2'-Deoxyuridines with a 5-heteroaromatic substituent: synthesis and biological evaluation

I. Luyten,¹ L. Jie,¹ A. Van Aerschot,¹ C. Pannecouque,¹ P. Wigerinck,¹ J. Rozenski,¹ C. Hendrix,¹ C. Wang,² L. Wiebe,² J. Balzarini,³ E. De Clercq³ and P. Herdewijn^{1,*}

¹Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

²Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

³Laboratory of Experimental Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Summary

A series of novel 2'-deoxyuridines with a thienyl substituent in the 5-position were synthesized as potential anti-HSV-1 agents. The brominated derivatives (1d, 1e and 3b) were obtained via halogenation reactions of the protected 5-(thien-2-yl)-2'-deoxyuridine and 5-(thien-3-yl)-2'-deoxyuridine, respectively. The palladium-catalysed cross-coupling reaction with stannylated thiophene was used for the synthesis of (E)-5-(2-thienylvinyl)-2'-deoxyuridine and 5-(5,2'-dithien-2-yl)-2'-deoxyuridine. These compounds show moderate to good activity against herpes simplex virus type 1 (HSV-1) in the order of decreasing activity 1d > 4 > 1e > 3b > 5. Finally, two substituted 5-isoxazol derivatives of 2'-deoxyuridine (6a and 6b) were obtained via a 1,3-dipolar cycloaddition of the protected 5-ethynyl-2'-deoxyuridine. These new compounds demonstrated poor affinity for the virus-specific enzyme thymidine kinase.

Key-words: HSV-1; nucleoside transport; 5-substituted 2'-deoxyuridines; thiophene analogues.

Introduction

The synthesis of 2'-deoxyuridine analogues having a heteroatomic substituent in the 5-position (Fig. 1) has led to the discovery of several biologically active molecules; 5-(5-chlorothien-2-yl)-2'-deoxyuridine, 5-(5-bromothien-2-

yl)-2'-deoxyuridine, 5-(thien-3-yl)-2'-deoxyuridine, 5-(thien-2-yl)-2'-deoxyuridine and 5-(furan-2-yl)-2'-deoxyuridine (Wigerinck *et al.*, 1991). All these molecules show highly selective activity against the replication of HSV-1 in cell culture, and are also inhibitory to the proliferation of HSV-1 thymidine kinase (TK) gene-transfected tumour cells (Bohman *et al.*, 1994). Both these effects rely on the selective phosphorylation of the compounds by the herpes simplex virus type-1 thymidine kinase. For activity against HSV-1-infected cells, the 5'-triphosphate of the compounds is probably the active metabolite. For activity against HSV TK gene-transfected tumours, the 5'-monophosphate seems to be the most important metabolite (Bohman *et al.*, 1994). This metabolite is an inhibitor of thymidylate synthase.

In studies of the physicochemical properties of these molecules, a relationship was found between the structure of the 5-substituent and the substrate specificity of the molecules for the purified herpes simplex virus type-1 thymidine kinase (Herdewijn, 1994; Olivier *et al.*, 1994). X-ray structure analysis revealed that the oxygen atom of the furanosyl ring of **2** is oriented in the opposite direction to the sulfur atom of the thienyl ring of **1a** and **1c**. A clear difference was found between the molecular potential isocontour maps of 5-heteroaromatic substituted 2'-deoxyuridines that are good and poor substrates, respectively, for the viral TK. A large negative region is located above the 5-membered heterocycle and a large positive region above the uracil ring of the compounds that are good substrates for the viral TK. In contrast, the positive region is situated above the entire molecule of the compounds that are poor substrates for the viral TK. In the light of the importance of the presence of an electron-withdrawing group on the thienyl ring, we synthesized some other thienyl derivatives, namely **1d**, **1e**, **3b**, **4** and **5**. Two substituted 5-isoxazolyl derivatives of 2'-deoxyuridine (**6a** and **6b**) were also synthesized in an effort to increase the biological activity of the parent compound [5-(isoxazol-5-yl)-2'-deoxyuridine].

Results

Synthesis

The 3',5'-dibrominated compound **1e** (Fig. 2) could be obtained from 5-(thien-2-yl)-1-(3,5-di-O-acetyl-β-D-2-deoxyribofuranos-1-yl)uracil (Wigerinck *et al.*, 1991).

Received 3 January, 1995; revised 17 January, 1995; accepted 2 March, 1995. *For correspondence. Tel. 32-16-3373 41; Fax 32-16-3373 40.

Fig. 1. Representative examples of 5-heteroaromatic substituted 2'-deoxyuridines with anti-HSV-1 activity.

Reaction of **7** with one equivalent of bromine in CCl_4 gave 85% of the 5-bromothien-2-yl compound **8**. Further addition of a second equivalent of bromine in CCl_4 gave quantitatively the 3,5-dibromothien-2-yl compound **9** from which **1e** was isolated after deprotection of the hydroxyl groups. When **9** was subjected to reductive conditions using Zn in a mixture of Ac_2O , AcOH and DMF at 90°C , the 2-bromo substituent was selectively removed (Van Aerschot *et al.*, 1990). The position of the bromine atom could be deduced from the $J_{4'',5''}$ values in the ^1H NMR spectrum. Should the 5-bromo substituent have been removed, the starting compound **7** would have been obtained. Removal of the acetyl groups of **10** gave **1d**. 5-(2-Bromothien-3-yl)-2'-deoxyuridine **3b** (Fig. 3) was synthesized from **11** (Wigerinck *et al.*, 1993). When **11** was treated with one equivalent of bromine in CCl_4 , substitution only took place in the 2''-position in 87% yield leaving the 4'' and 5'' positions unchanged. The location of bromination could also be deduced here from the $J_{4'',5''}$ coupling constant. Treatment of **12** with ammonia in methanol gave **3b**. The thienylvinyl analogue **4** (Fig. 4) was synthe-

sized from **13**. When **13** was reacted with 2-tributylstannylthiophene in NMP catalysed by a mixture of $\text{Pd}(\text{OAc})_2$, Ph_3As and Et_3N , a 43% yield of **14** was obtained. Removal of the acetyl protecting groups in the usual way gave (*E*)-5-(2-thienylvinyl)-2'-deoxyuridine **4**. The 5-(5,2'-dithien-2-yl)-2'-deoxyuridine **5** (Fig. 5) was prepared from **1b**. Analogous cross-coupling with 2-tributylstannylthiophene afforded 40% of the protected **5**. Deprotection and crystallization from MeOH gave green fluorescent needles in 85% yield.

The isoxazolyl substituted nucleosides **6a** and **6b** were obtained from 3',5'-di-*O*-toluoyl-5-ethynyl-2'-deoxyuridine (Robins and Barr, 1983) by a 1,3-dipolar cycloaddition reaction using *in situ* synthesized thiophene-2-carbonitrile *N*-oxide (Iwakura *et al.*, 1968) and furane-2-carbonitrile *N*-oxide (Ghabrial *et al.*, 1987). Both reagents can be obtained from thiophene 2-carboxaldoxime and furane-2-carboxaldoxime, respectively, via the carbohydroxamoyl chlorides (Liu *et al.*, 1980; Larsen and Torssell, 1984; Ghabrial *et al.*, 1987) (Fig. 6).

The final compounds (**1e**, **1d**, **3b**, **4** and **5**) were fully identified by ^1H NMR (Table 1; see 'Materials and Experimental Procedures'), ^{13}C NMR (these data are available as supplementary material from the authors), UV, MS spectroscopy and elementary analysis ('Materials and Experimental Procedures').

Antiviral and cytostatic activity

The antiviral activities of the compounds are shown in Table 2. As compounds **6a** and **6b** were totally inactive, they were not included in the table. All compounds show moderate to good activity against herpes simplex virus

Fig. 2. Synthetic scheme for 5-(3-bromothien-2-yl)-2'-deoxyuridine and 5-(3,5-dibromothien-2-yl)-2'-deoxyuridine. i: Br_2 , CCl_4 ; ii: NH_3 , MeOH; iii: Zn, Ac_2O , DMF.

Fig. 3. Synthesis of 5-(2-bromothiophen-3-yl)-2'-deoxyuridine. i : Br_2 , CCl_4 ; ii : NH_3 , MeOH .

Fig. 4. Synthetic scheme leading to 5-E-(2-thienylvinyl)-2'-deoxyuridine. i : $\text{Pd}(\text{OAc})_2$, Ph_3As , Et_3N , NMP ; ii : NH_3 , MeOH .

Fig. 5. Synthetic scheme leading to 5-E-(5,2'-dithien-2-yl)-2'-deoxyuridine. i : $\text{Pd}(\text{OAc})_2$, Ph_3As , Et_3N , NMP ; ii : NH_3 , MeOH , dioxane.

Fig. 6. Synthesis of 3-substituted 5-(isoxazol-5-yl)-2'-deoxyuridines. i : Et₃N; ii : 0.1 M NaOMe, MeOH.

Table 1. ¹H NMR data of the 2'-deoxyuridine analogues 1e, 1d, 3b, 4 and 5

	1e	1d ^a	3b	4	5	6a	6b
H-1'	6.25(t)	6.51(t)	6.25(t)	6.25(t)	6.21(t)	6.18(t)	6.17(dd)
H-2'	2.17	2.46	2.17	2.20	2.25	2.22(t)	2.20
H-3'	4.25	4.60	4.27	4.37	4.33	4.28	4.34
H-4'	3.85	4.10	3.82	4.04	3.86	3.85	3.85
H-5'	3.55	3.89	3.55	3.30	3.69	3.63	3.62
3'-OH	5.30(br)			5.25	5.29	5.29(d)	5.28(d)
5'-OH	5.00(br)			5.25	5.29	5.20(t)	5.17
H-6	8.48(s)	8.57(s)	8.11(s)	8.26(s)	8.63(s)	8.75(s)	8.73(s)
H-4''	7.32(s)	7.19(d)	7.06(d)			7.22(s)	7.11(s)
³ J _{1',2'} ^b	6.9	6.6	6.7		6.4	6.4	6.2 and 4.8
³ J _{4'',5''} ^b		5.3	5.7				

Only the signals are given.

Spectra were taken in DMSO-d₆ with tetramethylsilane as internal standard. δ values:

(s) = singlet, (d) = doublet, (t) = triplet.

^a Spectrum was taken in CD₃OD with tetramethylsilane as internal standard.

^b Hz values.

Table 2. Cytotoxicity and antiviral activity of the 5-heteroaromatic substituted 2'-deoxyuridines in human embryonic skin-muscle fibroblast (E₆SM) cells

	Minimum cytotoxic concentration ^a (µg ml ⁻¹)	Minimum inhibitory concentration ^b (µg ml ⁻¹)		
		Herpes simplex virus type 1 (KOS)	Herpes simplex virus type 1 TK ⁻ (B2006)	Vaccinia virus
1a^c	>400	0.45	300	>400
1b^c	>400	0.03	150	70
3a^c	>400	0.3	150	>40
1e	400	0.7	20	150
1d	>400	0.04	300	>400
3b	>400	2	>400	>400
4	>400	0.07	20	70
5	≥10	2	>10	>10
BrVdUrd	>400	0.004	>200	0.04

^a Required to cause a microscopically detectable alteration of normal cell morphology.^b Required to reduce virus-induced cytopathic by 50%.^c Data taken from Wigerinck *et al.* (1991).

type 1 (HSV-1), the order of decreasing activity being **1d>4>1e>3b~5**. The compounds were inactive against several other viruses, including herpes simplex virus type 2, vesicular stomatitis virus, Coxsackie B4 virus, polio virus type 1, Semliki forest virus, reovirus type 1, parainfluenza virus type 3 Sindbis virus, and human immunodeficiency virus type 1 (III_B) and type 2 (ROD) at subtoxic concentrations. The low activity against a TK⁻ strain of herpes simplex type 1 virus suggests that these compounds must be phosphorylated to express their antiviral activity. Only compound **5**, with a dithienyl moiety in the 5-position, had a CC₅₀ (50% cytostatic concentration of 16 µg ml⁻¹). No marked cytotoxicity was observed for the other compounds.

When a bromo substituent was introduced in the 5-position of **1a**, the activity against HSV-1 increased. In contrast, when a bromo substituent was introduced in **3a** (Wigerinck *et al.*, 1993) (giving **3b**) a modest drop in activity was observed. Substitution of **1a** (Wigerinck *et al.*, 1991) in the 3-position with bromine gave compound **1d** with similar activity to **1b**, while dibromination (**1e**) led to decreased antiviral activity. The 2-thienyl vinyl compound **4** was as active as **1d** and the dithienyl compound was as active as **3b**.

In a first effort to unravel the mode of action of the new 5-thienyl substituted 2'-deoxyuridine derivatives, their inhibitory effects on HSV-1 TK activity were determined (Table 3). From this table it is clear that, in contrast to 5-(thienyl-2-yl)-2'-deoxyuridine (IC₅₀=2.9 µM) and 5-(5-bromothien-2-yl)-2'-deoxyuridine (IC₅₀=3.5 µM) (Bohman *et al.*, 1994), the new compounds demonstrated poor affinity for the virus-specific thymidine kinase. In fact, the affinity of the HSV-1 TK for **1e**, **1d**, **3b**, **4** and **5** was similar to that of the 5-halogenated furan-2-yl compounds [5-(5-bromofuran-2-yl)-2'-deoxyuridine (IC₅₀=51 µM) and 5-(5-chlorofuran-2-yl)-2'-deoxyuridine (IC₅₀=28 µM)].

Before these nucleosides can exert their antiviral activity they must be taken up into the cell. We therefore studied the uptake of compound **1c** [5-(5-chlorothien-2-yl)-2'-deoxyuridine] in mouse erythrocytes by facilitated diffusion. This compound was selected because it is the most active congener of the 5-heteroaromatic substituted 2'-deoxyuridine series. The concentration dependence of the zero-trans influx of thymidine was plotted according to Hanes (1953), yielding kinetic parameters of $K_m=0.11 \pm 0.01$ mM and $V_{max}=4.9 \pm 0.1$ nmol (10¹⁰ cells s⁻¹) (Fig. 7a). Competition of 5-(5-chlorothien-2-yl)-2'-deoxyuridine with thymidine for the transport site was measured in an influx competition experiment with (methyl-³H)thymidine. The data were plotted according to Dixon (1953), as shown in Fig. 7b. The K_i value (inhibition constant) was determined as 0.0075 mM.

Compared with 2'-deoxyuridine (K_i=0.11 mM), the test compound had a very small K_i (0.0075 mM), from which we conclude that **1e** is a very good substrate for the binding site of the transporter.

Discussion

The brominated derivatives of **1a** and **3a** showed decreasing activity in the order **1b>1d>1e>3b**. Compound

Table 3. Inhibitory effect against HSV-1 thymidine kinase

	IC ₅₀ ^a (µM)
1e	62.5 ± 6.7
1d	61.4 ± 0.4
3b	143 ± 80
4	50.4 ± 17.9
5	71 ± 17.9

^a Concentration (µM) required to inhibit HSV-1 TK: [methyl-³H] thymidine is used at 1 µM.

Fig. 7. (a) Hanes plot of thymidine. (b) Dixon plot of 5-(5-chlorothien-2-yl)-2'-deoxyuridine (CITDU).

3b differs from **1b**, **1d** and **1e** because the heterocyclic ring is connected with its 3-position. This could explain its lower activity.

Introduction of one bromo substituent in the thienyl ring of **1a** increases the activity against HSV-1, while dibromination of **1a** leads to a decrease in antiviral activity. Because of the steric hindrance of the two bromine atoms it is possible that the quasi-coplanar geometry between the thienyl ring and the uracil base is destroyed, resulting in lower activity.

The antiviral activities of both **1b** and (*E*)-5-(2-bromovinyl)-2'-deoxyuridine were diminished when the bromine was replaced with a thiophene ring (see **5** and **4**). Both the bromine and the thienyl ring are hydrophobic and electron-withdrawing, but the thiophene ring is bulkier and aromatic, and this may influence the biological activity. We conclude that introduction of a thiophene

ring increased the activity less than introduction of a bromine.

The molecular potential isocontour map of 5-(isoxazol-5-yl)-2'-deoxyuridine shows a positive region situated above the entire molecule (except for the nitrogen atom of the isoxazolyl ring). This compound is a poor substrate for the viral TK and does not inhibit HSV-1 (Olivier *et al.*, 1994). The two substituted 5-isoxazolyl derivatives (**6a** and **6b**) are also inactive. To determine whether the presence of a positive region is correlated with antiherpesvirus activity, it would be of interest to investigate whether or not there is a positive region located above the entire molecule in the latter two compounds.

Degradation of the test compounds by phosphorylases may be expected to restrict the therapeutic potential of the 5-substituted 2'-deoxyuridine. The susceptibility of the prototype compound **1a** for hydrolysis by bacterial and human phosphorylase has therefore been investigated (Bohman *et al.*, 1994). It was found that **1a** was a markedly less efficient substrate for bacterial dThd phosphorylase than dThd or BrVDU, whereas it proved fully resistant to phosphorolytic cleavage by human dThd phosphorylase. These properties may be beneficial in that the compounds may prove relatively stable in human serum.

This study provides a better insight into the structure-activity relationship of 5-heteroaromatic substituted 2'-deoxyuridines and this information may be used for further drug design. Moreover, development of these types of compound should be pursued in the light of the potent anti-herpetic activity in cell culture and the resistance to phosphorylase degradation. The toxicity and resistance profile of the most active substance should be investigated and compared with that of acyclovir (Zovirax), the current drug of choice for treatment of HSV-1 infections.

Materials and Experimental Procedures: Chemistry

Melting points were determined in capillary tubes with a Büchi-Tolloli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8700 UV/vis spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined with a Varian gemini 200 MHz spectrometer with tetramethylsilane as internal standard for the ^1H NMR spectra and DMSO- d_6 (39.6 ppm) or CDCl_3 (76.6 ppm) for the ^{13}C NMR spectra (s=singlet, d=doublet, dd=double doublet, t=triplet, br s=broad signal, m=multiplet). Liquid secondary ion mass spectra (LSIMS), chemical ionization (CI) and electron impact (EI) mass spectra (MS) and accurate mass measurements (HRMS) were obtained using a KRATOS Concept IH mass spectrometer (Kratos, Manchester, UK). Precoated Macherey-Nagel Alu-gram Sil G) UV_{254} plates were used for TLC, and the spots were examined with UV light and sulfuric acid-anisaldehyde spray. Column chromatography was performed on Janssen Chimica silica gel (0.060–0.200 nm).

5-(3,5-dibromothienyl-2-yl)-3',5'-di-O-acetyl-2'-deoxyuridine 9 and 5-(3,5-dibromothien-2-yl)-2'-deoxyuridine 1e

To a solution of 473 mg (1 mmol) of 5-(5-bromothien-2-yl)-3',5'-di-O-acetyl-2'-deoxyuridine in 10 ml of dichloroethane was added, at 0 °C, 15 ml of a 0.74 M solution of bromine in CCl₄. The mixture was stirred for 1 h at 0 °C, diluted with CH₂Cl₂ (15 ml) and washed with saturated NaHCO₃ and water. The organic layer was dried (Na₂SO₄) and evaporated. The resulting oil was purified by column chromatography (CH₂Cl₂: MeOH 98:2) yielding 550 mg (1 mmol, 100%) of the dibrominated compound which was crystallized from MeOH. m.p.: 157–158 °C. MS (LSIMS, thioglycerol doped with NaCl) 575 [M + Na]⁺. UV (MeOH) λ_{max}: 261 and 311 nm, λ_{min}: 288 nm.

The acetyl groups were removed by treatment of the compound with 25% ammonia in methanol-dioxane at room temperature overnight, followed by evaporation to dryness and crystallization from MeOH. This gave 82.6% yield of the title compound. m.p.: 113–114 °C. MS (CI) 353 [base + 2H]⁺; 117 [sugar]⁺. Elemental analysis: C₁₃H₁₂Br₂N₂O₅S 2/3 equiv. H₂O, calc. C 32.53, H 2.80, N 5.84; found C 32.82, H 3.01, N : 5.56.

5-(3-Bromothien-2-yl)-3',5'-di-O-acetyl-2'-deoxyuridine 10 and 5-(3-bromothien-2-yl)-2'-deoxyuridine 1d

A mixture of 552 mg (1 mmol) of 5-(3,5-dibromothien-2-yl)-3',5'-di-O-acetyl-2'-deoxyuridine, 130 mg (2 mmol) of zinc, 0.1 ml of acetic acid and 50 µl of acetic anhydride in 1 ml of anhydrous DMF was heated at 90 °C for 3.5 h. The reaction mixture was diluted with diethyl ether (30 ml) and filtered, and the filtrate was washed with saturated NaHCO₃ and water. The organic layer was dried (Na₂SO₄) and evaporated to yield an oil which was purified by column chromatography (CH₂Cl₂-MeOH 99:1) giving 205 mg (0.43 mmol) of the acetylated title compound (43% yield). HRMS (EI): 471.9941 [M]⁺, calculated 471.9940 for C₁₇H₁₇O₇N₂SBr. Deprotection was carried out as described for the previous compound in a yield of 62%. m.p.: 155–156 °C. MS (LSIMS, thioglycerol after silylation): 533.0602 [M + 2 Me₃Si-H]⁺, calculated 533.0598. ¹H NMR (CD₃OD) δ: 7.19 (d, ³J_{4',5'} = 5.3 Hz) and 7.66 (d, ³J_{4',5'} = 5.3 Hz) (H-4' and H-5'); 8.57 (s, H-6). Elemental analysis (C₁₃H₁₃BrN₂O₅S): calculated C 40.12, H 3.37, N 7.20; found C 40.56, H 3.67, N 6.91.

5-(2-Bromothien-3-yl)-3',5'-di-O-acetyl-2'-deoxyuridine 12 and 5-(2-bromothien-3-yl)-2'-deoxyuridine 3b

To a solution of 369 mg (0.94 mmol) of 5-(thien-3-yl)-3',5'-di-O-acetyl-2'-deoxyuridine in 10 ml of dichloroethane was added dropwise 1.35 ml of a solution of 0.74 M of bromine (1 mmol) in CCl₄ at –30 °C. The temperature of the reaction mixture was allowed to increase to room temperature over a period of 1 h. The mixture was diluted with CH₂Cl₂ (20 ml), and washed with saturated NaHCO₃ and water. The organic layer was dried (Na₂SO₄) and evaporated, and the resulting oil was diluted with MeOH giving 384 mg (0.81 mmol, 87% yield) of the acylated title compound as crystalline material. MS (LSIMS, thioglycerol) 473 and 475 [M + H]⁺, 273 and 275 [base + 2H]⁺.

Removal of the acetyl protecting groups was carried out with 25% of ammonia overnight at room temperature in a mixture of

MeOH-dioxane and the title compound was crystallized from MeOH : 71% yield. m.p.: 154 °C (dec). MS (LSIMS, thioglycerol, after silylation): 533 and 535 [M + 2Me₃Si-H]⁺; 461 and 463 [M + Me₃Si]⁺. ¹H NMR (DMSO-d₆) δ: 7.06 (d, ³J_{4',5'} = 5.7 Hz) and 7.59 (d, ³J_{4',5'} = 5.7 Hz) (H-4' and H-5"); ppm. Elemental analysis (C₁₃H₁₃N₂O₅BrS): calculated C 40.12, H 3.37, N 7.20; found C 39.83, H 3.76, N 7.52.

(E)-5-(2-Thienylvinyl)-3',5'-di-O-acetyl-2'-deoxyuridine 14 and (E)-5-(2-thienylvinyl)-2'-deoxyuridine 4

To a mixture of 834 mg (2 mmol) of *E*-5-(2-bromovinyl)-3',5'-di-O-acetyl-2'-deoxyuridine, 45 mg (0.2 mmol) of Pd(OAc)₂, 123 mg (0.4 mmol) of Ph₃As and 0.28 ml (2.8 mmol) of triethylamine in 15 ml of NMP was added a solution of 2-tributylstannylthiophene in 5 ml of NMP. The mixture was heated at 110 °C for 3 h, cooled to room temperature, diluted with Et₂O, filtered through celite, and washed with saturated NaHCO₃ and H₂O. The organic layer was dried (Na₂SO₄) and evaporated, and the residual oil was purified by column chromatography (Et₂O : hexane 8:2 followed by CH₂Cl₂ : MeOH 95:5) yielding 360 mg (0.86 mmol, 43% yield) of the acetylated title compound, which was crystallized from MeOH. UV (λ_{max}): 325 nm. HRMS (LSIMS, thioglycerol): 421.1063 [M + H]⁺, calculated 421.1069. After deacetylation with ammonia, as previously described, 77% of the title compound was obtained. It was crystallized from MeOH. m.p.: 203 °C (decomp.) MS (LSIMS, thioglycerol, after silylation) : 481 [M + 2Me₃Si-H]⁺; 221 [base + 2H]⁺; 171 [sugar]⁺. HRMS : 481.1651 [M + H]⁺, calculated 481.1649 for C₂₁H₃₃N₂O₅SSi₂. UV (MeOH) λ_{max}: 332 nm (ε = 22400). ¹H NMR (DMSO-d₆) δ: 6.62 (d, ³J = 17.5 Hz, vinylic H); 6.96–7.10 (m, 2H), 7.26 (d, 1H) (H-3", H-4", H-5"), 7.62 (d, ³J = 16 Hz, vinylic H); 11.43 (br, NH) ppm. Elemental analysis (C₁₅H₁₆N₂O₅S 0.8 H₂O): calculated C 51.36, H 5.06, N 7.99; found C 51.55, H 5.24, N 7.65.

5-(5,2'-dithien-2-yl)-3',5'-di-O-acetyl-2'-deoxyuridine and 5-(5,2'-dithien-2-yl)-2'-deoxyuridine 5

Nitrogen was bubbled through a solution of 473 mg (1 mmol) of 5-(5-bromothien-2-yl)-3',5'-di-O-acetyl-2'-deoxyuridine in 5 ml of NMP for 5 min, after which 44 mg (0.2 mmol) of Pd(OAc)₂, 61 mg (0.2 mmol) of Ph₃As and 0.14 ml of triethylamine were added. The mixture was heated for 10 min at 60 °C under a nitrogen blanket, after which 745 mg (2 mmol) of 2-tributylstannylthiophene was added. After heating for a further 3 h, the mixture was diluted with 50 ml of EtOAc and filtered through celite. The filtrate was washed with water (2 × 50 ml) and with brine (50 ml), dried (Na₂SO₄), evaporated and purified on silica gel, affording 190 mg (0.4 mmol, 40 %) of the protected analogue. MS (EI, m/z) 476 [M]⁺, 276 [Base + 2H]⁺.

The obtained material was deprotected overnight in 30 ml of a mixture of concentrated ammonia (30%): dioxane: methanol (1:1:1). After evaporation, the residue was crystallized from methanol affording 133 mg (0.34 mmol, 85 %) of green fluorescent needles. m.p. ~250 °C (dec. mp.) UV (MeOH) λ_{max}: 356 nm (ε = 20600). HRMS (LSIMS): 392.0500 [M]⁺, calculated 392.0500 for C₁₇H₁₆N₂O₅S₂. ¹H NMR (DMSO-d₆) δ: 7.00–7.51 (m, thienyl-H); 11.70 (br, NH) ppm. Elemental analysis:

$C_{17}H_{16}N_2O_5S_2$: calculated C 52.03, H 4.11, N 7.14; found C 52.21, H 4.22, N 7.29.

5-[3-(2-Thienyl)-isoxazol-5-yl]-2'-deoxyuridine **6a and 5-[3-(2-furanyl)-isoxazol-5-yl]-2'-deoxyuridine **6b****

A mixture of 130 mg (1 mmol) of thiophene-2-carboxaldoxime, 0.74 mmol (133 mg) of *N*-chlorosuccinimide and 3 drops of pyridine in 20 ml of $CHCl_3$ was stirred for 15 min at room temperature. To this mixture was added 250 mg (0.55 mmol) of 3',5'-di-*O*-toluoyl-5-ethynyl-2'-deoxyuridine and 150 μ l of triethylamine in 5 ml of $CHCl_3$. The solution of triethylamine in $CHCl_3$ was added dropwise over a period of 30 min. After refluxing for 2 h, the reaction mixture was washed two times with H_2O , dried and evaporated. The residual oil was treated with 20 ml of 0.1 M NaOMe in MeOH, neutralized with 0.01 M HCl, evaporated and purified by column chromatography (CH_2Cl_2 : MeOH 94:6) yielding 100 mg (0.26 mmol, 48 %) of the title compound **6a**. m.p. >250 °C (decomp.). UV (MeOH) λ_{max} : 247 (ϵ = 18020) and 300 nm (ϵ = 22500). HRMS (LSIMS): 378.0750 [M+H]⁺, calculated 378.0760 for $C_{16}H_{16}N_3O_6S$. ¹H NMR (DMSO-*d*₆) δ : 7.19 (dd, H-4''); 7.72 (d, ³J = 4.9 Hz, H-3''); 7.78 (d, ³J = 3.0 Hz, H-5'') ppm. Starting from furane-2-carboxaldoxime and using the same *modus operandi* as described for **6a**, **6b** was obtained in 40 % yield (80 mg, 0.22 mmol). m.p. >250 °C (decomp.). UV (MeOH) λ_{max} : 244 (ϵ = 20600) and 301 nm (ϵ = 24300). HRMS (LSIMS): 362.0970 [M+H]⁺, calculated 362.0988 for $C_{16}H_{16}N_3O_7$. ¹H NMR (DMSO-*d*₆) δ : 6.67 (dd, H-4''); 7.16–7.24 (m, H-3''); 7.88 (br s, H-5''); 8.73 (s, H-6) ppm. Note: The NMR data of the acetylated compounds **9**, **10**, **12** and **14** are available as supplementary material.

Materials and Experimental Procedures: Transport Studies

Mice

Male B₆D₂F₁ mice were obtained from the Health Sciences Small Animal Program, University of Alberta. 5-(5-Chlorothien-2-yl)-2'-deoxyuridine was synthesized as described previously (Wigerinck *et al.*, 1991). [Methyl-³H]thymidine (1.81 TBq mmol⁻¹, radiochemical purity 98.6%) was purchased from Amersham Corporation (Arlington Hts, IL). ScintiVerse II TM scintillation fluor was purchased from Fisher Scientific Company (Chemical Manufacturing Division, Fair Lawn, NJ). The transport inhibitor nitrobenzylthioinosine (NBMPR) was purchased from the Aldrich Chem. Co. (Milwaukee WI).

Methods

Male B₆D₂F₁ mice were asphyxiated with CO₂. Blood was immediately collected into a syringe containing 3.8 % sodium citrate solution by cardiac puncture and used the same day. Mouse erythrocytes were obtained by centrifugation at 12 800 r.p.m. for 1 min followed by 4 washes with cold buffered saline. The mouse erythrocytes obtained were suspended in the same medium to a 10% haematocrit. The transport study was performed at room temperature according to Gati *et al.* (1983, 1984). Nucleoside influx was initiated by the rapid addition

of buffered saline containing (methyl-³H)thymidine at three concentrations (0.053–0.53 mM) alone and together with various concentrations of 5-(5-chlorothien-2-yl)-2'-deoxyuridine. After 3 s, transport was terminated by adding NBMPR at a final concentration of 10 μ M. Cells were pelleted and washed once with NBMPR solution (20 μ M). Cell pellets were extracted with ice-chilled 5% perchloric acid. After 30 min cooling in ice, aliquots were counted by liquid scintillation counting in ScintiVerse II. Radioactivity trapped in the extracellular space of the pellet was determined as a zero-time value by reversal of the order of addition of NBMPR and nucleoside to the cells. This value was subtracted from radioactivity in the influx samples. Duplicate samples were taken in each experiment.

Antiviral and cytostatic activity assays

Assays for activity against herpesvirus were performed as described previously (De Clercq *et al.*, 1980). The origin of the viruses [herpes simplex virus type 1 (HSV-1) (strain KOS), thymidine kinase-deficient (TK⁻) HSV-1 (strain B 2006) and vaccinia virus] has been described previously (De Clercq *et al.*, 1986). Cytotoxicity measurements were based on either a microscopically detectable alteration of normal cell morphology or inhibition of cell growth. The antiviral activity and cytotoxicity assays were performed in human embryonic skin-muscle (E₆SM) cells seeded in 96-well microtitre trays.

Acknowledgements

We are indebted to Professor G. Janssen for coordinating the mass spectra analysis. We thank M. Vandekinderen for fine editorial help. This research was supported by a grant from the Belgian National Fund of Scientific Research (Project no 3.0076.92).

References

- Bohman, C., Balzarini, J., Wigerinck, P., Van Aerschot, A., Herdewijn, P., and De Clercq, E. (1994) Mechanism of cytostatic action of novel 5-(thien-2-yl)- and 5-(furan-2-yl)-substituted pyrimidine nucleoside analogues against tumor cells transfected by the thymidine kinase of simplex virus. *J Biol Chem* **269**: 8036–8043.
- Cass, C.E., and Paterson, A.R.P. (1972) Mediated transport of nucleosides in human erythrocytes. *J Biol Chem* **247**: 3314.
- Cass, C.E., and Paterson, A.R.P. (1973) Mediated transport of nucleosides by human erythrocytes specificity toward purine nucleosides as permeants. *Biochem Biophys Acta* **291**: 734.
- De Clercq, E., Descamps, J., Verhelst, G., Walker, R.T., Jons, A.S., Torrence, P.F., and Shugar, D. (1980) Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *J Infect Dis* **141**: 563–574.
- De Clercq, E., Holý, A., Rosenberg, I., Sakuma, T., Balzarini, J., and Maudgal, P.C. (1986) A novel selective broad-spectrum anti-DNA virus agent. *Nature* **323**: 464–467.
- Dixon, M. (1953) The determination of enzyme inhibitor constants. *Biochem J* **55**: 170–171.
- Gati, W.P., and Knauss, E.E. (1983) Interaction of 2' halogeno-2'-deoxyuridine with the human erythrocyte nucleoside transport mechanism, Wiebe, L.I., *Molec Pharmacol* **23**: 146.

- Gati, W.P., Misra, H.K., Kanuss, E.E., and Wiebe, L.I. (1984) Structural modifications at the 2' and 3'-positions of some pyrimidine nucleosides as determinants of their interactions with the mouse erythrocyte nucleoside transpo. *Biochem. Pharmacol.* **33**: 3325–3331.
- Ghabrial, S.S., Thomsen, I., and Torssell, K.B.G. (1987) Synthesis of Biheteroaromatic compounds via the isoxazoline route. *Acta Chem Scand* **B41**: 426–434.
- Hanes, C.S. (1932) The effect of starch concentration upon the velocity of hydrolysis by the amylase of germinated Barley. *Biochem J* **26**: 1406–1421.
- Herdewijn, P. (1994) 5'-Substituted-2'-deoxyuridine as anti-HSV-1 agents : synthesis and structure activity relationship. *Antiviral Chem Chemother* **5**(3): 131–146.
- Iwakura, Y., Uno, K., Shiraishi, and S., Hongu, T. (1968) 1,3-Dipolar cycloaddition reaction of thiophenecarbonitrile N-oxides with various dipolarophiles. *Bull Chem Soc Jpn* **41**: 2954–2959.
- Larsen, K.E., and Torssell, K.B.G. (1984) An improved procedure for the preparation of 2-isoxazolines. *Tetrahedron* **40**: 2985.
- Liu, K.-C., Shelton, B.R., and Howe, R.K. (1980) A particularly convenient preparation of benzohydroximinoyl chlorides (nitrile oxide precursors). *J Org Chem* **45**: 3916–3918.
- Olivier, A., Creuven, I., Evrard, C., Evrard, G., Van Aerschot, A., Wigerinck, P., Herdewijn, P., and Durant, F. (1994) The stereoelectronic properties of five 2'-deoxynucleoside analogues with heterocyclic substituents in the 5-position : a comparison with BrVdUrd. *Antiviral Res.* **24**: 289–304.
- Robins, M.J., and Barr, P.J. (1983) Nucleic acid related compounds 39. Efficient conversion of 5-iodo to 5-alkynyl and derived 5-substituted uracil bases and nucleosides. *J Org Chem* **48**: 1854–1862.
- Van Aerschot, A., Everaert, D., Balzarini, J., Augustyns, K., Jie, L., Janssen, G., Peeters, O., Bleton, N., De Ranter, C., De Clercq, E., and Herdewijn, P. (1990) Synthesis and anti-HIV evaluation of 2',3'-dideoxyribo-5-chloropyrimidine analogues: reduced toxicity of 5-chlorinated 2',3'-dideoxy nucleosides. *J Med Chem* **33**: 1833–1839.
- Wigerinck, P., Pannecouque, C., Snoeck, R., Claes, P., De Clercq, E., and Herdewijn, P. (1991) 5-(5-Bromothien-2-yl)-2'-deoxyuridine and 5-(5-chlorothien-2-yl)-2'-deoxyuridine are equipotent to (E)-5-(2-bromovinyl)-2'-deoxyuridine in the inhibition of herpes simplex virus type 1 replication. *J Med Chem* **34**: 2383–2389.
- Wigerinck, P., Kerremans, L., Claes, P., Snoeck, R., Maudgal, P., De Clercq, E., and Herdewijn, P. (1993) Synthesis and antiviral activity of 5-thien-2-yl-2'-deoxyuridine analogues. *J Med Chem* **36**: 538–543.