Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2507–2510

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Bicyclic Anti-VZV Nucleosides: *Thieno* Analogues Retain Full Antiviral Activity

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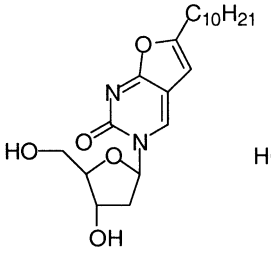
Received 25 May 2001; revised 3 July 2001; accepted 5 July 2001

Abstract—Thieno analogues of the potent and selective furo-pyrimidine anti-VZV nucleoside family are herein reported. The compounds retain full antiviral potency in comparison to the *furo* parent.

Since our discovery of the 2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one nucleosides (**1**) as a new class of anti-Varicella–Zoster virus (VZV) nucleosides,[1] several studies have been made in our group in order to investigate the structure–activity relationships of these compounds and increase the antiviral activity.[2] We have studied modifications in the side chain,[3–5] in the sugar moiety[6] and in the bicyclic base.[7] In particular, the *pyrrolo* analogues (**2**) have shown a marked decrease in potency.[7]



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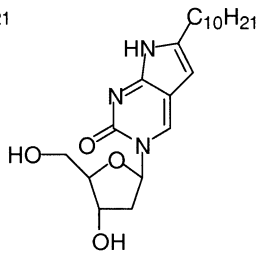

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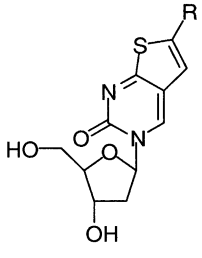
As a continuation of that work, we here report the synthesis and the biological evaluation of some *thieno* analogues, where the oxygen atom in the 7-position of **1** is replaced by a sulfur atom (**3**).

Several synthetic methods have been tried in order to achieve these modified nucleosides,[8] one route is summarised in Scheme 1.[9] The starting 5-alkynyl deoxyuridines (**4a–e**) were prepared as reported previously.[1] The first step is the protection of the free nucleosides by chlorotrimethylsilane in the presence of triethylamine. After 2 h, TLC showed the complete conversion of the starting material. Phosphorous oxychloride and triazole were added at 0 °C and the reaction was stirred for a further 5 h. After this time, the solution was treated with a saturated solution of sodium bicarbonate and then extracted with dichloromethane. TLC revealed the presence of an intense fluorescent spot, corresponding to compounds **5a–e**. The gum obtained after the evaporation of the organic solvent was dissolved in acetonitrile and treated with thiolacetic acid.



3

The mixture was stirred for 19 h at room temperature. Visible on TLC was an intense fluorescent slower running spot. After purification by silica column, ^{1}H NMR, ^{13}C NMR and mass spectrometry revealed that the molecule was the unprotected *thieno* derivative (**3a–e**).[10]

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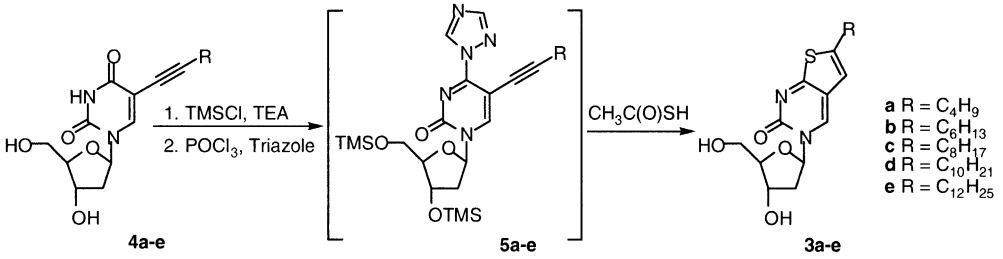
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00471-1



Scheme 1.

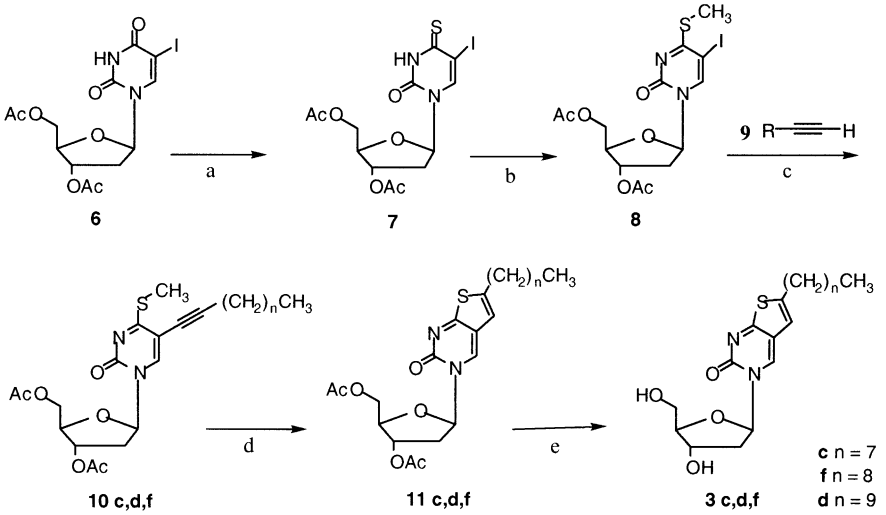
This result was unexpected; in all the cases, no uncyclised compound was isolated. At the present moment, there is no clear explanation for the cyclisation of the 5-alkynyl deoxythiouridine that occurred in an acidic media and without any copper catalyst, while the parent nucleosides need basic conditions and the presence of copper (I) in order to cyclise.[1]

To reach the target molecules, another synthetic route was explored starting from, 3′,5′-di-*O*-acetyl-5-iodo-2′-deoxyuridine **6** (Scheme 2).[11] Thus, **6** was converted into the corresponding 4-thio derivative **7** upon treatment with phosphorous pentasulfide. Subsequently, this compound was methylated with methyl iodide in the presence of triethylamine to give the 4-methylthio derivative **8**. We were pleased to find that this 5-iodopyrimidine nucleoside, in contrast to its precursor **7**, could undergo a Pd-catalysed coupling reaction with a number of terminal alkynes **9** (R = *n*-C_8H_{17}, *n*-C_9H_{19} and *n*-$C_{10}H_{21}$) to give the desired derivatives **10** in moderate yields (70–75%).[12] Conventional removal of the *S*-methyl group of **10** by treatment of a solution of **10** in DMF with an excess of sodium hydrogen sulfide gave directly the cyclised derivative **11**.[13] In this instance, cyclisation of the presumed 4-thio intermediate occurred in basic medium as observed with the oxygen analogue, albeit in the absence of copper iodide. Finally, the remaining *O*-acetyl groups of **11** were conveniently eliminated by treament of the protected nucleosides **11** with a methanolic solution of aqueous ammonia to give analogues **3**.[14]

Compounds **3a**–**f** 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), and cytomegalovirus. Data 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-induced 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 **3a**–**f** proved to be highly active against VZV, with a biological activity of compound **3c** that was superior to the lead compound **1**. No cytotoxicity was observed for the short chain compounds (**3a**–**b**) at the highest concentration tested (50–200 μM) whilst the longer chain analogues were cytotoxic at 5 to 50 μM (**3c**–**e**). As with all the other compounds of this class,[2] the nucleosides **3a**–**e** displayed no significant activity against thymidine kinase-deficient VZV strains confirming their dependence on VZV thymidine kinase-mediated activation for their biological activity. They also did not show activity against HSV-1, HSV-2 and CMV (data not shown). It is worth noting that the



Scheme 2. (a) P_2S_5, dioxane, 120 °C, 1 h, 80%; (b) MeI, TEA, DCM, rt, 1 h, 93%; (c) **9**, TEA, CuI, $Pd(PPh_3)_2Cl_2$, dioxane, 80 °C, 1 h, 70–75%; (d) excess NaSH, DMF, rt, 18 h, 75%; (e) NH_4OH, MeOH, rt, 18 h, 85%.

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)	TK^- VZV[d] (07/1)	TK^- VZV[d] (YS/R)		
3a	0.15±0.07	0.16±0.07	≥200	153±9	≥100	>200
3b	0.14±0.06	0.14±0.06	>50	≥50	125	>200
3c	0.005±0.003	0.002±0.002	≥5	>5	20±0.0	53
3f	0.01	0.01	>20	>20	>20	>20
3d	0.06	0.03	>5	>5	20	54
3e	0.3±0.1	0.2±0.05	>5	>5	12±12	49
1	0.008	0.015	>50	>50	>50	>50
ACV	1.5±0.6	1.1±0.1	40±5	44±3	>200	>400

[a]EC_{50}, 50% effective concentration, required to reduce virus 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.

results obtained with these new nucleosides follow the correlation between chain length and antiviral activity, typical of the analogues of this class (optimal activity at a chain length of 8–10 carbon atoms).[2] Finally, the potent activity herein reported may suggest the possibility of other heteroatom substitutions in the heterocycle.[7]

Acknowledgements

The authors are grateful to Mrs. Ann Absillis, Mrs. Anita Camps, Mrs. Frieda De Meyer, Miss Lies Vandenheurck and Mrs. Anita Van Lierde for excellent technical assistance. The research was supported by grants from the Belgian Fonds voor Wetenschappelijk Onderzoek Vlaanderen and the Belgian Geconcerteerde Onderzoeksacties Vlaamse Gemeenschap. We also thank Helen Murphy for excellent secretarial assistance.

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10. Preparation of **3c**: To a solution of 5-decynyl-2′-deoxyuridine (**4c**) (144 mg, 0.38 mmol) in acetonitrile (5 mL), TMSCl (1.91 mmol, 207.86 mg, 0.24 mL) and triethylamine (0.6 mL) were added. The reaction was stirred for 2 h at room temperature, under a nitrogen atmosphere. $POCl_3$ (0.76 mmol, 117 mg, 0.071 mL) and triazole (3.48 mmol, 240 mg) were added at 0 °C, and the reaction was left stirring for 5 h under a nitrogen atmosphere at 0 °C. $NaHCO_3$ satd. soln. 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 (5 mL) and thiolacetic acid (0.1 mL) was added. The mixture was stirred for 19 h at room temperature under a nitrogen atmosphere. The solvent was evaporated and the crude was 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 (64 mg, 44%). ^1H NMR (DMSO-d_6; 300 MHz) δ (ppm): 8.84 (1H, s, H-4), 6.79 (1H, s, H-5), 6.10 (1H, dd, ^3J=6.07 Hz, H-1′), 5.30 (1H, d, ^3J=4.0 Hz, 3′-OH), 5.15 (1H, t, ^3J=4.3 Hz, 5′-OH), 4.22 (1H, m, H-3′), 3.92 (1H, m, H-4′), 3.62 (2H, m, H-5′), 2.72 (2H, t, ^3J=7.3 Hz, α-CH_2), 2.41 and 2.06 (2H, m, 2-H′a and 2-H′b), 1.57 (2H, m, CH_2), 1.22 (10H, m, 5×CH_2), 0.83 (3H, t, ^3J=7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6; 75 MHz) δ (ppm): 14.3 (CH_3), 22.4, 28.6, 28.9, 29.0, 29.9, 30.4, 31.6 (7×CH_2), 41.5 (C-2′), 61.0 (C-5′), 69.8 (C-3′), 88.0, 88.7 (C-1′ and C-4′), 116.6 (C-5), 118.5 (C-4a), 138.6 (C-4), 141.8 (C-6), 152.0 (C-2), 178.4 (C-7a). Mass spectrum [ES-MS (+ve)]; *m/z* 403 (100%, $[M+Na]^+$), 287 (80%, $[base+Na]^+$). FAB *m/e* 403.1663 (MNa^+ $C_{19}H_{28}N_2O_4SNa$ requires 403.1667).
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12. Preparation of **10f**: To a solution of 3′,5′-di-*O*-acetyl-5-iodo-4-thiomethyl-2′-deoxyuridine **8** (400 mg, 0.85 mmol) in dry dioxane (10 mL) was added successively: $Pd(PPh_3)_2Cl_2$ (30 mg, 0.04 mmol), copper iodide (32 mg, 0.17 mmol), triethylamine (0.24 mL, 1.7 mmol) and undecyne (0.25 mL, 1.25 mmol). The reaction mixture was heated at 80 °C under stirring for 2h and finally poured into an ice-water solution (20 mL). The oily residue, obtained after methylene chloride extraction of the aqueous phase, was purified by silica gel column chromatography using ethyl acetate/heptane (30/70) as eluent to give the pure product **10** (R=C_9H_{19}) (345 mg,

82%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ (ppm): 7.83 (1H, s, H-6), 6.24 (1H, dd, $^3J = 5.9$ Hz, H-1′), 5.20 (1H, dt, $^3J = 6.5$, 2.8 Hz, H-3′), 4.32 (3H, m, H-5′ and H-4′), 2.43 (1H, 2dd, $^3J = 5.9$ and 2.8 Hz, H-2′), 2.50 (3H, s, SCH_3), 2.73 (2H, t, $^3J = 7.1$ Hz, CH_2), 2.12 (1H, m, H-2′), 2.09 (3H, s, CH_3), 2.07 (3H, s, CH_3), 1.56 (2H, m, CH_2), 1.10–1.50 (14H, m, 7×CH_2), 0.84 (3H, t, $^3J = 6.8$ Hz, CH_3). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ (ppm): 179.66, 170.36, 170.15, 152.04, 140.35, 101.97, 98.49, 87.08, 82.88, 73.83, 71.14, 63.59, 39.03, 31.87, 29.27, 29.48, 29.10, 28.89, 28.46, 22.68, 20.74, 20.88, 19.52, 14.11, 13.44. Mass spectrum (IE) m/z 492 $[M]^+$.

13. Preparation of **11f**: A solution of 3′,5′-di-*O*-acetyl-4-methylthio-5-undecenyl-2′-deoxyuridine **10f** (R = C_9H_{19}) (220 mg, 0.55 mmol) and NaSH (300 mg, 4.4 mmol) in dry DMF (10 mL) was stirred at room temperature for 16 h under a nitrogen atmosphere. The solvent was removed in vacuo and the resulting oil was purified by silica gel chromatography using ethyl acetate/heptane (1:1) as eluent, yielding the pure product **11** (R = C_9H_{19}) (162 mg, 75%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ (ppm): 8.30 (1H, s, H-4), 6.55 (1H, s, H-5), 6.30 (1H, dd, $^3J = 5.7$ Hz, H-1′), 5.23 (1H, d, $^3J = 6.4$ Hz, H-3′), 4.42 (2H, s, H-5′), 4.41 (1H, m, H-4′), 2.94 (1H, 2dd, $^3J = 5.7$ and 2.3 Hz, H-2′), 2.73 (2H, t, $^3J = 7.4$ Hz, CH_2), 2.11 (3H, s, CH_3), 2.09 (1H, m, H-2′), 2.05 (3H, s, CH_3), 1.65 (2H, m, CH_2), 1.50–1.10 (14H, m, 7×CH_2), 0.85 (3H, t, $^3J = 6.7$ Hz, CH_3). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ (ppm): 179.90, 170.36, 170.26, 152.39, 144.51, 134.81, 119.39, 114.59, 88.45, 83.38, 74.10, 63.64, 39.30, 31.84, 31.11, 29.95, 29.25, 28.96, 22.64, 20.85, 14.10. Mass spectrum (ES^+) m/z 501 (100%, $[M + Na]^+$), 479 (30%, $[M + H]^+$).

14. Preparation of **3f**: Treatment for 16 h, at room temperature, of the nucleoside derivative **11** (R = C_9H_{19}) (120 mg, 0.25 mmol) in a solution (5 mL) of ethanolic ammonia (aq NH_3 (32%)/EtOH 1/1) gave, after evaporation of the solvent, the crude deprotected product **3** (R = C_9H_{19}) which was purified by silica gel chromatography using methylene chloride/methanol (95:5) as eluent (72 mg, 73%). ^{1}H NMR ($CDCl_3$/CD_3OD, 250 MHz) δ (ppm) 8.98 (1H, s, H-4), 6.75 (1H, s, H-5), 6.27 (1H, dd, $^3J = 5.6$ Hz, H-1′), 4.42 (1H, m, H-3′), 4.20–3.70 (5H, m, H-5′, H-4′, OH), 2.75 (2H, t, $^3J = 7.5$ Hz, CH_2), 2.60 (1H, m, H-2′), 2.35 (1H, m, H-2′), 1.67 (2H, m, CH_2), 1.50–1.10 (14H, m, 7×CH_2), 0.86 (3H, t, $^3J = 6.6$ Hz, CH_3). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ (ppm): 174.69, 164.67, 153.19, 144.07, 137.86, 120.24, 115.60, 88.33, 88.04, 69.47, 60.83, 41.36, 31.37, 30.87, 29.79, 29.39, 19.14, 28.88, 22.50, 21.96, 17.12, 13.88. Mass spectrum (ES^+) m/z: 417 (100%, $[M + Na]^+$).