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Bicyclic Nucleoside Inhibitors of Varicella-Zoster Virus (VZV): The Effect of a Terminal Halogen Substitution in the Side-Chain

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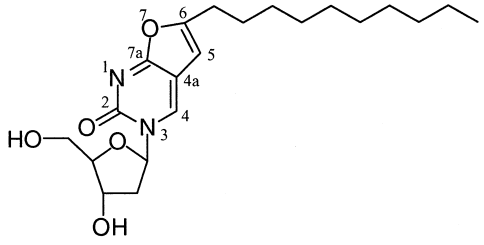
Abstract—Preliminary SAR studies on the side chain of a new class of antiviral nucleosides have shown that terminal substitution in the side-chain, with a halogen atom, lead to potent and highly specific anti-VZV agents. © 2000 Elsevier Science Ltd. All rights reserved.

We have recently reported[1] the discovery of an entirely new category of potent anti-VZV agents based on novel deoxynucleoside analogues with an unusual fluorescent bicyclic base bearing a long alkyl side-chain, with an optimum length of C8–C10 for antiviral activity (**1**). The role of this peculiar structural feature for the antiviral activity is still not clear and our groups are carrying out several structure–activity relationship studies, which involve modifications of the lipophilic chain of this new class of anti-VZV nucleosides.



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The fact that a minimum length of eight carbon atoms in the side chain is fundamental for potent anti-VZV activity,[1] suggests the presence of a lipophilic pocket in relation to that position, potentially either in the thymidine kinase or the DNA polymerase of VZV. In order to probe this, we planned to prepare several analogues bearing different groups at the end of the chain. We report here the synthesis and the biological evaluation of several new compounds, which contain a halogen atom in the terminal position of the side chain. All the compounds of this new class of anti-VZV nucleosides were prepared by a Pd-catalysed coupling reaction of 5-iodo-2′-deoxyuridine with terminal alkynes, followed by treatment of the 5-alkynyl nucleosides thus obtained, with copper (I) iodide, leading to the desired fluorescent derivatives.[1,2] These two steps (coupling and cyclisation) can be conducted in one flask without isolating the 5-alkynyl derivatives but, in the present case, the intermediates were isolated and biologically evaluated.

The preparation of the appropriate terminal alkyne precursors was achieved by converting 10-undecyn-1-ol (**2**) into the desired halides **3a–d** (Scheme 1). The fluoroalkyne **3a** was obtained by treatment of the alcohol **2** with DAST,[3] while **3b** was prepared by reaction with thionyl chloride, in the presence of pyridine.[4]

Carbon tetrabromide and triphenylphosphine[5] were used to obtain the bromo-alkyne **3c**, and the reaction of **2** with iodine, in the presence of triphenylphosphine and imidazole,[6] gave the iodo derivative **3d**. All of these terminal alkynes (**3a–d**) were prepared in quantitative yields and were successfully coupled to 5-iodo-2′-deoxyuridine, leading to the 5-alkynyl nucleosides **4a–d**[7] which, after the cyclisation step, gave the desired nucleosides **5a–d**[8,9] (Scheme 2). Compounds **4a–d** and **5a–d** were characterized by high-field heteronuclear NMR and mass spectrometry.

Compounds **4a–d** and **5a–d** were evaluated as inhibitors of a variety of herpes viruses in vitro, including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus, cytomegalovirus and HIV-1. Data

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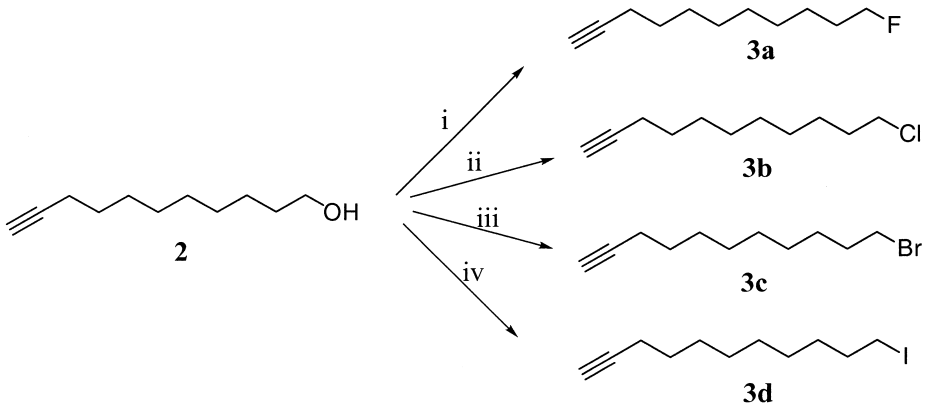
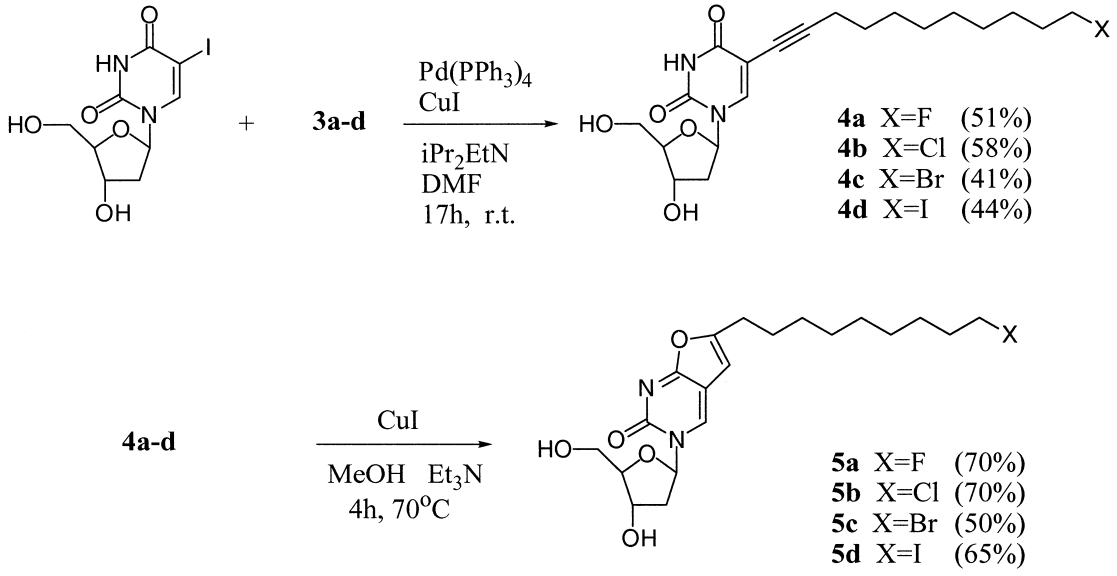
for VZV in human embryonic lung (HEL) cells are shown in Table 1. The antiviral activity (EC_{50}) was measured as the effective concentration required to reduce virus plaque formation by 50%. In Table 1 the activity of these new nucleosides is compared with that of the lead compound **1** and the reference compound acyclovir (ACV).

The target compounds **5a–d** did not prove significantly different from the lead compound **1** in their anti-VZV potency. No cytotoxicity was observed for these compounds at the highest concentration tested, while the 5-alkynyl nucleosides **4a–d** showed some cytotoxicity, with a markedly lower anti-VZV activity, but still in the same order as that of ACV. It cannot be excluded that some



Scheme 1. (i) DAST, DCM; −78 °C to rt 1 h; (ii) $SOCl_2$, pyridine, CH_2Cl_2; rt 1 h; (iii) CBr_4, PPh_3, Et_2O; rt 2 h; (iv) I_2, PPh_3, imidazole, THF; rt 1 h.



Scheme 2.

Table 1.

Compound	EC_{50} (μM)[a] VZV YS Strain	EC_{50} (μM)[a] VZV OKA Strain	EC_{50} (μM)[a] TK^- VZV[d] 07/1 Strain	EC_{50} (μM)[a] TK^- VZV[d] YS/R Strain	MCC (μM)[b]	CC_{50} (μM)[c]
4a	22	26	33	23	200	116
4b	2.8	3.6	10	9.6	200	74
4c	0.8	1.1	16	2.5	50	97
4d	6	13	36	31	>50	68
5a	0.014	0.022	>20	>20	>50	200
5b	0.012	0.007	15	13	>200	200
5c	0.031	0.026	>50	50	>200	>50
5d	0.034	0.061	>50	>50	50	>200
1	0.008	0.015	>50	>50	>50	>50
ACV	1.0	2.9	74	125	>200	>200

[a]EC_{50}, 50% effective concentration, required to reduce virus plaque formation 50%.
[b]MCC, minimal cytotoxic concentration, required to alter microscopically detectable cell morphology.
[c]CC_{50}, 50% cytotoxic concentration, required to inhibit Hel cell growth by 50%
[d]TK^-, thymidine kinase-deficient.

of the observed activity of the alkynyl precursors arises from in situ cyclisation to the corresponding bicyclic system. As all the other compounds of this class,[1] the nucleosides **5a**–**d** displayed no significant activity against thymidine kinase deficient-VZV strains assays confirming their dependence on VZV thymidine kinase-mediated activation, for their biological activity, and did not show activity against HSV-1, HSV-2, CMV or HIV-1 (data not shown). The results obtained with these new nucleosides are extremely encouraging and more extensive work is currently in progress in our laboratories to delineate the effect of side-chain modifications.

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4. Preparation of **3b**: To a stirred solution 10-undecyn-1-ol (2.0 g, 11.88 mmol) in dichloromethane (10 mL) was added thionyl chloride (20.7 mmol, 2.46 g, 1.5 mL) and pyridine (11.88 mmol, 0.94 g, 0.96 mL). The reaction was then left stirred for 1 h at room temperature. The solvent was evaporated, water was added to the reaction mixture and then extracted with dichloromethane. The organic layer was dried on $MgSO_4$, filtered and the solvent removed in vacuo, yielding to the product **2b** as a colourless oil in quantitative yield.
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7. Preparation of **4b**: To a stirred solution of 5-iodo-2′-deoxyuridine (800 mg, 2.26 mmol) in dry dimethylformamide (8 mL), at room temperature under a nitrogen atmosphere, was added diisopropylethylamine (584 mg, 0.80 mL, 4.52 mmol), **2b** (1.26 g, 6.78 mmol), *tetrakis*(triphenylphosphine)palladium(0) (261 mg, 0.226 mmol) and copper (I) iodide (86 mg, 0.452 mmol). The reaction mixture was stirred at room temperature for 19 h, after which time the solvent was removed in vacuo. The resulting residue was dissolved in dichloromethane:methanol (1:1) (6 mL) and an excess of Amberlite IRA-400 (HCO_3^- form) was added and the mixture was stirred for 30 min. The resin was then filtered, washed with methanol and the combined filtrate was evaporated to dryness. The crude product was purified by silica gel column chromatography using ethyl acetate as eluent. The appropriate fractions were combined and the solvent removed in vacuo to yield **4b** as a white solid (540 mg, 58% yield).
8. Preparation of **5b**: to a stirred solution of **4b** (200 mg) in methanol:triethylamine (7:3) (20 mL), at room temperature under a nitrogen atmosphere, was added copper (I) iodide (20 mg). The reaction mixture was then heated to reflux and stirred for 4 h. The solvent was removed in vacuo and the crude product purified by silica column chromatography, using an initial eluent of ethyl acetate, followed by an eluent of ethyl acetate:methanol (9:1). The appropriate fractions were combined and the solvent removed in vacuo yielding the pure product **5b** (140 mg, 70% yield).
9. Significant spectroscopic data for **5b**: ^{1}H NMR (d_6-DMSO; 300 MHz): 8.68 (1H, s, H-4), 6.44 (1H, s, H-5), 6.17 (1H, dd, ^3J = 6.1 Hz, H-1′), 5.29 (1H, d, ^3J = 4.3 Hz, 3′-OH), 5.13 (1H, t, ^3J = 5.3 Hz, 5′-OH), 4.24 (1H, m, H-3′), 3.92 (1H, m, H-4′), 3.65 (4H, m, H-5′, $C\underline{H}_2$Cl), 2.65 (2H, t, ^3J = 7.2 Hz, base-$C\underline{H}_2$), 2.38, 2.05 (2H, m, H-2′), 1.74–1.30 (14H, m, 7×CH_2). ^{13}C NMR (d_6-DMSO; 75 MHz): 26.6, 26.7, 27.7, 28.5, 28.7, 28.9, 29.1, 32.4 (8×CH_2), 41.6 (C-2′), 45.8 ($\underline{C}H_2$Cl), 61.1 (C-5′), 70.0 (C-3′), 87.7, 88.4 (C-1′, C-4′), 100.1 (C-5), 106.7 (C-4a), 137.1 (C-4), 154.1 (C-2), 158.7 (C-6), 171.5 (C-7a). Mass spectrum (ES–MS (+ve) MNOBA matrix); *m*/*z* 435 (10%, $[MNa]^+$), 413 (50%, $[MH]^+$), 297 (100%, $[baseH]^+$). FAB *m*/*e* 435.1680 (MNa^+ $C_{20}H_{23}Cl\,N_2O_5Na$ requires 435.1663).