A series of hitherto unknown acyclic 5,5-difluoro-5-phosphono-pent-2-en-1-yl pyrimidines (9a, b, 13a, b), purines (16a, b) and -(1,2,4)-triazolo-3-carboxamide (19) were successfully synthesized from (E)-1-bromo-5-diethoxypent-2-en-1-yl-phosphonate-5,5-difluoro-pent-2-ene in a stereoselective manner. All the synthesized compounds were assayed for antiviral activity against various viruses, but were found to be neither active nor toxic.

Introduction

Viruses are infectious agents that can replicate their genome within host cells. Many antiviral drugs are nucleoside or nucleotide analogs. Acyclic nucleoside phosphonates (ANPs) are a class of nucleotide analogs, originally developed by A. Holy’s group, which exhibit a broad spectrum of antiviral activities. ANPs possess a common structure, a nucleobase attached to an aliphatic side chain containing a phosphonate moiety (C-P), and have an increased metabolic stability and resistance to chemical and biological degradation. Their activities are reliant on their diphosphorylation by NDP and NTP kinases, and further incorporation into the viral DNA where they can act as chain terminators. Three ANPs are in current clinical use for the treatment of serious viral infections, adefovir (PMEA), tenofovir [(R)-PMPA] and cidofovir [(S)-CDV] against hepatitis B virus (HBV), human immunodeficiency virus (HIV) and cytomegalovirus (CMV), respectively, (Fig. 1).

During our investigations, we have demonstrated that the N^1-[(E)-4-phosphono-but-2-en-1-yl]-thymine is a substrate of human TMPK and that the (E)-but-2-enyl moeity mimics the conformation of the C1’-O4’-C4’-C5’ atoms from the natural substrate, the thymidine 5’-monophosphate, (Fig. 2). However, unlike natural nucleotide, our molecule missed the oxygen of the phosphate group (e.g., -O=P). Thus, we turn our attention to the introduction of a gem-difluoromethylphosphonate moiety (e.g., -CF2=P), which is isopolar and isosteric to the phosphate group. In fact, due to specific properties of fluorine (high electronegativity, small steric size, hydrogen bond acceptor, ...), its introduction into biologically active molecules could lead to major changes in their biological properties, such as reported by Halazy et al. for the 9-(5,5-difluoro-5-phosphonopentyl)-
guanine, an inhibitor of purine nucleoside phosphorylase (PNP), a key enzyme in the purine metabolism. Therefore, based on these findings, it was interesting to design and synthesize a new type of acyclic nucleoside phosphonate, the 5,5-difluoro-5-phosphono-pent-2-en-1-yl-pyrimidines, purines and -triazole, and to evaluate their inhibitory activity against several viruses.

**Results and discussion**

Based on our previous work on the preparation of unsaturated acyclic nucleoside phosphonate using olefin cross-metathesis as key step, we decided to utilize this reaction between the unsaturated gem-difluorophosphonate and N,N-crotylated N3-protected thymine. The key intermediate was synthesized from (diethoxyphosphinyl)difluoromethyl zinc bromide, allylic iodide, under CuBr catalysis, following the procedure introduced by Burton et al. Compound 3 was then engaged in the reaction of cross metathesis in presence of N3-benzylo-N4- crotylthymine 4 with Grubbs-Nolan catalyst in dichloromethane. If cross-metathesis reactions were reported with fluorinated substrates, despite all our attempts and contrary to our results with non-fluorinated phosphonate derivatives, we never obtained the desired product (Scheme 1); this could be due to the strong electron-withdrawing effect of the 2-gem-difluoro group and to the low reactivity of both cross partners with the metal alkylidene complex.

Alternatively we decided to introduce the nucleobase moiety through direct N-alkylation of protected and unprotected nucleobases with the corresponding (E)-1-bromo-5,5-dioethoxyphosphoryl-5,5-difluoro-pent-2-ene (5). Starting from bromodifluoromethylphosphonate, the previously described organo-zinc intermediate 2 was reacted with (E)-1,4-dibromobut-2-ene at 0 °C, to yield the desired compound 5 in 80% with no observed isomerization of the double bond (Scheme 2).

**Scheme 1** Reagents and conditions: (a) Znact, 1,2-dibromoethane, TMSCI, THFanh, 40 °C, 12 h; (b) CuBr, LiClact, allyl iodide, rt, 24 h, 35%; (c) 4 (1.5 eq.), Nolan–Grubb’s II catalyst (10 mol%), CH2Cl2anh, reflux, 24 h.

**Scheme 2** Reagents and conditions: (a) Znact, 1,2-dibromoethane, TMSCI, THFanh, 40 °C, 12 h; (b) CuBr, LiClact, trans-1,4-dibromobutene, 0 °C, 4 h, 80%.

Then uracil 6a was converted to its N3-benzoyl derivative 7a through a two steps procedure involving first the formation of N4,N3-dibenzoyl derivative in presence of an excess of benzoyl chloride in CH3CN/pyridine mixture, then its selective N3-deprotection by treatment with potassium carbonate in 1,4-dioxane, (Scheme 3).

Similarly, thymine 6b was converted to its N3-protected derivative bromide their 7b. Finally, the successful N4-alkylation of 7a and 7b on 5 in the presence of cesium carbonate in DMF proceeded in good yields and excellent regioselectivities and afforded 8a and 8b, in 86% and 75% yields, respectively. Simultaneous deprotection of the N3-benzoyl group and phosphonic esters with TMSBr/CH2Cl2 afforded analogues 9a and 9b, in good yields, respectively. These coupling conditions were extended to other nucleic bases. Cytosine 10a and its fluorinated analogue 10b were converted to N4-bis(Boc)-cytosine derivatives 11a and 11b, respectively, in good yields, through N4,N3,N4-tris-Boc forms followed by regioselective N3 deprotection with saturated solution of NaHCO3 in methanol. Alkylation at N1 position of 11a and 11b in presence of derivative 5, according to the same previous conditions using Cs2CO3, afford 12a and 12b, in 83% and 77% yield, respectively. Deprotection with TMSBr afforded the expected free phosphonates 13a and 13b, respectively, in quantitative yield (Scheme 4).

**Scheme 3** Reagents and conditions: (a) (i) BzCl, CH3CN/pyridine, rt (ii) K2CO3 (0.5 M), dioxane, 70 °C, 90% (for R = H) and 96% (for R = CH3); (b) (E)-1-bromo-5-dioethoxyphosphoryl-5,5-difluoro-pent-2-ene (5), Cs2CO3, dry DMF, 85% (for R = H) and 81% (for R = CH3); (c) TMSBr, CH2Cl2, rt, 72 h, 90% (for R = H) and 96% (for R = CH3).

**Scheme 4** Reagents and conditions: (a) (i) Boc2O, DMAP, dry THF (ii) saturated NaHCO3, MeOH 50 °C, 62%; (b) (E)-1-bromo-5-dioethoxyphosphoryl-5,5-difluoro-pent-2-ene (5), Cs2CO3, dry DMF, 85% (for R = H) and 77% (for R = F); (c) TMSBr, CH2Cl2, rt, 72 h, quantitiative for R = H and for R = F.
Finally, with respect to the broad-spectrum antiviral drug ribavirin, which possess a 1,2,4-triazole-3-carboxamide nucleo-
base, its acyclic difluorinated phosphonate 19 was synthesized from 17, in a similar pathway, (Scheme 6).

All the synthesized 5,5-difluoro-5-phosphono-pent-2-en-1-yl nucleosides, 9a, b, 13a, b, 16a, b and 19, were evaluated against a wide variety of viruses, to determine their antiviral activity (EC50) in HEL, MDCK, Vero and HeLa cell lines, as the effective concentration required to reduce virus-induced cytopathicity or plaque formation by 50%. Compounds were evaluated against vaccinia virus (VV), herpes simplex virus 1 (HSV-1) (KOS strain), herpes simplex virus 2 (HSV-2) (G strain), thymi-
dine kinase deficient (TK-) HSV-1, vesicular stomatitis virus (VSV), varicella-zoster virus (VZV) (TK' and TK strains), human cytomegalovirus (HCMV) (AD-169 and Davis strains) in HEL, vesicular stomatitis virus (VSV), Coxsackie B4, respiratory syncytial virus in HeLa cell cultures, parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4 in Vero cells and influenza virus in MDCK cells. All of the synthesized compounds did not exhibit promising antiviral activity.

**Conclusions**

In summary, a series of hitherto unknown acyclic 5,5-di-
fluoro-5-phosphono-pent-2-en-1-yl-pyrimidines (9a, b, 13a, b), purines (16a, b) and -(1,2,4)-triazolo-3-carboxamide (19) were successfully synthesized from (E)-1-bromo-5-diethoxyporphosphoryl-5,5-
difluoro-pent-2-ene (5) in a convergent stereoselective manner. Surprisingly, it was discovered that cross-metathesis, in our hand, cannot afford the desired difluorinated phosphonate compounds. However, the final nucleosides were obtained, in good yields, by N-alkylation of various nucleobases with (E)-diethy-5-bromo-1,1-difluoropent-3-ylphosphonate.

However, none of the synthesized compounds showed significant antiviral activities. One plausible explanation could be due to a poor penetration to the cell and to the lack of next phosphorylation steps which could be dependent to the length of the acyclic chain.

**Experimental section**

**General methods**

Commercially available chemicals were of reagent grade and used as received. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60F254, E. Merck). Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). The 1H and 13C NMR spectra were recorded on a Varian InovaUnity 400 spectrometer (400 MHz) in (d4) methanol, CDCl3, shift values in parts per million relative to SiMe₄ as internal reference. High resolution mass spectra were performed on a Bruker maXis mass spectrometer by the “Fédération de Recherche” IOCA/ CBM (FR2708) platform. The following products are known products or previously reported by our group: N3-benzoyluracil (7a), CAS registration 2775-87-3; N3-benzylthymine (7b), CAS registration 4330-20-5; N4,N4-bis(Boc)-cytosine (11a) CAS registration 1108637-28-0; 5-fluoro-N4,N4-bis(Boc)-cytosine (11b) CAS registration: 1450880-36-0.

(E)-1-Bromo-5-diethoxyporphosphoryl-5,5-difluoro-pent-2-ene (5)

A suspension of zinc powder (441.3 mg, 6.75 mmol, 99.99% purity) in THF (0.4 mL) was added to a solution of 1,2-dibromo-
moethane (0.96 mL, 1.13 mmol) in THF (5 mL) at room temperature, and was then warmed up to 65 °C. After 1 min, chlorotrimethylsilane (0.12 mL, 0.8 mmol) was added and the resulting mixture was stirred at 25 °C. After 15 min, the suspension was added dropwise to a solution of diethyl (bromodiethoxyporphosphoryl) methylphosphonate (0.8 mL, 4.5 mmol) in THF (2 mL), then the reaction mixture was stirred 12 h at 45 °C. CuBr (1.16 g, 8.1 mmol), activated LiCl (344.0 mg 8.1 mmol) and THF (4 mL) was added to the yellow solution at 0 °C under nitrogen, and then the resulting blue solution was stirred at 0 °C. After 10 min, trans-1,4-dibromo-2-buten (1.44 g, 6.75 mmol) was added and the reaction was stirred during 4 h at 0 °C. The mixture was filtered through celite and the filtrate was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO4, and concentrated under vacuum. The residue was purified by silica gel column chromatography with petroleum ether–EtOAc (4:1) to give 2 (1.15 g, 80%) as a colourless oil. 1H NMR (400 MHz, CDCl3) δ 5.88–5.76 (m, 1H), 5.29–5.22 (m, 1H), 4.27 (q,J = 7.3 Hz, 2H), 4.25 (q,J = 7.3 Hz, 2H), 2.81 (m, 2H), 1.36 (t,J = 7.3 Hz, 6H); 13C NMR (100 MHz, CDCl3) δ 125.2, 127.1, 127.0, 126.9, 121.4, 111.1, 111.4. 31P NMR (162 MHz, CDCl3) δ 6.99; HRMS (ESI): m/z [M + H]+ calcd for C₉H₇BrF₂O₃P: 321.006487, found: 321.006126.

**General procedure A: alkylation with nucleobases**

A solution of nucleobase (1.3 equiv.) in dry DMF (3 mL), Cs₂CO₃ (1.3 equiv.) and (E)-diethyl(5-bromo-1,1-difluoropent-3-ylphosphonate (1 equiv.) was stirred at room temperature...
under argon for 16 h. After removal of DMF under vacuum, the residue was purified by silica gel column chromatography with CHCl₃-MeOH (99:1 to 96:4) to the desired product.

**N⁴-Benzoyl-1-[\{E\}-5-diethoxyphosphoryl-5,5-difuoro-pent-2-en-1-yl]-uracil** (8a). The title compound was prepared from N⁴-benzoyluracil 7a following procedure A to give 8a (86%) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 7.90 (dd, J = 8.0, 1.2 Hz, 2H), 7.62 (dd, J = 8.0, 1.2 Hz, 1H), 7.47 (t, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 1H), 5.78 (m, 2H), 5.77 (d, J = 8.0 Hz, 1H), 4.35 (d, J = 6.0 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 4.24 (q, J = 7.2 Hz, 2H), 2.84 (m, 2H), 1.35 (t, J = 7.2 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 168.9, 162.5, 149.8, 143.7, 135.2, 131.5, 130.5, 129.6, 129.3, 125.7, 102.4, 64.8, 49.5, 37.7, 37.5, 37.4, 37.1, 16.5; ¹⁹F NMR (376 MHz, CDCl₃) δ −111.1, −111.4; ³¹P NMR (162 MHz, CDCl₃) δ 6.3; HRMS (ESI): m/z [M + H⁺] calcd for C₂₆H₂₆F₂N₂O₅P: 457.134078, found: 457.133456.

**N⁴-Benzoyl-1-[\{E\}-5-diethoxyphosphoryl-5,5-diﬂuoro-pent-2-en-1-yl]-thymine** (8b). The title compound was prepared from N⁴-benzoylthymine with typical procedure A to give 8b (81%) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 7.91 (dd, J = 8.0, 1.2 Hz, 2H), 7.63 (dd, J = 8.0, 1.2 Hz, 1H), 7.48 (t, J = 8.0 Hz, 2H), 7.17 (s, 1H), 5.79 (m, 2H), 4.35 (d, J = 6.0 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 2.87 (m, 2H), 1.35 (t, J = 7.2 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 169.2, 163.2, 149.9, 139.5, 135.1, 131.7, 130.5, 130.0, 129.2, 125.7, 125.6, 125.5, 111.2, 64.7, 49.2, 37.8, 37.6, 37.4, 37.2, 29.8, 16.5, 12.4; ³¹P NMR (376 MHz, CDCl₃) δ −110.0 (t, J = 18.8 Hz), −111.0 (t, J = 18.8 Hz); ³¹P NMR (162 MHz, CDCl₃) δ 6.4; HRMS (ESI): m/z [M + H⁺] calcd for C₂₂H₂₆F₂N₂O₅P: 471.149900, found: 471.149106.

General procedure B: deprotection of diethylphosphonate nucleosides

A septum-sealed microwave tube charged with diethylphosphonate derivative and trimethylsilylbromide (10.0 equiv.) in DMF (10 mL) was added to a solution of nucleoside 471.149106. The mixture was stirred under an argon atmosphere for 3 h. The resulting mixture was then diluted with EtOAc (2 × 20 mL), washed with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, and concentrated under vacuum. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (98:2 to 1:2) to give product 12a (279 mg, 85%) as a colourless oil; ¹H NMR (400 MHz, CDCl₃) δ 7.57 (d, J = 7.4 Hz, 1H), 6.99 (d, J = 7.4 Hz, 1H), 5.79 (m, 2H), 4.48 (d, J = 4.8 Hz, 2H), 2.47 (q, J = 7.3 Hz, 2H), 2.45 (q, J = 7.3 Hz, 2H), 2.83 (m, 2H), 1.37 (t, J = 7.2 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 162.3, 147.1, 130.2, 124.9, 96.5, 84.8, 64.5, 51.1, 37.4 (t, J = 21.5, 15.4 Hz), 27.7, 16.4; ¹⁹F NMR (376 MHz, CDCl₃) δ −110.8 (t, J = 19.0 Hz), −111.1 (t, J = 19.0 Hz); ³¹P NMR (162 MHz, CDCl₃) δ 7.09 (s), 6.44 (s), 5.78 (s); HRMS (ESI): m/z [M + H⁺] calcd for C₂₆H₂₆F₂N₂O₅P: 552.2281, found: 552.2284.

**N⁴-Bis(Boc)-1-[\{E\}-5-diethoxyphosphoryl-5,5-diﬂuoro-pent-2-en-1-yl]-5-fluorouracil** (12b). To a solution of N⁴,Bis(Boc)-5-fluorouracil 11b (113.5 mg, 0.34 mmol, 1.0 equiv.) in dry DMF (1.5 mL) was added Cs₂CO₃ (123 mg, 0.38 mmol, 1.1 equiv.) and the gem difluorinated phosphate 5 (211 mg, 0.65 mmol, 1.1 equiv.) at room temperature and stirred under an argon atmosphere for 3 h. The resulting mixture was then diluted with EtOAc (2 × 15 mL), washed with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, and concentrated under vacuum. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (98:2 to 1:2) to give product 12a (149 mg, 77%) as a yellow oil; ¹H NMR (400 MHz, CDCl₃) δ 7.74 (d, J = 4.5 Hz, 1H), 5.90−5.76 (m, 2H), 4.53 (d, J = 5.4 Hz, 2H), 4.27 (q, J = 7.1 Hz, 2H), 4.25 (q, J = 7.1 Hz, 2H), 2.87 (m, 2H), 1.47 (s, 18H), 1.37 (t, J = 7.1 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 155.95 (d, J = 14.3 Hz), 153.84, 149.02, 142.02, 139.56, 133.07 (d, J = 14.3 Hz), 129.21, 126.94 (m, 84.89, 64.80 (d, J = 8.5 Hz), 51.57, 37.60 (m), 29.82, 27.86, 16.53 (d, J = 5.5 Hz); ¹⁹F NMR (376 MHz, CDCl₃) δ −110.8, −111.1, −156.23; ³¹P NMR (162 MHz, CDCl₃) δ 6.23 (t, J = 105.8 Hz) HRMS (ESI): m/z [M + H⁺] calcd for C₂₂H₂₆F₂N₂O₅P: 570.2189, found 570.2187.

**N⁴-[\{E\}-5-Difuoro-5-phosphono-pent-2-en-1-yl]-cytosine** (13a). The title compound was prepared from phosphate 12a following procedure B to give 13a (>98%) as a colourless oil; ¹H NMR (250 MHz, MeOD) δ 7.95 (d, J = 5.8 Hz, 1H), 6.14 (d, J = 5.8 Hz, 1H), 5.87 (m, 2H), 4.44 (d, J = 8.50 Hz, 2H), 2.87 (m, 2H); ¹³C NMR (101 MHz, MeOD) δ 153.7, 153.4, 146.6, 136.0, 134.0, 133.7, 120.6, 36.9 (d, J = 22.1, 15.6 Hz); ¹⁹F NMR (376 MHz, MeOD) δ −113.19, −113.46, −170.84; ³¹P NMR (162 MHz, MeOD) δ 4.79 (t, J = 106.9 Hz); HRMS (ESI): m/z [M + H⁺] calcd for C₂₆H₂₆F₂N₄O₅P: 532.06365 found 532.0645.
The title compound was prepared from phosphonate following procedure B to give 13b (>98%) as a colourless oil; 1H NMR (250 MHz, MeOD) δ 7.95 (dJ = 7.7 Hz, 1H), 6.17–6.08 (m, 1H), 5.88 (dJ = 4.3 Hz, 1H), 4.50 (dJ = 5.0 Hz, 2H), 3.02–2.77 (m, 2H); 13C NMR (101 MHz, MeOD) δ 153.7, 154.4, 146.6, 136.0, 134.0, 133.7, 120.6, 36.9 (td, dJ = 22.1, 15.6 Hz); 19F NMR (376 MHz, MeOD) δ –113.3 (tJ = 19.0 Hz), –113.6 (tJ = 18.9 Hz); 31P NMR (162 MHz, MeOD) δ 5.86 (s), 5.04 (s), 4.40 (s); HRMS (ESI) m/z [M + H]+ calcd for C13H14F3N3O4P: 395.0606, found 395.0607.

N1-[15]-3,5-Difluoro-5-phosphono-2-pent-2-en-1-yl]-5-fluorocytosine (13b). The title compound was prepared from phosphonate following procedure B to give 13b (>98%) as a colourless oil; 1H NMR (400 MHz, MeOD) δ 8.49 (sJ = 1H), 6.04–5.96 (m, 1H), 5.85–5.71 (m, 1H), 4.93 (dJ = 6.1 Hz, 2H), 4.34–4.22 (m, 4H), 2.99–2.80 (m, 2H), 1.36 (tJ = 7.1 Hz, 6H); 13C NMR (101 MHz, MeOD) δ 159.4, 149.6, 149.5–149.1 (mJ = 145, 159, 130.7, 132.3 (dd, dJ = 10.9, 6.0 Hz), 120.4, 118.2, 64.8 (dJ = 7.0 Hz), 51.6, 45.7, 36.9 (ddJ = 21.6, 6.3 Hz), 15.4; 19F NMR (376 MHz, MeOD) δ –111.8 (tJ = 19.2 Hz), –112.1 (tJ = 19.2 Hz); 31P NMR (162 MHz, MeOD) δ 7.21 (dtJ = 15.7, 7.9 Hz), 6.54 (dtJ = 15.8, 7.9 Hz), 5.87 (dtJ = 15.9, 7.9 Hz); HRMS (ESI) m/z [M + H]+ calcd for C12H20F3N4O4P: 354.1185, found 354.1187.

The antiviral activity assays.

The antiviral activity assays were based on inhibition of virus-induced cytopathicity or plaque formation in HEL 299 (ATCC® CCL-137™) cell culture against herpes simplex virus 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV), and varicella-zoster virus (VZV). Moreover, the Vero (ATCC® CCL-81™) cell culture was utilized to test such compounds against parainfluenza-3, reovirus-1, Sindbis virus and Cossackie B4. Furthermore, the novel compounds were evaluated in HeLa cell culture against vesicular stomatitis virus, Cossackie virus B4, and respiratory syncytial virus or MDCK (ATCC® CCL-34™) [influenza A (H1N1; H3N2) and influenza B]. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 CCID50 of the virus (1 CCID50 being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU). After 1–2 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, 1.6, 0.32 μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC50 or concentration required reducing virus-induced cytopathogenicity or viral plaque (VZV) plaque formation by 50%. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. Alternatively, cytotoxicity of the test compounds was measured.
based on inhibition of cell growth. HEL cells were seeded at a rate of $5 \times 10^3$ cells per well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter.

The cytostatic concentration was calculated as the CC50, or the compound concentration required reducing cell proliferation by 50% relative to the number of cells in the untreated controls.

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References

1. E. De Clercq, Biochem. Pharmacol., 2007, 73, 911–922.
2. E. De Clercq, A. Holý, I. Rosenberg, T. Sakuma, J. Balzarini and P. C. Maudgal, Nature, 1986, 323, 464–467.
3. S. Eriksson, B. Munch-Petersen, K. Johansson and H. Eklund, Cell. Mol. Life Sci., 2002, 59, 1327–1346.
4. F. Pertusati, M. Serpi and C. McGuigan, Antiviral Chem. Chemother., 2012, 22, 181–203, review.
5. O. Baszczynski and Z. Janeba, Med. Res. Rev., 2013, 33, 1304–1344, review.
6. D. Topalis, U. Pradère, V. Roy, C. Caillat, A. Azzouzi, J. Broggi, R. Snoeck, G. Andrei, J. Lin, S. Eriksson, J. A. C. Alexandre, C. El Amri, D. Deville-Bonne, P. Meyer, J. Balzarini and L. A. Agrofoglio, J. Med. Chem., 2011, 54, 222–232.
7. V. D. Romanenko and V. P. Kukhar, Chem. Rev., 2006, 106, 3868–3935.
8. S. Halazy, A. Ehrhard and C. Danzí, J. Am. Chem. Soc., 1991, 113, 315–317.
9. S. A. Diab, C. De Schutter, M. Muzard, R. Plantier-Royon, E. Pfund and T. Lequeux, J. Med. Chem., 2012, 55, 2758–2768.
10. H. Kumamoto, D. Topalis, J. Broggi, U. Pradère, V. Roy, S. Berteina-Raboin, S. P. Nolan, D. Deville-Bonne, R. Snoeck, D. Garin, J. M. Crance and L. A. Agrofoglio, Tetrahedron, 2008, 64, 3517–3526.
11. O. Sari, M. Hamada, V. Roy, S. P. Nolan and L. A. Agrofoglio, Org. Lett., 2013, 15, 4390–4393.
12. M. Bessières, O. Sari, V. Roy, D. Warszycki, A. J. Bojarski, S. P. Nolan, R. Snoeck, G. Andrei, R. F. Schinazi and L. A. Agrofoglio, ChemistrySelect, 2016, 1, 3108–3113.
13. D. J. Burton and Z. Yang, Tetrahedron, 1992, 48, 189–275.
14. D. J. Burton and L. G. Sprague, J. Org. Chem., 1989, 54, 613–617.
15. J. Huang, E. D. St, S. P. Nola and J. L. Petersen, J. Am. Chem. Soc., 1999, 121, 2674–2678.
16. S. Fustero, A. Simon-Fuentes, P. Barrio and G. Haufe, Chem. Rev., 2015, 115, 871–930, review.
17. M. Bessières, V. Roy and L. A. Agrofoglio, RSC Adv., 2014, 4, 59747–59749.
18. A. Porcheddu, G. Giacomelli, I. Pirreda, M. Carta and G. Nieddu, Eur. J. Org. Chem., 2008, 5786–5797.
19. M. Hamada, V. Roy, T. R. Mcbrayer, T. Whitaker, C. Urbina-Blanco, S. P. Nolan, J. Balzarini, R. Snoeck, G. Andrei, R. F. Schinazi and L. A. Agrofoglio, Eur. J. Med. Chem., 2013, 67, 398–402.
20. J. H. Hong, S.-Y. Kim, C.-H. Oh, K. H. Yoo and J.-H. Cho, Nucleosides, Nucleotides Nucleic Acids, 2006, 25, 341–350.
21. S. Hikishima, M. Isobe, S. Koyanagi, S. Soeda, H. Shimeno, S. Shibuya and T. Yokomatsu, Bioorg. Med. Chem., 2006, 14, 1660–1670.