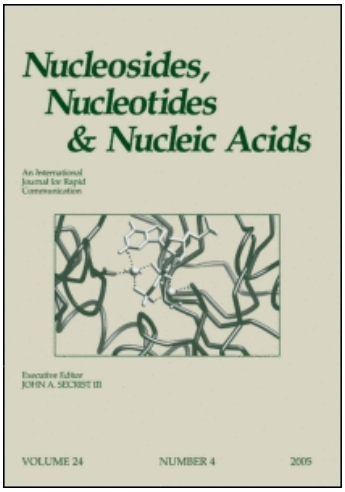

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NOVEL ARYL SUBSTITUTED BICYCLIC FURO NUCLEOSIDES AS EXTREMELY POTENT AND SELECTIVE ANTI-VZV AGENTS

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INTRODUCTION

Whilst recently pursuing the concept that judicious chemical phosphorylation of certain long chain 5-alkynyl-2′-deoxyuridines might confer on them some useful biological properties, by way of 'kinase by-pass'(1–5), we noted for the first time that a synthetic by-product in the preparation of the parent 5-alkynyl nucleosides displayed prominent potency and exclusive selectivity for VZV (Varicella-Zoster Virus) (6). This unusual compound represented the first in an entirely new family of potent antiviral nucleosides which may have considerable potential as new antiviral drugs with high inherent lipophilicity, and moreover whose intrinsic fluorescence, may lead to their wider utility in biochemistry.

The conventional synthesis of 5-alkynyl-2′-deoxyuridines involves the Pd-catalysed coupling of terminal alkynes with 5-iodo nucleoside (7–8). A generally unwanted by-product in such coupling reactions is the fluorescent furano pyrimidine (**1**) [Fig. 1] noted by a number of researchers as a slower spot on TLC (7–9).

Previously, the only well characterised examples of the fluorescent by-product in this 2′-deoxy series have been the parent compound (**1a**) (10–12) [Fig. 1] and

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the n-butyl homologue (7). Prior to our recent reports, biological evaluation of this cyclic by-product has not been extensive. However, the parent compound (**1a**) was noted to be inactive against herpes simplex viruses type 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV) and VZV (12). Ribo-(13) and xylofuranosyl (14) analogues of compound (**1**) have also been reported, although no antiviral activity noted. In 1999 we first described the synthesis and biological evaluation of novel compounds of type (**1**) bearing long alkyl side-chains (6). Such materials had not been previously reported and we noted them to have a surprising and unique biological profile. Thus, reaction of 5-iodo-2′-deoxyuridine with medium- to long chain alkyl acetylenes in DMF under co- catalysis of Pd and CuI (8–9) gave the corresponding 5-alkynyl-2′-deoxyuridines in moderate yield. These could be cyclised, either after purification, or in situ, by heating with CuI in methanol/triethylamine to give bicyclic fluorescent furo compounds such as **1b–d** [Fig. 1].

The target bicyclic systems were evaluated for their ability to inhibit the replication of HSV-1, HSV-2, VV, CMV and it being noted that long chain bicyclic nucleosides (**1b–d**) are potent and selective inhibitors of VZV, with activity crucially depending on the length of the alkyl side-chain; chain lengths of C8-C10 being optimal (6). The lead compounds display EC_{50} values against VZV of 7–20 nM (compounds **1b–d**), and are thus ca. 300-fold more potent against VZV than the reference compound acyclovir (**2**) and are ca 10-fold less potent than BVDU (**3**). Furthermore, compounds (**1b–d**) show little or no cytotoxicity and thus the lead compounds display extremely high values of SI (selectivity index = ratio of MCC to EC50); in the region of >5,000. The compounds emerge as unusual antiviral nucleosides in respect of this unique requirement for the long alkyl chain. Furthermore, this structural feature has a significant impact on the physical properties of these compounds; their water solubility is in general low, whilst their lipophilicity is rather high. Calculated octanol-water logP [ClogP] values for lead structures **1b–d** are in the range of 2.5 to 3.5 (15).

This may have important repercussions for membrane, skin, and blood brain barrier penetration by the lead compounds, an early assessment being that topical application may be a promising approach for these new agents.

The precise mechanism of action of these novel compounds remains unclear. However, in keeping with previous antiviral nucleosides we would anticipate the necessity for the VZV thymidine kinase-mediated activation to their 5′-phosphate forms. This would appear to be supported the data from thymidine kinase-deficient VZV assays (6). Thus, the complete absence of antiviral activity in the VZV TK^- systems strongly implicates TK-mediated phosphorylation of compounds **1b–d** to give bio-active nucleotides. Whilst activity as the corresponding 5′-triphosphate would appear most likely, present data cannot exclude activity as the mono- or diphosphate.

The novelty of the lead structures presents much scope for structural elucidation and further exploitation of this series. Given the apparent necessity for the long alkyl chain we sought to retain this moiety, but to substitute at the terminus to probe SARs in this region.

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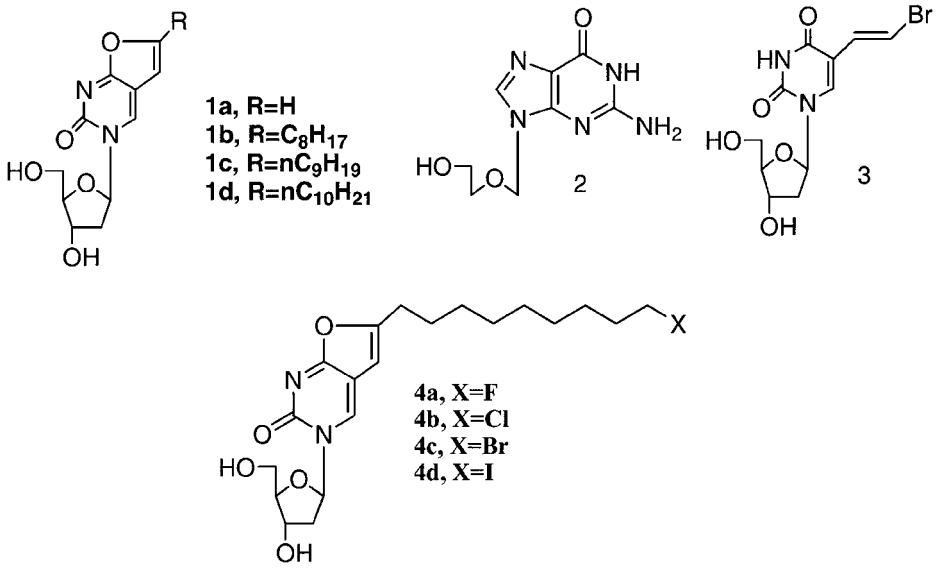

Figure 1.

Thus, as series of ω-halogenated nonyl analogues were prepared by similar chemistry to that described above. The preparation of the appropriate terminal alkyne precursors was achieved by converting 10-undecyn-1-ol into the desired halides. All of these terminal alkynes were prepared in quantitative yields and were successfully coupled to the 5-iodo-2′-deoxyuridine, leading to the corresponding ω-halo-5-alkynyl nucleosides which, after the CuI-catalysed cyclisation step, gave the desired nucleosides **4a–d** (Fig. 1) (16). Target compounds **4a–d** did not prove significantly different from the corresponding lead compounds **1b–d** in their anti-VZV potency. No cytotoxicity was observed for these compounds at the highest concentration tested, and as all the other compounds of this class, the nucleosides **4a–d** displayed no significant activity against HSV-1, HSV-2, or CMV. Also, as with the lead series **1b–d**, the halo analogues **4a–d** displayed no significant activity against thymidine kinase deficient-VZV strains, confirming their dependence on VZV thymidine kinase-mediated activation, for their biological activity. Given the apparent correlation noted above between ClogP and antiviral potency for the parent alkyl systems, it is interesting to note that the introduction of the terminal halogen atom in **4a–d** does not significantly perturb the ClogP value, with the range being from 2.3 (F) to 3.2 (I), and thus roughly within the apparent optimal values of 2.5–3.5 noted above.

RESULTS

In order to retain high lipophilicity whilst introducing some conformational constraints in the alkyl side chain of the abovementioned analogues we sought the

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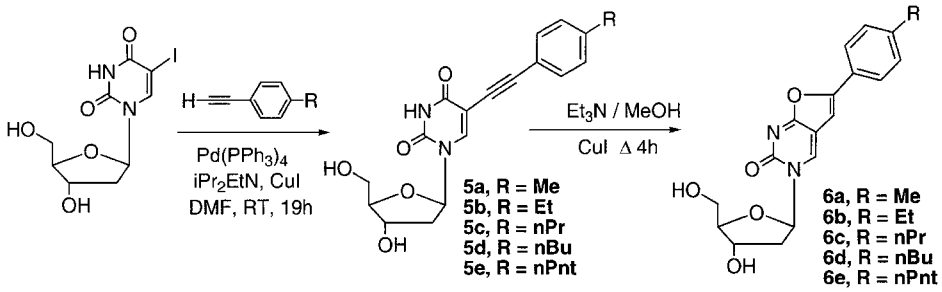

Scheme 1.

synthesis and evaluation of some p-alkylaryl compounds. Using entirely analogous synthetic procedures to those outlined above we prepared intermediate alkynes **5a–e** which were converted smoothly to target compounds **6a–e** (Scheme 1). These analogues were then evaluated for anti-VZV activity, with data outlined in Table 1 (17).

The data in Table 1 clearly show the exquisite antiviral potencies of these alkylaryl systems; the pentyl analogue (**6e**) was active at 0.1–0.5 nanomolar concentration, and thus being ca. 100-times more potent than the alkyl leads and ca 10,000-times more potent than acyclovir, the current drug of choice for the treatment of VZV infection. Indeed, **6e** is ca 10–50 fold more potent than BVDU (**3**), itself one of the most potent anti-VZV compounds reported to date (18). It is interesting to note a similar apparent dependence on ClogP for antiviral potency, as noted above, the optimal ClogP value in this series being ca. 3.0. However, optimal ClogP values here were achieved at C4–C6 (versus C8–C10 in the alkyl series **1b–d**), and led to markedly increased potency compared to compounds of corresponding ClogP.

Table 1. Anti-VZV Activity and Cytotoxicity for Bicyclic Pyrimidines **6a–d** and Reference Compounds **2–3** and **1b–d**

	R	EC_{50} VZV OKA (μM)	EC_{50} VZV YS (μM)	EC_{50} VZV TK$^-$ 07 (μM)	EC_{50} VZV TK$^-$ YS-R (μM)	MCC (μM)	CC_{50} (μM)
6a	Me	0.06	0.06	103	>200	>200	>200
6b	Et	0.09	0.07	>50	>50	200	123
6c	Pr	0.010	0.008	>50	>20	≥50	188
6d	Bu	0.002	0.0005	≥20	>20	≥20	>200
6e	Pnt	0.0003	0.0001	>20	>5	≥20	>200
2 (Acyclovir)	–	2.9	1	74	125	>200	>200
3 (BVDU)	–	0.003	0.003	>150	>150	>150	>400
1b	Oct	0.008	0.024	>50	>50	>50	≥50
1c	Non	0.02	0.02	>200	>200	≥200	>200
1d	Dec	0.015	0.008	>50	>50	>50	>50

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CONCLUSIONS

The exquisite potencies and high slectivities of the p-alkylaryl series of furo pyrimidine nucleosides, coupled with their ease of synthesis, facile detection via fluoresecence, and promising physical properties lead us to conclude that they may represent promising clinical candidates for early evaluation as new treatments for herpes zoster. In this regard, the recent disclosure of a small animal model for VZV infection may be particularly useful (19).

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 aluminium 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 nm 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 mm, Phase Sep, UK). Samples were applied as a concentrated solution, in the same eluent, or pre-absorbed 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 MHz and 75 MHz respectively) and auto-calibrated to the deuterated solvent reference peak. All 13C-NMR spectra were proton decoupled. Mass spectra were performed by the service at the University of Birmingham, UK. Elemental Analyses were performed by the service at the University of Wales, Swansea, UK. All solvents used were anhydrous, and used as supplied from Aldrich. All nucleosides and solid reagents were dried whilst 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-Propyl-phenylacetylene)-2′-deoxyuridine (5c). To a stirred solution of 5-iodo-2′-deoxyuridine (800 mg, 2.26 mmol) in anhydrous dimethylformamide (8 ml), was added diisopropylethylamine (584 mg, 0.8 ml, 4.52 mmol), 4-n-propyl-phenylacetylene (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 hours, 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 minutes. The resin was filtered and washed with methanol, and the combined filtrate was evaporated

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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-propyl-phenylacetylene)-2′-deoxyuridine (**5c**) as the pure product (350 mg, 41%): ^{1}H- NMR (d_6-DMSO; 300 MHz); 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 (d_6-DMSO; 75 MHz): 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 (6c). To a solution of 5-(4-n-propyl-phenylacetylene)-2′-deoxyuridine (**5c**) (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 hours. 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 recrystallised from methanol to give pure 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-n-propylphenylacetylene)-2,3-dihydrofuro- [2,3-d]pyrimidin-2-one (**6c**) (86 mg, 43%): ^{1}H-NMR (d_6-DMSO; 300 MHz); 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 (d_6-DMSO; 75 MHz): 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%. $C_{20}H_{22}N_2O_5$. H_2O requires: C, 61.85%; H, 6.23%; N, 7.21%.

General Procedure for the 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 (800 mg, 2.26 mmol) in anhydrous dimethylformamide (8 ml), was added diisopropylethylamine (584 mg, 0.8 ml, 4.52 mmol), the 4-n-alkyl-phenylacetylene (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 hours, at room temperature, under a nitrogen atmosphere, after which time tlc (ethyl acetate/methanol 9:1), showed complete conversion of

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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 hours. 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 minutes. 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-(4-n-methylphenyl)-2,3-dihydrofuro-[2,3-d]Pyrimidin-2-one(6a). 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-methyl)-phenylacetylene-2,3-dihydrofuro-[2,3-d]pyrimidin-2-one (**6a**) (131 mg, 17%), after purification by column chromatography.

^{1}H-NMR (d_6-DMSO; 300 MHz); 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 (d_6- DMSO; 75 MHz): 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 (6b). The procedure was carried out using 4-n-ethyl-phenylacetylene (0.885 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-n-ethyl)-phenylace-tylene-2,3-dihydrofuro-[2,3-d]pyrimidin- 2-one (**6b**) (115 mg, 14%), after purification by column chromatography.

^{1}H-NMR (d_6-DMSO; 300 MHz); 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 (d_6-DMSO; 75 MHz): 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%. $C_{19}H_{20}N_2O_5 . 0.5\ H_2O$ requires: C, 62.46%; H, 5.79%; N, 7.67%.

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3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-n-butylphenyl)-2,3-dihydrofuro-[2, 3-d]Pyrimidin-2-one (6d). The procedure was carried out using 4-n-butyl-phenylacetylene (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 (**6d**) (140 mg, 16%), after purification by column chromatography.

^{1}H-NMR (d_d-DMSO; 300 MHz); 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 (d_6-DMSO; 75 MHz): 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 (6e). The procedure was carried out using 4-n-pentyl-phenyllacetylene (1.15 g, 6.76 mmol), which gave 3-(2′-deoxy-β-D-ribofuranosyl)-6-(4-n-pentylphenylace-tylene)-2,3-dihydrofuro-[2,3-d]pyrimidin-2-one (**6e**) (137 mg, 15%), after purification by column chromatography.

^{1}H-NMR (d_6-DMSO; 300 MHz); 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 (d_6-DMSO; 75 MHz): 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%. $C_{22}H_{26}N_2O_5$. 1/2 H_2O requires: C, 64.85%; H, 6.68%; N, 6.87%.

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

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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) per well or with CMV at an input of 100 PFU per well with HSV at 100 $CCID_{50}$ (50% cell culture infective doses) per well. After a 1 to 2-hour 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 after 5 days (VZV) or virus-induced cytopathicity (CMV after 7 days and HSV, 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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