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Short communication

Branched-chain C-cyano pyranonucleosides: Synthesis of 3’-C-cyano & 3’-C-cyano-3’-deoxy pyrimidine pyranonucleosides as novel cytotoxic agents

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**A B S T R A C T**

This report describes the total and facile synthesis of 3’-C-cyano & 3’-C-cyano-3’-deoxy pyrimidine pyranonucleosides. Reaction of 3-keto glucoside 1 with sodium cyanide gave the desired precursor 3-C-cyano-1,2,5,6-di-O-isopropylidene-a-D-glucopyranose (2). Hydrolysis followed by acetylation led to the 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-glucopyranose (4). Compound 4 was condensed with silylated 5-fluorouracil, uracil, thymine and N\(^4\)-benzoylcytosine, respectively and deacetylated to afford the target 1-(3’-cyano-β-D-glucopyranosyl)nucleosides 6a–d. Routine deoxygenation at position 3’ of cyanoxydrin 2, followed by hydrolysis and acetylation led to the 3-C-cyano-3-deoxy-1,2,4,6-tetra-O-acetyl-β-D-allopyranose (10). Coupling of sugar 10 with silylated pyrimidines and subsequent deacetylation yielded the target 1-(3’-C-cyano-3’-deoxy-β-D-allopyranosyl)nucleosides 12a–d. The new analogues were evaluated for their antiviral and cytostatic activities. It was found that 6a was endowed with a pronounced anti-proliferative activity that was only 2- to 8-fold less potent than that shown for the parental base 5-fluorouracil. None of the compounds showed activity against a broad panel of DNA and RNA viruses.

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1. Introduction

Modified nucleosides constitute a major class of biologically active compounds, especially as antitumor and antiviral agents [1–4]. Cytotoxic nucleoside analogues were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer [5]. Nucleoside chemistry has also evolved to facilitate efficient routes to effective agents for the treatment of viral diseases caused by HIV [6] and herpes viruses [7]. Subsequently, nucleosides, which are frequently altered in the carbohydrate or base moiety, became the focus for the development of novel chemotherapeutic agents.

During the last decades, several branched-chain sugar nucleosides have been extensively studied for their potential antitumor or antiviral properties [8–11]. Attachment of the cyano group to the sugar moiety has been an attractive object for nucleoside chemists due to its small size and its great electron withdrawing character. Thus, cyano ribonoside nucleosides have been reported as interesting antiviral agents [12–14], while replacement of the hydroxyl group of 1-β-D-arabinofuranosylcytosine (ara-C) by the cyano group led to a new biologically active compound [15,16], with a novel mechanism of anticancer action [17].

Lately, nucleosides bearing pyranosyl rings have been evaluated for their potential antiviral [18–20], antioxidant [21] and antibiotic [22] properties and as building blocks in nucleic acid synthesis [23,24]. As part of our efforts to develop novel biologically active agents, we recently reported that new classes of uncommon 3’-flourinated pyranonucleosides have a promising potential in combating the rotaviral infections and in the treatment of colon cancer, and are efficient as antitumor growth inhibitors [25–29]. Experimental data also revealed that human poly(A)-specific ribonuclease [30] and glycogen phosphorylase [31] are among the molecular targets of these compounds.

In view of the interesting biological activity of the fluorinated pyranonucleosides, we decided to extend our studies to the synthesis of novel molecules in which an electron withdrawing cyano group replaces the fluorine atom. Therefore, we report the stereocontrolled synthesis of novel branched-chain C-cyano pyrimidine pyranonucleosides, i.e. 3’-C-cyano-β-D-glucopyranosides and 3’-C-cyano-3’-deoxy-β-D-allopyranosides, bearing 5-fluorouracil, uracil, thymine and cytosine as heterocyclic bases, in order to assess their biological activity. The chemical synthesis and biological activity of these compounds are presented herein.
2. Results and discussion

2.1. Chemistry

3′-C-Cyano-β-D-glucopyranonucleosides 6a–d were prepared according to the synthetic route outlined in Scheme 1. Treatment of the 1,2,5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (1) [32] with sodium cyanide in a two-phase ethylether/H2O system, in the presence of sodium bicarbonate, afforded the thermodynamically more stable gluco cyanohydrin epimer 2, in a virtually quantitative yield [33,34]. The assignment of its positions of the sugar moiety. hydrogen atom enters from the less hindered, of the furanose ring. In this type of radical deoxygenation, the cyano- and the presence of two leaving groups (OAc) at the 2- and 4-positions of the sugar moiety.

Hydrolysis of 2 using Amberlite IR 120 (H⁺) resin in methanol followed by acetylation using acetic anhydride (Ac2O) in pyridine [32] led to the 1,2,3,4,6-penta-O-acetyl-3′-C-cyano-D-glucopyranose (4). The protected, 1-(2′,3′,4′,6′-tetra-O-acetyl-3′-C-cyano-β-D-glucopyranosyl) pyrimidine nucleosides 5a–d were obtained upon coupling of the precursor material 4 with silylated 5-fluorouracil, uracil, thymine and N⁵-benzoylcytosine, respectively, in the presence of trimethylsilyl trifluoromethane-sulfonate (Me3SiOSO2CF3), in refluxing acetonitrile [35]. The 1H NMR spectra obtained for the protected nucleosides 5a–d, showed large coupling constants between protons H-1’ and H-2’ (J1,2 = 9.4–9.6 Hz), indicating an axial orientation of both protons and equatorially oriented base rings. Fully deprotection of 5a–d, performed by saturated methanolic ammonia [36], gave the desired nucleosides 6a–d.

Compound 2 was the starting material for the synthesis of 3′-cyano-β-D-allopyranonucleosides 12a–d (Scheme 1). Phenoxathiocarbonylation of 2 under a commonly used condition, phenyl chlorothionoformate, 4-((dimethylamino)pyridine (DMAP) and triethylamine (Et3N) in CH3CN [16], afforded the 3′-O-phenoxathiocarbonyl derivative 7, which proved to be unstable during the purification process. Therefore, crude 7 was directly submitted to deoxygenation with Bu3SnH in the presence of 2,2′-azobis(isobutyronitrile) (AIBN), to give the 3′-deoxy derivative sugar 8, in 76% overall yield. In order to elucidate the structure of the newly synthesized 8, NOE measurements were performed, as depicted in Fig. 1. The mutual NOE enhancements observed between H-2 with both H-1 and H-3 show that all these protons are in the same β face of the furanose ring. In this type of radical deoxygenation, the hydrogen atom enters from the less hindered, β-face of the planar radical intermediate, opposite to the bulky 1,2-O-isopropylidene group.

Hydrolysis of 8 using Amberlite IR 120 (H⁺) resin in methanol followed by direct standard acetylation led to the 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranose (10). Condensation of cyano sugar 10 with per-O-silylated 5-fluorouracil, uracil, thymine and N⁵-benzoylcytosine using Me3SiOSO2CF3 as activator, afforded the 1-(2′,3′,4′,6′-tri-O-acetyl-3′-C-cyano-3′-deoxy-β-D-allopyranosyl) pyrimidine nucleosides 11a–d, respectively [35]. 1H NMR data obtained for the newly synthesized nucleosides 11a–d (J1,2 = 9.4–9.7 Hz, J2,2 = 5.0–5.2 Hz), revealed the β-configuration of the sugar moiety and an axial oriented cyano group, respectively. Finally, treatment of 11a–d with hydrazine hydrate in buffered acetic acid (AcOH)–pyridine gave the fully deprotected nucleosides 12a–d, in yields that varied from 55% to 75%. Interestingly, attempts to deprotect 11a–d by NH4OH/methanol (MeOH) under the subsequent basic conditions produced complex mixtures, containing β-elimination products, probably due to the acidity of H-3′ and the presence of two leaving groups (OAc) at the 2′- and 4′-positions of the sugar moiety.

2.2. Biological activity

The cytostatic activity of 6a–d and 12a–d was determined against murine leukemia L1210, human lymphocyte CEM and human cervix carcinoma HeLa cell cultures. The test compounds showed poor, if any cytostatic activity against the three cell lines, except the 3′-cyano-5-fluorouracil pyranonucleoside 6a that showed pronounced anti-proliferative activity against all three cell lines. Its cytostatic activity spectrum was similar to that of the parent base 5-fluorouracil. It is currently unclear whether 6a is biologically active as such, or, alternatively, acts as a prodrug of 5-fluorouracil, from which the free base may be released by the action of phosphorolytic enzymes and/or by a spontaneous release (Table 1).

None of the compounds was endowed with activity against a broad panel of DNA and RNA viruses in cell culture at 100 μM.

3. Conclusion

In conclusion, the stereocontrolled synthesis of the 3′-cyano & 3′-cyano-3′-deoxy pyranonucleoside analogues bearing 5-fluorouracil, uracil, thymine and cytosine, respectively was undertaken. The target nucleosides were tested for their inhibitory effects on the proliferation of murine leukemia L1210, human lymphocyte CEM and human cervix carcinoma HeLa cell cultures. 3′-C-Cyano-5-fluorouracil pyranonucleoside 6a showed a similar cytostatic activity spectrum as the free base 5-fluorouracil.

4. Experimental part

4.1. Chemistry

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Merck precoated 60F244 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography was performed using silica gel (240–400 mesh, Merck). 1H and 13C NMR spectra were obtained at room temperature with a Bruker 400 spectrometer at 400 and 100 MHz, respectively, using CDCl3 and methanol-d4 (CD3OD) with internal tetramethylsilane (TMS). UV–Vis spectra were recorded on a PG T70 UV–VIS spectrometer and mass spectra were obtained with a Micromass Platform LC (ESI-MS). Optical rotations were measured using an Autopol 1 polarimeter. Infrared spectra were obtained with a Thermo Scientific Nicolet IR100 FT-IR spectrometer. Acetonitrile and toluene were distilled from calcium hydride and stored over 3E molecular sieves.

4.2. Synthesis of 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-glucopyranose (4)

4.2.1. Synthesis of 3-C-cyano-1,2,5,6-di-O-isopropylidene-α-D-glucuronic acid (2)

A mixture of 12,5,6-di-O-isopropylidene-α-D-ribo-hexofurananos-3-ulose (1) (4 g, 15.5 mmol), H2O (62 mL), ethylether (124 mL), sodium bicarbonate (2.6 g, 15.5 mmol) and sodium cyanide (0.76 g, 7.75 mmol) was stirred vigorously at room temperature overnight. The organic phase was separated, and the aqueous phase was washed with ethylether (2 x 124 mL). The combined ether phases were dried over Na2SO4, filtered and evaporated to dryness. The residue was purified by flash chromatography [(ethylacetate) EtOAc/hexane, 3:7] to give compound 2 (4.28 g, 97%, RF = 0.40 in EtOAc/hexane, 3:7) as a white solid, mp 98–100 °C [34], [α]D20 + 46 (c 0.3, CHCl3); 1H NMR (CDCl3); δ 5.97 (d, 1H, J1,2 = 3.4 Hz, H-1), 4.59 (d, 1H, H-2), 4.36–4.32 (m, 1H, H-5),...
4.2.2. Synthesis of 3-C-cyano-D-glucopyranose (3)

To a solution of 2 (2 g, 7.01 mmol) in MeOH (10.9 mL) and H₂O (62.2 mL) was added Amberlite IR 120 (H⁺) resin and the mixture was refluxed overnight. The reaction mixture was filtered and

Scheme 1. (i) H₂O, ethylether, NaHCO₃, NaCN; (ii) H₂O, MeOH, Amberlite IR 120 (H⁺); (iii) Ac₂O, pyridine; (iv) Silylated base, CH₃CN, Me₃SiOSO₂CF₃; (v) Methanolic ammonia; (vi) Phenyl chlorothionoformate, Et₃N, DMAP, CH₃CN, 0 °C; (vii) Bu₃SnH, AIBN, toluene, 100 °C; (viii) N₂H₄·H₂O, AcOH, pyridine.

4.24—4.21 (m, 2H, H-6a, H-4), 4.11 (m, 1H, H-6b), 4.04 (s, 1H, 3-ÔH), 1.58, 1.55, 1.39, 1.37 (4s, 12H, 4CH₃); Anal. Calcd for C₁₃H₁₉NO₆: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.84; H, 6.77; N, 4.82; ESI-MS (m/z): 286.32 (M + H⁺).
4.3.1. Synthesis of 1-(2-

4.2.4. Synthesis of 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-

4.2.3. Synthesis of 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-

4.2.2. Synthesis of 1-(2-

4.2.1. Synthesis of 1-(2-

4.2. Synthesis of 1-(3'-cyano-

4.1. Synthesis of 1-(2',3',4',6'-teta-O-acetyl-3'-C-cyano-

4.3. Synthesis of 3'-C-cyano-

4.3. Synthesis of 1-(3'-cyano-

4.3.4. Synthesis of 1-(3'-cyano-

4.3.3. Synthesis of 1-(2',3',4',6'-teta-O-acetyl-3'-C-cyano-

4.3.2. Synthesis of 1-(3'-cyano-

4.3.1. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

 evaporated to dryness to give compound 3 (1.25 g, 87%, RF = 0.33 in

4.3. Synthesis of 3'-C-cyano-

Fig. 1. NOE enhancements measured on compounds 2 and 8.

Table 1

| Compound | IC50 (μM) |
|----------|-----------|
| L1210    | CEM       | Hela     |
| 6a       | 1.9 ± 0.0 | 32 ± 9.6 | 4.5 ± 1.2 |
| 6b       | 417 ± 376 | > 500    | > 500     |
| 6c       | 535 ± 85  | > 500    | > 500     |
| 6d       | 147 ± 14  | > 500    | 377 ± 0   |
| 12a      | 550 ± 10  | > 500    | 819 ± 44  |
| 12b      | > 500     | > 500    | 811 ± 40  |
| 12c      | > 750     | > 750    | > 750     |
| 12d      | > 750     | > 750    | > 750     |
| F-Uracil | 0.49 ± 0.13 | 18 ± 5 | 0.54 ± 0.12 |

4.3.5. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

Thymine derivative 5c was synthesized from 4 by the similar procedure as described for 5a. It was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure 5c (0.18 g, 47%). RF = 0.47 in EtOAc/hexane, 1:1 as a white solid, mp 114–116 °C. [α]D22 − 2 (c 0.37, CHCl3); δmax 262 nm (ε 7950); 1H NMR (CDCl3): δ 8.36 (br s, 1H, NH), 7.37 (d, 1H, J6,6' = 5.6 Hz, H-6), 6.05 (dd, 1H, J1,2' = 9.5 Hz, J6,6' = 1.2 Hz, H-1'), 5.70 (d, 1H, H-5'), 5.64 (d, 1H, J6,6' = 10.2 Hz, H-4'), 4.45–4.39 (m, 1H, H-5), 4.22–4.14 (m, 2H, H-6a', H-6b'); 2.16, 2.14, 2.11, 2.04 (4s, 12H, 4OAc); Anal. Calc. for C20H16N2O11: C, 47.02; H, 4.15; N, 8.66; Found: C, 47.22; H, 4.21; N, 8.79; ESI-MS (m/z) 486.39 (M + H+).

4.3.4. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

4.3.3. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

4.3.2. Synthesis of 1-(3'-cyano-

4.3.1. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

Table 1 Cytostatic activity of 6a–d and 12a–d against a panel of tumor cell lines.

4.3. Synthesis of 3'-C-cyano-

4.2. Synthesis of 1-(3'-cyano-

4.1. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

Fig. 1. NOE enhancements measured on compounds 2 and 8.

Table 1

| Compound | IC50 (μM) |
|----------|-----------|
| L1210    | CEM       | Hela     |
| 6a       | 1.9 ± 0.0 | 32 ± 9.6 | 4.5 ± 1.2 |
| 6b       | 417 ± 376 | > 500    | > 500     |
| 6c       | 535 ± 85  | > 500    | > 500     |
| 6d       | 147 ± 14  | > 500    | 377 ± 0   |
| 12a      | 550 ± 10  | > 500    | 819 ± 44  |
| 12b      | > 500     | > 500    | 811 ± 40  |
| 12c      | > 750     | > 750    | > 750     |
| 12d      | > 750     | > 750    | > 750     |
| F-Uracil | 0.49 ± 0.13 | 18 ± 5 | 0.54 ± 0.12 |

4.3.5. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

Thymine derivative 5c was synthesized from 4 by the similar procedure as described for 5a. It was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure 5c (0.16 g, 57%, RF = 0.34 in EtOAc/hexane, 1:1) as a white solid, mp 127 °C. [α]D22 + 7 (c 0.32, CD3OD); δmax 258 nm (ε 11306); 1H NMR (CD3OD): δ 7.70 (d, 1H, J6,6' = 8.0 Hz, H-6), 6.01 (d, 1H, J1,2' = 9.0 Hz, H-1'), 5.70 (d, 1H, H-5'), 3.97–3.54 (m, 5H, H-6, H-4', H-5'), 6.6a', H-6b'; 13C NMR (CD3OD): δ 164.12, 150.23, 141.44, 119.01, 102.6, 92.22, 79.68, 71.72, 70.68, 68.35, 61.32; IR (Nujol, cm−1): 2230 (CN); Anal. Calc. for C19H16N2O11: C, 47.02; H, 4.15; N, 8.66; Found: C, 47.22; H, 4.21; N, 8.79; ESI-MS (m/z) 486.39 (M + H+).

4.3.3. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

4.3.2. Synthesis of 1-(3'-cyano-

4.3.1. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

4.2. Synthesis of 1-(3'-cyano-

4.1. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-

4.0.5% inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%. Data are the mean of 2–3 independent experiments (±S.D.).
4.3.6. Synthesis of 1-(3’-C-cyano-3-deoxy-D-glucopyranosyl)thymine (6c)

Thymine derivative 6c was synthesized from 5c by the similar procedure as described for 6a. The residue was purified by flash chromatography (CH2Cl2/MeOH, 9:1) to afford pure 6c (114 mg, 71%, RF as 0.32 in CH2Cl2/MeOH, 8:2) as a white foam. \[^{0}13C\]NMR (CD3OD): \(\delta\) 108.1 (s, 1H); 110.5 (d, 1H; \(J_{1-H_{5}} = 9.0\) Hz, H-1); 4.28 (dd, 1H, \(J_{6-F_{5}} = 9.7\) Hz, H-5, H-6); 4.23 (dd, 1H, \(J_{3-F_{2}} = 5.6\) Hz, H-2, H-3); 4.21–4.34 (m, 4H, H-4, H-5, H-6a, H-6b); 1.51 (s, 3H, CH3); 1.42 (s, 3H, CH3); 1.33 (s, 6H, 2CH3); 0.33 in EtOAc/hexane, 1:1) as a white solid, mp 428.36 (M\(+\)).

4.4.2. Synthesis of 3-C-cyano-3-deoxy-a-D-allopyranosyl-5-fluorouracil (11a)

A mixture of 5-fluouracil (126 mg, 0.97 mmol), HMDMS (254 µL, 1.20 mmol) and saccharine (8 mg, 0.045 mmol) in anhydrous CH2CN (3.5 mL) was refluxed for 30 min under nitrogen. 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranosyl-5-fluorouracil (10) (0.25 g, 0.69 mmol) and Me3SiOSO2CF3 (152 µL, 0.84 mmol) were then added and the reaction mixture was refluxed for 4 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH2Cl2 (200 mL). The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure 11a (0.19 g, 64%, RF as 0.33 in EtOAc/hexane, 1:1) as a white solid, mp 108–110 °C. \[^{0}13C\]NMR (CD3OD): \(\delta\) 108.1 (s, 1H); 110.5 (d, 1H; \(J_{1-H_{5}} = 9.0\) Hz, H-1); 4.28 (dd, 1H, \(J_{6-F_{5}} = 9.7\) Hz, H-5, H-6); 4.23 (dd, 1H, \(J_{3-F_{2}} = 5.6\) Hz, H-2, H-3); 4.21–4.34 (m, 4H, H-4, H-5, H-6a, H-6b); 1.51 (s, 3H, CH3); 1.42 (s, 3H, CH3); 1.33 (s, 6H, 2CH3); 0.33 in EtOAc/hexane, 1:1) as a white solid, mp 428.36 (M\(+\)).

4.5.1. Synthesis of 1-(2’4’6’-tri-O-acetyl-3’-C-cyano-3-deoxy-D-allopyranosyl)-5-fluorouracil (11b)

A mixture of 5-fluouracil (126 mg, 0.97 mmol), HMDMS (254 µL, 1.20 mmol) and saccharine (8 mg, 0.045 mmol) in anhydrous CH2CN (3.5 mL) was refluxed for 30 min under nitrogen. 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranosyl-5-fluorouracil (10) (0.25 g, 0.69 mmol) and Me3SiOSO2CF3 (152 µL, 0.84 mmol) were then added and the reaction mixture was refluxed for 4 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH2Cl2 (200 mL). The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure 11a (0.19 g, 64%, RF as 0.33 in EtOAc/hexane, 1:1) as a white solid, mp 108–110 °C. \[^{0}13C\]NMR (CD3OD): \(\delta\) 108.1 (s, 1H); 110.5 (d, 1H; \(J_{1-H_{5}} = 9.0\) Hz, H-1); 4.28 (dd, 1H, \(J_{6-F_{5}} = 9.7\) Hz, H-5, H-6); 4.23 (dd, 1H, \(J_{3-F_{2}} = 5.6\) Hz, H-2, H-3); 4.21–4.34 (m, 4H, H-4, H-5, H-6a, H-6b); 1.51 (s, 3H, CH3); 1.42 (s, 3H, CH3); 1.33 (s, 6H, 2CH3); 0.33 in EtOAc/hexane, 1:1) as a white solid, mp 428.36 (M\(+\)).
4.5.4. Synthesis of 1-(3'-cyano-3-deoxy-β-D-allopyranosyl)cytosine (12d)

Cytosine derivative 12d was synthesized from 11d by the similar procedure as described for 12a. The residue was purified by flash chromatography (CH2Cl2/MeOH, 9:1) to afford pure 12d (33 mg, 55%, RF = 0.25 in CH2Cl2/MeOH, 7:3) as an orange foam, $\delta^1{H} = 6.42 (c 0.50, CD3OD); \lambda_{\text{max}} 266 nm (ε 12306); ^1{H} NMR (CD3OD): 6.768 (d, 1H, J=5.7 Hz, H-3d), 5.97 (d, 1H, J=15.6 Hz, H-3d, H-3e, H-3f), 5.42 (d, 1H, H-3g), 4.36-4.46 (m, 3H, 6a, 6b, 6c), 3.70 (t, 1H, H-2d), 1.91 (s, 3H, 5b), 1.16 (s, 3H, 5c); ^13{C} NMR (CD2OD): 165.79, 155.73, 143.46, 118.86, 95.36, 93.94, 86.01, 67.38, 63.71, 63.12, 27.83; IR (Nujol, cm$^{-1}$): 2235 (CN); Anal. Calcld for C11H13N3O6C: C, 46.81; H, 5.00; N, 19.85; Found: C, 46.56; H, 4.78; N, 19.41; ESI-MS (m/z) 283.28 (M+H$^+$).

4.5.5. Antiviral and cytostatic assays

The antiviral assays [except anti-human immunodeficiency virus (HIV) assays] were based on inhibition of virus-induced cytolysis in HEL [murine simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus], Vero (parainfluenza-1, reovirus-1, Coxackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Coxackie virus B4, and respiratory syncytial virus), MDCK (influenza A (H1N1; H3N2) and B virus) and CrFK (feline coronavirus (FIPV) and feline herpes virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 (CCID50) of virus (1 CCID50 being the virus dose to inhibit 50% of the cell cultures) in the presence of varying concentrations (5,000, 1,000, 200 ... nM) of the test compounds. Viral cytolysis was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

The anti-HIV activity and anti-proliferative activity were evaluated against HIV-1 strain 118B and HIV-2 strain ROD in human T-lymphocyte CEM cell cultures. Briefly, virus stocks were titrated in CEM cells and expressed as the 50% cell culture infective dose (CCID50). CEM cells were suspended in culture medium at ~3 x 10^5 cells/ml and infected with HIV at ~100 CCID50. Immediately after viral exposure, 100 µl of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4-day incubation period at 37 °C, the giant cell formation was microscopically determined. Compounds were tested in parallel for cytostatic effects in unaffected CEM cell cultures.

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References

[1] W. Zhou, G. Gumina, Y. Chong, J. Wang, R.F. Shinazi, C.K. Chu, J. Med. Chem. 47 (2004) 3399–3408.
[2] R.K. Robins, G.D. Kini, in: D.E.V. Wilman (Ed.), The Chemistry of Antitumor Agents, Chapman and Hall, New York, 1990, pp. 299–321.
[3] M. MacCoss, M.J. Robins, in: D.E.V. Wilman (Ed.), The Chemistry of Antitumor Agents, Chapman and Hall, New York, 1990, pp. 261–298.
[4] D. Komiotis, S. Manta, E. Tsoukala, N. Tzioumaki, Curr. Med. Chem. Anti-Infect. Agents 7 (2008) 219–244.
[5] C.M. Calmarini, J.R. Mackey, C. Dumontet, Lancet Oncol. 3 (2002) 415–424.
[6] E. De Clercq, Biochim. Biophys. Acta 1587 (2002) 258–275.
[7] R.C. Brady, D.I. Bernstein, Antivir. Res. 61 (2004) 73–81.
[8] K. Takenuki, A. Matsuda, T. Ueda, T. Sasaki, A. Fujii, K. Yamagami, J. Med. Chem. 31 (1988) 1063–1064.
[9] J.R. McCarthy, D.P. Matthews, D.M. Stemerick, E.W. Huber, P. Bey, B.J. Lippert, R.D. Snyder, P.S. Sunkara, J. Am. Chem. Soc. 113 (1991) 7439–7440.
[10] H. Hattori, M. Tanaka, M. Fukushima, T. Sasaki, A. Matsuda, J. Med. Chem. 39 (1996) 5005–5011.
[11] H. Hayakawa, S. Kohgo, K. Kitano, N. Ashida, E. Kodama, H. Mitsuya, H. Ohrui, Antiviral. Chem. Chemother. 15 (2004) 169–187.
[12] M.J. Camarasa, A. Diaz-Ortiz, A. Calvo-Mateo, F.G. De las Heras, J. Balzarini, E. De Clercq, J. Med. Chem. 32 (1989) 1732–1738.
[13] C. O-Yang, H.Y. Wu, E.B. Fraser-Smith, K.A.M. Walker, Tetrahedron Lett. 33 (1992) 37–40.
[14] W. Zhu, G. Gumina, R.F. Shinazi, C.K. Chu, Tetrahedron 59 (2003) 6423–6431.
[15] A. Matsuda, Y. Nakajima, A. Azuma, M. Tanaka, T. Sasaki, J. Med. Chem. 34 (1991) 2917–2919.
[16] A. Azuma, Y. Nakajima, N. Nishizono, N. Minakawa, M. Suzuki, K. Hanaoka, T. Kobayashi, M. Tanaka, T. Sasaki, A. Matsuda, J. Med. Chem. 36 (1993) 4183–4189.
[17] A. Azuma, P. Huang, A. Matsuda, W. Plunkett, Mol. Pharmacol. 59 (2001) 725–731.
[18] I. Verheggen, A. Van Aerschot, L. Van Meervelt, J. Rozenski, L. Wiebe, R. Snoeck, G. Andrei, J. Balzarini, P. Claes, E. De Clercq, P. Herdewijn, J. Med. Chem. 38 (1995) 826–835.
[19] T. Ostrowski, B. Wroblowska, R. Busson, J. Rozenski, E. De Clercq, M.S. Bennet, J.N. Champness, W.C. Summers, P. Herdewijn, J. Med. Chem. 41 (1998) 4343–4353.
[20] Y. Maurinsh, J. Schraml, H. De Winter, N. Blaton, O. Peeters, E. Lescrienier, J. Rozenski, A. Van Aerschot, E. De Clercq, R. Busson, P. Herdewijn, J. Org. Chem. 62 (1997) 2861–2871.
[21] C. Spanou, S. Manta, D. Komiotis, A. Dervishi, D. Kouretas, Int. J. Mol. Sci. 8 (2007) 695–704.
[22] A. Haouz, V. Vanheusden, H. Munier-Lechman, M. Froeyen, P. Herdewijn, S. Van Calenbergh, M. Delarue, J. Biol. Chem. 278 (2003) 4983–4971.
[23] K. Vastmans, S. Pochet, A. Peys, L. Kerremans, A. Van Aerschot, C. Hendrix, P. Marliere, P. Herdewijn, Biochemistry 39 (2000) 12757–12765.
[24] K. Vastmans, M. Froeyen, L. Kerremans, S. Pochet, P. Herdewijn, Nucleic Acids Res. 29 (2001) 3154–3163.
[25] S. Manta, G. Agelis, T. Botić, A. Cencić, D. Komiotis, Bioorg. Med. Chem. 15 (2007) 980–987.
[26] S. Manta, G. Agelis, T. Botić, A. Cencić, D. Komiotis, Eur. J. Med. Chem. 43 (2008) 420–428.
[27] S. Manta, E. Tsoukala, N. Tzioumaki, A. Goropevsek, R.T. Pamulapati, A. Cencić, D. Komiotis, Eur. J. Med. Chem. 44 (2009) 2696–2704.
[28] S. Manta, N. Tzioumaki, E. Tsoukala, A. Panagiotopoulos, M. Pelecanou, J. Balzarini, D. Komiotis, Eur. J. Med. Chem. 44 (2009) 4764–4771.
[29] E. Tsoukala, N. Tzioumaki, S. Manta, A. Riga, J. Balzarini, D. Komiotis, Bioorg. Chem. 38 (2010) 285–293.
[30] N.A.A. Balatsos, D. Vlachakis, P. Maragoudis, S. Manta, D. Anastasakis, A. Kyritsis, M. Vlassi, D. Komiotis, C. Stathopoulos, Biochemistry 48 (2009) 6044–6052.
[31] V.G. Tsirkone, I. Tsoukala, C. Lamprakis, S. Manta, J.M. Hayes, V.T. Skamnaki, C. Drakou, S.E. Zographos, D. Komiotis, D.D. Leonidou, Bioorg. Med. Chem. 18 (2010) 3423–3425.
[32] J. Elhalabi, K.G. Rice, Nucleosides Nucleotides Nucleic Acids 23 (2004) 195–205.
[33] J.M. Bourgeois, Helv. Chim. Acta 58 (1975) 365–372.
[34] J.A. Rossenthal, B.L. Cliff, Can. J. Chem. 54 (1976) 543–547.
[35] H. Vorbruggen, G. Ho, Chem. Ber 114 (1981) 1256–1268.
[36] V. Vanheusden, R. Busson, P. Herdewijn, S. Van Calenbergh, J. Org. Chem. 69 (2004) 4446–4453.