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Bicyclic anti-VZV nucleosides: thieno analogues bearing an alkylphenyl side chain have reduced antiviral activity

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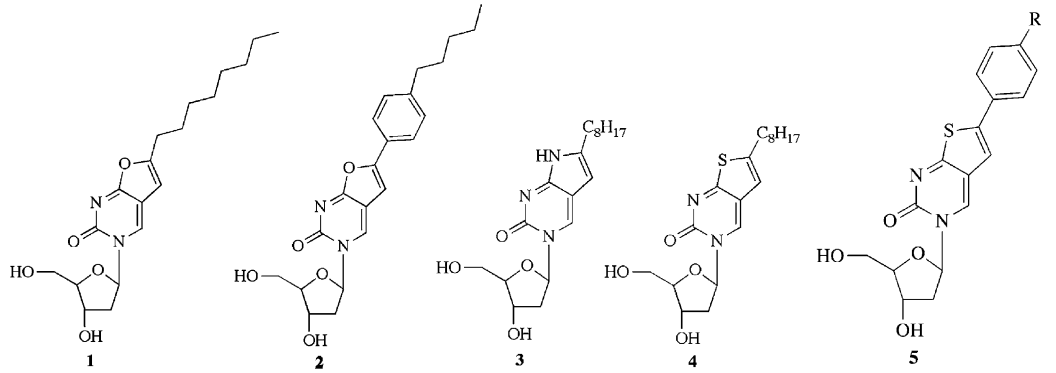
Abstract—Thieno analogues of the potent and selective furo-pyrimidine anti-VZV nucleoside family bearing a *p*-alkylphenyl side chain have been synthesised and tested for their antiviral activity against Varicella–Zoster virus (VZV). While the alkyl chain analogues were shown to retain full antiviral activity against VZV, these new analogues did not when compared to their furo parent nucleosides.

1. Introduction

Following the discovery of the 2,3-dihydrofuro[2,3-*d*]pyrimidine-2-one nucleosides (BCNAs) as a new class of potent anti-Varicella–Zoster virus (VZV) nucleosides[1] (**1**), a number of studies have been made to determine the structure–activity relationship (SAR) of these compounds and to increase the antiviral activity.[2] Investigations into modifications on the side chain and the introduction of a *p*-alkylphenyl moiety led to the discovery of the most potent BCNA to date, bearing a *p*-pentylphenyl side chain[3] (**2**). Other investigations on the bicyclic base moiety and replacement of oxygen with nitrogen and sulfur on the alkyl parent BCNAs, found that while the *pyrrolo* analogues (**3**) showed a marked decrease in potency,[4] the *thieno* analogues (**4**) retained full activity against VZV.[5] To further continue in this investigation we sought to synthesise *thieno* analogues of the most potent parent nucleosides bearing a *p*-alkylphenyl side chain (**5**). These analogues were synthesised via the corresponding 5-alkynyl nucleosides (**6a**–**g**) prepared as previously reported[1] (Scheme 1). The free hydroxyl groups are first protected with chlorotrimethylsilane in the presence of triethylamine, followed by



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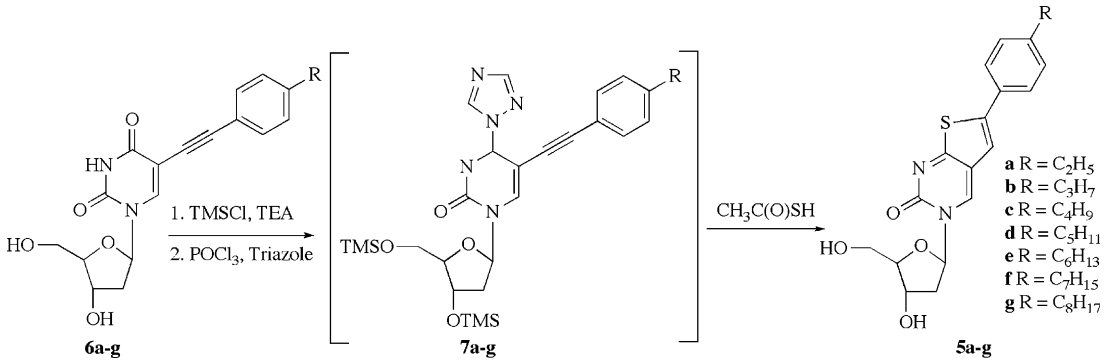

Scheme 1.

addition of phosphorus oxychloride and triazole to give the intermediates (**7a–g**), which were isolated following extraction with dichloromethane and used without further purification. As reported previously,[5] the desired products (**5a–g**) were obtained after an unexpected cyclisation, following treatment with thiol acetic acid.

2. Results and discussion

In Table 1, the activity of these new nucleosides is compared with that of the lead compound **2** and the reference compound acyclovir (ACV).

The target compounds (**5a–g**) were found to be active against VZV and more active than the current anti-VZV treatment of choice acyclovir. However, unlike the alkyl thieno analogues they did not retain the full antiviral activity of their furo parent nucleosides. Moreover, while the potency of the furo alkyl BCNAs was superseded with the introduction of an alkylphenyl side chain, this was not the case for the thieno analogues. The alkylphenyl thieno analogues have shown a reduction in activity of ca. 5-fold, when compared to their alkyl analogues. As with all the compounds of this class these analogues did not show any significant activity against thymidine kinase-deficient VZV, once again confirming their dependence on VZV thymidine kinase for their biological activity. The results obtained from these new analogues follow the correlation between chain length on the bicyclic base and antiviral activity, which seem typical of the analogues in this class (optimal chain length C5).[2]

3. Conclusion

The results reported herein suggest that while some heteroatom substitutions can be tolerated for the alkyl parent BCNAs, this is not the case for the *p*-alkylphenyl analogues.

4. Experimental

Preparation of **5e**: To a solution of 5-(4-*n*-hexylphenylacetylene)-2′-deoxyuridine (300 mg, 0.73 mmol) in acetonitrile (10.0 mL), TMSCl (0. 46 mL, 3.65 mmol) and triethylamine (1.15 mL) were added. The reaction was stirred for 2 h at room temperature, under a nitrogen atmosphere. $POCl_3$ (0.14 mL, 1.46 mmol), and triazole (462 mg, 6.69 mmol) were added at 0 °C, and the reaction was left stirring for 5 h under nitrogen atmosphere

Table 1. Antiviral and cytostatic activity of test compounds

Compound	EC_{50} (μM)[a]			MCC (μM)[b]	CC_{50} (μM)[c]
	VZV (YS)	VZV (OKA)	VZV TK-(07/1)[d]		
5a	0.15	0.2	20	>200	>200
5b	0.06	0.09	>16	80	>200
5c	0.020	0.028	>3.2	50	>200
5d	0.014	0.025	20	50	>200
5e	0.043	0.08	>50	200	>200
5f	0.18	0.27	>20	50	>200
5g	3.4	—	>20	⩾20	>200
2	0.0003	0.0001	>5	⩾20	>200
4	0.005	0.002	>5	20	53
ACV	1.5 ± 0.6	1.1 ± 0.1	40 ± 5	>200	>400

[a] EC_{50}, 50% effective concentration, required to reduce viral plaque formation by 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.

at 0 °C. $NaHCO_3$ saturated solution was added and the mixture was extracted with dichloromethane. The organic layer was dried on $MgSO_4$ and the solvent was evaporated. The residue was dissolved in acetonitrile (10.0 mL) and thiol acetic acid (0.19 mL) was added. The mixture was stirred for 19 h at room temperature under a nitrogen atmosphere. After which time, the precipitate was washed with CH_2Cl_2 and MeOH, yielding 180 mg (58%) of 6-(4-*n*-hexylphenyl)-3-(2′-deoxy-β-D-ribofuranosyl)-2,3-dihydrothieno[2,3-*d*]pyrimidin-2-one as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.98 (s, 1H, H-4); 7.60 (d, 2H, $J = 8.1$ Hz, Ha); 7.50 (s, 1H, H-5); 7.30 (d, 2H, $J = 8.1$ Hz, Hb); 6.13 (dd, 1H, $J_1 = 5.9$, $J_2 = 5.9$ Hz, H-1′); 5.33 (d, 1H, $J =$ 4.3 Hz, 3′-OH); 5.19 (t, 1H, $J = 5.1$ Hz, 5′-OH); 4.25 (m, 1H, H-3′); 3.97 (m, 1H, H-4′); 3.67 (m, 2H, H-5′); 2.61 (t, 2H, $J = 7.7$ Hz, α-CH_2); 2.47 and 2.13 (m, 2H, H-2′); 1.58 (m, 2H, β-CH_2); 1.28 (m, 6H, 3×CH_2); 0.88 (t, 3H, $J = 6.4$ Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 180.0 (C-7a), 154.2 (C-2), 146.0 (C-6), 142.0 (C-4), 140.4 (*para*-C), 132.6 (*ipso*-C), 131.7 (C-Hb), 128.2 (C-Ha), 121.6 (C-4a), 117.5 (C-5), 90.9 (C-4′), 90.4 (C-1′), 72.0 (C-3′), 63.2 (C-5′), 43.7 (C-2′), 37.3 (α-CH_2), 33.6 (CH_2), 33.3 (β-CH_2), 30.8, 24.6 (2×CH_2), 16.5 (CH_3). FABMS *m*/*e*, 451.1653, ($MNa^+C_{23}H_{28}N_2O_4NaS$ requires 451.1667).

4.1. Antiviral assay

Confluent Hel cells grown in 96-well microtiter plates were inoculated with VZV at an input of 20PFU (plaque forming units) per 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-Glutamine (1%) 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 by 50% compared to the untreated control.

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