, 20%). Accurate mass: $C_{20}H_{22}N_2O_5Na$ requires 393.1426; found 393.1422. Found: C, 61.69; H, 6.23%; N, 7.13. $C_{20}H_{22}N_2O_5 \cdot H_2O$ requires: C, 61.85; H, 6.23; N, 7.21.

General Procedure for Preparation of 3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-alkyllphenyl)-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one Analogues. To a stirred solution of 5-iodo-2′-deoxyuridine (**2**) (800 mg, 2.26 mmol) in anhydrous dimethylformamide (8 mL) were added diisopropylethylamine (584 mg, 0.8 mL, 4.52 mmol), the 4-*n*-alkylphenylacetylene (6.76 mmol), tetrakis(triphenylphoshine)palladium(0) (261 mg, 0.266 mmol) and copper(I) iodide (86 mg, 0.452 mmol). The mixture was stirred for 18 h, at room temperature, under a nitrogen atmosphere, after which time TLC (ethyl acetate/methanol 9:1), showed complete conversion of the starting material. Copper(I) iodide (80 mg, 0.40 mmol), triethylamine (15 mL) and methanol (20 mL) were then added to the mixture, which was subsequently refluxed for 4 h. The reaction mixture was then concentrated in vacuo, and the resulting residue was dissolved in dichloromethane and methanol (1:1) (6 mL), whereupon an excess of Amberlite IRA-400 (HCO_3^- form) was added and stirred for 30 min. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness. The crude product was purified by flash column chromatography (Initial eluent: ethyl acetate, followed by: ethyl acetate/methanol (9:1)). The appropriate fractions

were combined, and the solvent was removed in vacuo, to give the pure product.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-phenyl-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (4a). The procedure was carried out using phenylacetylene (0.689 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-phenylacetylene-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (**4a**) (123 mg, 16%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.84 (1H, s, H-4), 7.81 (2H, H_a) − 7.62 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.25 (1H, s, H-5), 6.28 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.41 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.23 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.33 (1H, m, H-3′), 3.91 (1H, m, H-4′), 3.72 (2H, m, H-5′), 2.40 and 2.15 (2H, m, 2-H′$_a$ and 2-H′$_b$); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 41.6 (C-2′), 60.9 (C-5′), 69.8 (C-3′), 87.9, 88.5 (C-1′, C-4′), 99.12 (C-5), 107.1 (C-4a), 124.8 (C−H_b), 128.4 (*ipso*-C), 129.7 (C−H_a), 140.2 (*para*-C), 138.5 (C-4), 154.1 (C-6), 160.3 (C-2), 171.4 (C-7a); MS (ES$^+$) *m/e* 351 (MNa$^+$, 100%). Accurate mass: $C_{17}H_{16}N_2O_5Na$ requires 351.0957; found 351.0961. Found: C, 60.44; H, 5.18; N, 8.25. $C_{17}H_{16}N_2O_5\cdot 0.5H_2O$ requires: C, 60.53; H, 5.08; N, 8.30.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-methylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4b). The procedure was carried out using 4-*n*-methylphenylacetylene (0.791 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-methylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4b**) (131 mg, 17%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.81 (1H, s, H-4), 7.79 (2H, H_a) − 7.48 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.31 (1H, s, H-5), 6.22 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.37 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.19 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.29 (1H, m, H-3′), 3.87 (1H, m, H-4′), 3.65 (2H, m, H-5′), 2.41 and 2.19 (2H, m, 2-H′$_a$ and 2-H′$_b$), 2.15 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 15.2 (CH_3), 40.5 (C-2′), 61.3 (C-5′), 70.3 (C-3′), 87.2, 89.1 (C-1′, C-4′), 100.2 (C-5), 106.3 (C-4a), 125.3 (C−H_b), 127.4 (*ipso*-C), 128.8 (C−H_a), 138.2 (*para*-C), 137.9 (C-4), 155.1 (C-6), 159.3 (C-2), 170.8 (C-7a); MS (ES$^+$) *m/e* 365 (MNa$^+$, 100%), 249 (baseNa$^+$, 10%). Accurate mass: $C_{18}H_{18}N_2O_5Na$ requires 365.1113; found 365.1107. Found: C, 62.87; H, 5.39; N, 7.88. $C_{18}H_{18}N_2O_5$ requires: C, 63.15; H, 5.30; N, 8.18.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-ethylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4c). The procedure was carried out using 4-*n*-ethylphenylacetylene (0.885 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-ethylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4c**) (115 mg, 14%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.91 (1H, s, H-4), 7.76 (2H, H_a) − 7.49 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.25 (1H, s, H-5), 6.26 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.39 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.24 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.34 (1H, m, H-3′), 3.98 (1H, m, H-4′), 3.71 (2H, m, H-5′), 2.71 (2H, t, ^3J = 6.9 Hz, α-CH_2) 2.48 and 2.12 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.2 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 15.7 (CH_3), 27.9 (CH_2), 40.6 (C-2′), 61.0 (C-5′), 69.8 (C-3′), 87.9, 88.5 (C-1′, C-4′), 99.1 (C-5), 107.2 (C-4a), 124.9 (C−H_b), 126.5 (*ipso*-C), 128.8 (C−H_a), 145.7 (*para*-C), 138.2 (C-4), 154.1 (C-6), 162.1 (C-2), 171.4 (C-7a); MS (ES$^+$) *m/e* 379 (MNa$^+$, 100%), 263 (baseNa$^+$, 10%). Accurate mass: $C_{19}H_{20}N_2O_5Na$ requires 379.1270; found 379.1272. Found: C, 62.77; H, 5.93; N, 7.61. $C_{19}H_{20}N_2O_5\cdot 0.5H_2O$ requires: C, 62.46; H, 5.79; N, 7.67.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-butylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4e). The procedure was carried out using 4-*n*-butylphenylacetylene (1.072 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-butylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4e**) (140 mg, 16%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.76 (1H, s, H-4), 7.46 (2H, H_a) − 7.31 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.20 (1H, s, H-5), 6.21 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.37 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.31 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.31 (1H, m, H-3′), 3.75 (1H, m, H-4′), 3.48 (2H, m, H-5′), 2.65 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.31 and 2.12 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.62 (4H, m, CH_2), 0.87 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.2 (CH_3), 20.1, 22.3, 27.9 (C_3H_6), 42.5 (C-2′), 63.7 (C-5′), 73.6 (C-3′), 83.5, 88.7 (C-1′, C-4′), 100.8 (C-5), 108.4 (C-4a), 125.3 (C−H_b), 128.4 (*ipso*-C), 131.8 (C−H_a), 141.2 (*para*-C), 138.5 (C-4), 154.6 (C-6), 159.1 (C-2), 170.9 (C-7a); MS (ES$^+$) *m/e* 407 (MNa$^+$, 100%), 291 (baseNa$^+$, 20%). Accurate mass: $C_{21}H_{24}N_2O_5Na$ requires 407.1583; found 407.1575. Found: C, 65.41; H, 6.48; N, 7.40. $C_{21}H_{24}N_2O_5$ requires: C, 65.61; H, 6.29; N, 7.29.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4f). The procedure was carried out using 4-*n*-pentylphenylacetylene (1.15 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-pentylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4f**) (137 mg, 15%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.81 (1H, s, H-4), 7.51 (2H, H_a) − 7.35 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.18 (1H, s, H-5), 6.23 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.37 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.31 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.34 (1H, m, H-3′), 3.79 (1H, m, H-4′), 3.41 (2H, m, H-5′), 2.67 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.34 and 2.14 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.67 (2H, m, CH_2), 1.51−1.32 (4H, m, CH_2), 0.84 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.2 (CH_3), 20.1, 22.3, 27.9, 28.4, (C_4H_8), 41.3 (C-2′), 62.6 (C-5′), 71.8 (C-3′), 83.4, 86.4 (C-1′, C-4′), 100.4 (C-5), 107.4 (C-4a), 125.4 (C−H_b), 127.4 (*ipso*-C), 131.8 (C−H_a), 138.5 (C-4), 141.3 (*para*-C), 154.6 (C-6), 161.1 (C-2), 170.9 (C-7a); MS (ES$^+$) *m/e* 421 (MNa$^+$, 100%), 305 (baseNa$^+$, 40%). Accurate mass: $C_{22}H_{26}N_2O_5Na$ requires 421.1739; found 421.1733. Found: C, 64.69; H, 6.67; N, 6.82. $C_{22}H_{26}N_2O_5\cdot 0.5H_2O$ requires: C, 64.85; H, 6.68; N, 6.87.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-hexylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4g). The procedure was carried out using 4-*n*-hexylphenylacetylene (1.26 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-hexylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4g**) (124 mg, 13%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.85 (1H, s, H-4), 7.53 (2H, H_a) − 7.29 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.23(1H, s, H-5), 6.24 (1H, dd, ^3J = 6.15 Hz, H-1′), 5.58 (1H, d, ^3J = 4.0 Hz, 3′-OH), 5.29 (1H, t, ^3J = 5.01 Hz, 5′-OH), 4.54 (1H, m, H-3′), 3.79 (1H, m, H-4′), 3.51 (2H, m, H-5′), 2.72 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.31 and 2.10 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.62 (2H, m, CH_2), 1.42−1.22 (6H, m, CH_2), 0.87 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.2 (CH_3), 20.1, 22.3, 27.9, 29.5, 30.2 (C_5H_{10}), 41.6 (C-2′), 62.3 (C-5′), 769.8 (C-3′), 83.5, 88.7 (C-1′, C-4′), 99.1 (C-5), 107.2 (C-4a), 124.3 (C−H_b), 126.4 (*ipso*-C), 129.3 (C−H_a), 138.5 (C-4), 141.2 (*para*-C), 154.6 (C-6), 160.9 (C-2), 171.3 (C-7a); MS (ES$^+$) *m/e* 435 (MNa$^+$, 100%), 319 (baseNa$^+$, 40%). Accurate mass: $C_{23}H_{28}N_2O_5Na$ requires 435.1896; found 435.1897. Found: C, 64.28; H, 7.10; N, 6.47. $C_{23}H_{28}N_2O_5\cdot H_2O$ requires: C, 64.17; H, 7.02; N, 6.51.

3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-heptylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4h). The procedure was carried out using 4-*n*-heptylphenylacetylene (1.35 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-heptylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4h**) (129 mg, 13%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.91 (1H, s, H-4), 7.62 (2H, H_a) − 7.35 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.26 (1H, s, H-5), 6.28 (1H, dd, ^3J = 6.17 Hz, H-1′), 5.62 (1H, d, ^3J = 4.1 Hz, 3′-OH), 5.32 (1H, t, ^3J = 5.12 Hz, 5′-OH), 4.52 (1H, m, H-3′), 3.81 (1H, m, H-4′), 3.62 (2H, m, H-5′), 2.71 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.35 and 2.14 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.59 (2H, m, CH_2), 1.48−1.21 (8H, m, CH_2), 0.82 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.2 (CH_3), 20.1, 22.3, 27.9, 28.5, 29.5, 30.2 (C_6H_{12}), 41.6 (C-2′), 61.5 (C-5′), 69.8 (C-3′), 87.9, 88.5 (C-1′, C-4′), 99.1 (C-5), 107.2 (C-4a), 124.3 (C−H_b), 126.2 (*ipso*-C), 129.3 (C−H_a), 138.2(C-4), 144.2 (*para*-C), 154.6 (C-6), 160.7 (C-2), 170.6 (C-7a); MS (ES$^+$) *m/e* 449 (MNa$^+$, 100%), 333 (baseNa$^+$, 30%). Accurate mass: $C_{24}H_{30}N_2O_5Na$ requires 449.2052; found 449.2057. Found: C, 67.27; H, 7.26; N, 6.38. $C_{24}H_{30}N_2O_5$ requires: C, 67.59; H, 7.09; N, 6.57.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-octylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4i). The procedure was carried out using 4-*n*-octylphenylacetylene (1.45 g, 6.76 mmol),

which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-octylphenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4i**) (111 mg, 11%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.92 (1H, s, H-4), 7.61 (2H, H_a) − 7.33 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.25 (1H, s, H-5), 6.21 (1H, dd, ^3J = 6.19 Hz, H-1′), 5.59 (1H, d, ^3J = 4.1 Hz, 3′-OH), 5.272 (1H, t, ^3J = 5.12 Hz, 5′-OH), 4.39 (1H, m, H-3′), 3.75 (1H, m, H-4′), 3.62 (2H, m, H-5′), 2.71 (2H, t, ^3J = 6.9 Hz, α-CH_2), 2.34 and 2.13 (2H, m, 2-H′$_a$ and 2-H′$_b$), 1.61 (2H, m, CH_2), 1.51−1.19 (10H, m, CH_2), 0.82 (3H, t, ^3J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 13.2 (CH_3), 20.1, 21.39, 22.3, 27.9, 28.5, 29.5, 30.2 (C_7H_{14}), 41.7 (C-2′), 61.1 (C-5′), 69.8 (C-3′), 87.9, 88.7 (C-1′, C-4′), 99.0 (C-5), 107.2 (C-4a), 124.8 (C−H_b), 126.2 (*ipso*-C), 129.3 (C−H_a), 138.2(C-4), 144.2 (*para*-C), 154.2 (C-6), 160.7 (C-2), 171.6 (C-7a); MS (ES$^+$) *m/e* 463 (MNa^+, 100%), 347 (baseNa^+, 30%). Accurate mass: $C_{25}H_{32}N_2O_5Na$ requires 463.2209; found 463.2215. Found: C, 67.83; H, 7.41; N, 6.28. $C_{25}H_{32}N_2O_5$ requires: C, 68.16; H, 7.32; N, 6.36.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-chlorophenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4j). The procedure was carried out using 4-*n*-chlorophenylacetylene (0.92 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-chlorophenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4j**) (474 mg, 58%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.91 (1H, s, H-4), 7.88 (2H, H_a) − 7.57 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.37 (1H, s, H-5), 6.19 (1H, dd, ^3J = 6.17 Hz, H1′), 5.35 (1H, d, ^3J = 4.1 Hz, 3′-OH), 5.24 (1H, t, ^3J = 5.12 Hz, 5′-OH), 4.26 (1H, m, H-3′), 3.95 (1H, m, H-4), 3.70 (2H, m, H-5′), 2.41 and 2.13 (2H, m, 2-H′$_a$ and 2-H′$_b$); ^{13}CNMR (DMSO-d_6; 75 MHz) δ 41.6 (C-2′), 60.9 (c-5′), 69.8 (C-3′), 88.0, 88.5, (C-1′, C-4′), 100.8 (C-5), 107.0 (C-4a), 126.6 (C−Hb), 127.6 (*ipso*-C), 129.6 (C−Ha), 134.2 (C-4), 152.8 (*para*-C), 154.1 (C-6), 161.2 (C-2), 171.8 (C-7a); MS (ES$^+$) *m/e* 385 (MNa^+, 100%), 269 (baseNa^+, 10%). Accurate mass: $C_{17}H_{15}N_2O_5ClNa$ requires 385.0567; found 385.0575. Found: C, 56.02; H, 4.39; N, 7.67. $C_{17}H_{15}ClN_2O_5$ requires: C, 56.29; H, 4.17, N, 7.72.

3-(2′-Deoxy-β-D-ribofuranosyl)-6-(4-*n*-bromophenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (4k). The procedure was carried out using 4-*n*-bromophenylacetylene (1.22 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-bromophenylacetylene)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (**4k**) (174 mg, 19%), after purification by column chromatography: ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.88 (1H, s, H-4), 7.78 (2H, H_a) − 7.66 (2H, H_b) (AB system, ^3J = 7.89 Hz, ^4J = 2.3 Hz), 7.34 (1H, s, H-5), 6.14 (1H, dd, ^3J = 6.17 Hz, H1′), 5.31 (1H, d, ^3J = 4.1 Hz, 3′-OH), 5.19 (1H, t, ^3J = 5.12 Hz, 5′-OH), 4.65 (1H, m, H-3′), 3.92 (1H, m, H-4), 3.67 (2H, m, H-5′), 2.48 and 2.19 (2H, m, 2-H′$_a$ and 2-H′$_b$); ^{13}CNMR (DMSO-d_6; 75 MHz) δ 41.6 (C-2′), 60.9 (c-5′), 69.8 (C-3′), 88.1, 88.5, (C-1′, C-4′), 100.9 (C-5), 107.0 (C-4a), 122.9 (C−Hb), 126.8 (*ipso*-C), 127.9 (C−Ha), 139.0 (C-4), 152.8 (*para*-C), 154.1 (C-6), 160.9 (C-2), 171.3 (C-7a); MS (ES$^+$) *m/e* 429 (MNa^+, 100%), 431 (MNa^+, 100%), 313 (baseNa^+, 25%), 315 (baseNa^+, 25%). Accurate mass: $C_{17}H_{15}N_2O_5{}^{79}BrNa$ requires 429.0062; found 429.0061; $C_{17}H_{15}N_2O_5{}^{81}BrNa$ requires 431.0042; found 431.0052. Found: C, 49.89; H, 3.88; N, 6.63. $C_{17}H_{15}BrN_2O_5{\cdot}0.5H_2O$ requires: C, 49.04; H, 3.88, N, 6.73.

Materials and Experimental Procedures: Virology. Cells. Human embryonic lung (HEL) fibroblasts and E6SM cells were grown in minimum essential medium (MEM) supplemented with 10% inactivated fetal calf serum (FCS), 1% L-glutamine and 0.3% sodium bicarbonate.

Viruses. The laboratory wild-type VZV strains OKA and YS, the thymidine kinase-deficient VZV strains 07-1 and YS-R, HSV-1 (KOS), HSV-2 (G), the thymidine kinase-deficient HSV-1 strains B-2006 and VMW 1837, cytomegalovirus strains Davis and AD-169, and vaccinia virus were used in the virus inhibition assays.

Antiviral Assays. Confluent HEL cells grown in 96-well microtiter plates were inoculated with VZV at an input of 20 PFU (plaque-forming units)/well or with CMV at an input of 100 PFU/well with HSV at 100 $CCID_{50}$ (50% cell culture infective doses)/well. After a 1−2-h incubation period, residual virus was removed and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 1% L-glutamine and 0.3% sodium bicarbonate) containing varying concentrations of the compounds. Antiviral activity was expressed as EC_{50} (50% effective concentration), or compound concentration required to reduce viral plaque formation (VZV after 5 days) or virus-induced cytopathicity (CMV after 7 days; HSV and VV after 3 days) by 50% compared to the untreated control.

Cytotoxicity Assays. Confluent monolayers of HEL cells as well as growing HEL cells in 96-well microtiter plates were treated with different concentrations of the experimental drugs. Cell cultures were incubated for 3 (growing cells) or 5 (confluent cells) days. At the indicated times, the cells were trypsinized and the cell number was determined using a Coulter counter. The 50% cytostatic concentration (CC_{50}) was defined as the compound concentration required to reduce the cell number by 50%.

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