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Exploring the purine core of 3′-*C*-ethynyladenosine (EAdo) in search of novel nucleoside therapeutics

Fabian Hulpia [a], Jan Balzarini [b], Dominique Schols [b], Graciela Andrei [b], Robert Snoeck [b], Serge Van Calenbergh [a,*]

[a] *Laboratory for Medicinal Chemistry, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium*
[b] *KU Leuven, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

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ABSTRACT

A series of new nucleoside analogues based on a C-3 branched ethynyl sugar derivative as present in 3′-*C*-ethynylcytidine (ECyd) and -adenosine (EAdo), combined with modified purine bases was synthetized and evaluated against a broad array of viruses and tumour cell lines. The pronounced cytostatic activity of EAdo was confirmed. EAdo and its 2,6-diaminopurine analogue showed inhibitory activity against vaccinia virus (EC_{50}: 0.31 and 51 μM, respectively). Derivative **10** on the other hand was found active against varicella zoster virus (EC_{50}: 4.68 μM).

Nucleoside analogues have been used as therapeutics for the treatment of cancer and viral infections for over 50 years. Despite this fact, new agents with improved efficacy and tolerability have still been discovered over the past decade.[1]

Recently, a strategy relying on building nucleosides with a fixed sugar and varying heterocyclic bases has shown to reveal intriguing biological activity.[2,3] Additionally, previous focussed profiling (e.g., only HIV, HCV or anticancer) might have overlooked starting points for other indications (e.g., cytotoxicity in antiviral assays or activity against other viruses). Furthermore, re-investigating certain sugar modifications has already shown to be a fruitful approach with some derivatives ultimately being advanced into clinical trials.[4]

With this in mind, research was initiated to explore the untapped potential of certain nucleoside scaffolds. We therefore became interested in a C-3 branched sugar motif, as is present in a former Phase-II candidate, 3′-*C*-ethynylcytidine (**2**, ECyd). After the discovery in the mid-90s by Matsuda et al.,[5] SAR of its sugar[6] as well as its heterocyclic[7] part has been reported, but failed to produce analogues with improved activity. The adenosine (**1**, EAdo) congener of ECyd however, which also showed antitumour properties,[5] only received scarce follow-up[8,9] (see Fig. 1).

Based on these findings, the C-3 ethynyl ribofuranose unit was believed a good sugar starting point for the 'mix-and-match' strategy mentioned above. In this study we concentrated on the poorly explored purine analogues.

In order to prioritize, we focused on substituents present in FDA-approved nucleosides as well as clinical phase analogues. This translated into modifications of EAdo at both the C-2 and C-6 position of the purine ring to give a heterocycle pattern as is, for example, found in the two FDA-approved nucleosides nelarabine **3** and clofarabine **4**.[1] Additionally, two modifications (cyclopentylamine, 3-chlorobenzylamine) were included as they are preferred in purinergic receptor ligands.[10]

The synthesis of the key ribofuranose building block **4** was accomplished using known literature procedures.[7,11] Next, three different purines were subjected to 'classical' Vorbrüggen conditions to achieve glycosylation with **4**[12] (Scheme 1). Deprotection or nucleophilic aromatic substitution with either NH_3 in MeOH or NaOMe in MeOH yielded the corresponding target nucleosides **1**,[5] **7**, **8**, **12** and **13**. Compounds **9** and **10** were obtained by nucleophilic aromatic substitution with the appropriate amine, immediately followed by deprotection. Initially, synthesis of **14** and **15** was also attempted from **11** and **6**, respectively. However, due to prolonged exposure and elevated temperature to force nucleophilic aromatic ring substitution, the desired product could not be isolated. Instead, the product formed was the enol ether resulting from a 5-*exo* dig cyclization of the 5′-OH onto the alkyne (**16** and **17**). Therefore, ring substitution was performed before glycosylation (Scheme 2), resulting in **20** and **21**. Final deprotection of these intermediates furnished **14** and **15**.

* Corresponding author. Tel.: +32 9 264 81 24; fax: +32 9 264 81 46.
E-mail address: Serge.VanCalenbergh@ugent.be (S. Van Calenbergh).

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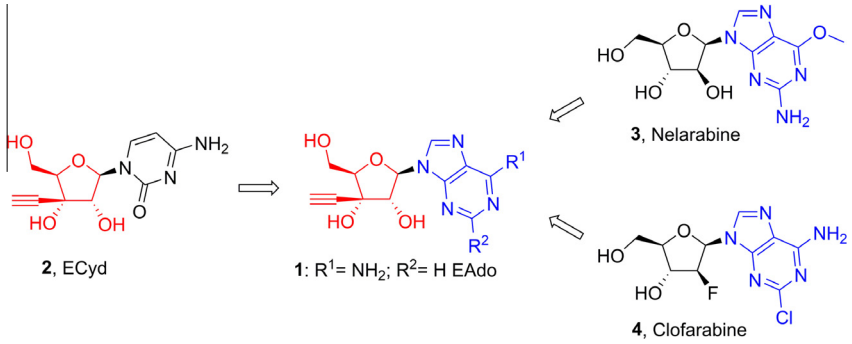
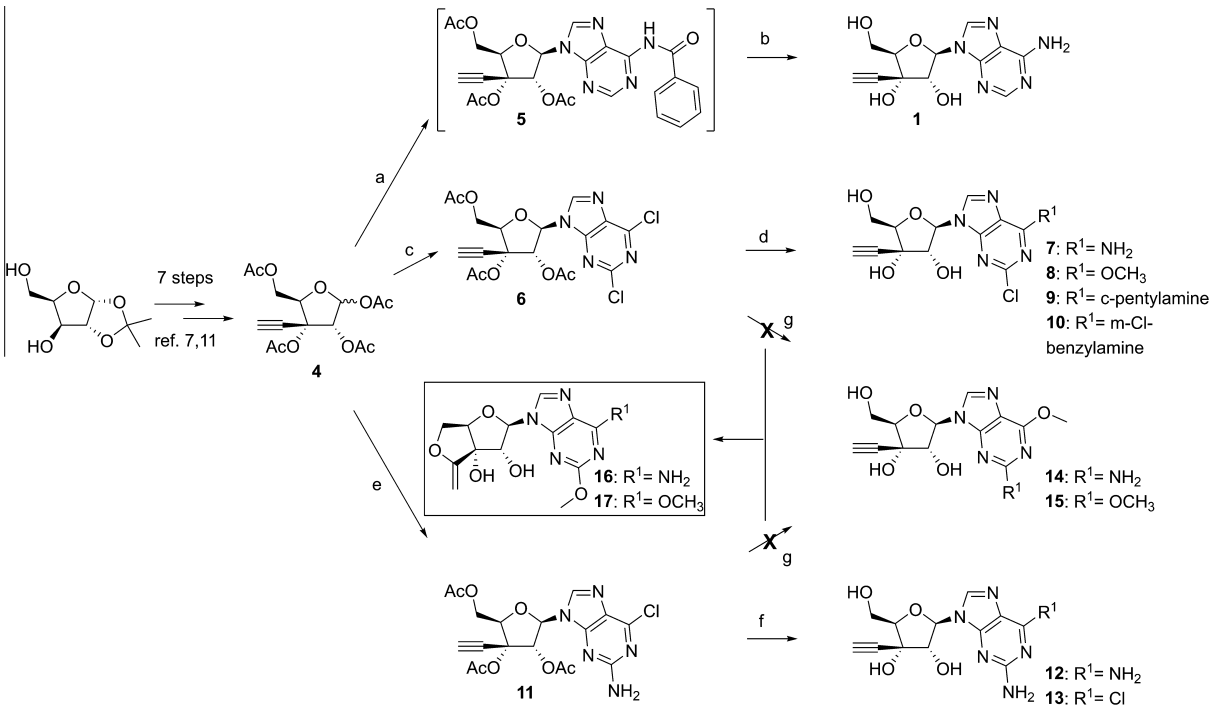
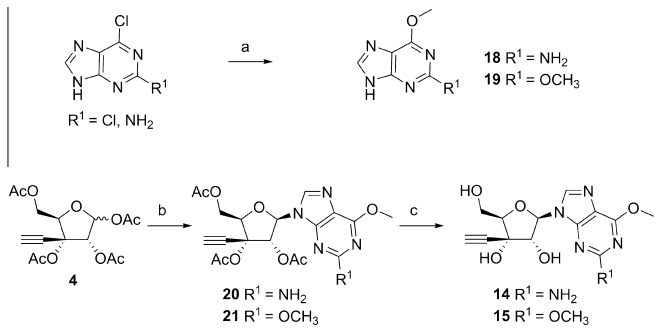


Figure 1.



Scheme 1. Reagents and conditions: (a) (1) *N*-benzoyladenine, HMDS, cat. $(NH_4)_2SO_4$, reflux, (2) TMSOTf, MeCN, reflux; (b) 7 N NH_3 in MeOH (40% over 2 steps (a + b)); (c) (1) 2,6-dichloropurine, HMDS, cat. $(NH_4)_2SO_4$, reflux, (2) TMSOTf, 1,2-dichloroethane, reflux (72%); (d) 7 N NH_3 in MeOH for **7** (31%), NaOMe in MeOH for **8** (72%), for **9**: (1) c-pentylamine, EtOH, reflux, (2) 7 N NH_3 in MeOH (85%); for **10**: (1) 3-chlorobenzylamine, EtOH, reflux, (2) 7 N NH_3 in MeOH (77%); (e) (1) 2-amino-6-chloropurine, HMDS, cat. $(NH_4)_2SO_4$, reflux, (2) TMSOTf, 1,2-dichloroethane, reflux (43%); (f) 7 N NH_3 in MeOH for **12** (25%) and NaOMe in MeOH for **13** (63%); (g) NaOMe in MeOH, rt to reflux, 38% for **16** and 29% for **17**.



Scheme 2. Reagents and conditions: (a) Na, MeOH, reflux, 91% for **18**; 83% for **19**; (b) (1) 2-amino-6-methoxypurine (**18**) or 2,6-dimethoxypurine (**19**), HMDS, cat. $(NH_4)_2SO_4$, reflux, (2) TMSOTf, 1,2-dichloroethane, reflux; 29% for **20**; 36% for **21**; (c) NaOMe in MeOH; 62% for **14**; 66% for **15**.

All final compounds were investigated for their potential activity against a broad array of viruses including herpes simplex virus-1 and -2 (HSV), cytomegalovirus (CMV), varicella zoster virus (VZV), vaccinia virus (VV), adenovirus-2, influenza A virus (H1N1, H3N2), influenza B virus, feline corona virus, feline Herpes virus, para-influenza virus, reovirus-1, sindbis virus, Coxsackie virus B4, Punta Toro virus, vesicular stomatitis virus, respiratory syncytial virus (RSV), HIV-1 and HIV-2. Additionally, inhibition of cell proliferation of murine leukemia (L1210), human CD_4^+ T-lymphocyte (CEM) and human cervix carcinoma cells (HeLa) was also evaluated (Table 1).

EAdo **1** was confirmed to exhibit potent cytostatic activity, while all other derivatives were found to be poorly cytostatic (IC_{50}: 104 > 250 μM). Compound **10** was found to be moderately cytotoxic (22–50 μM) in the three cell lines tested, but at least 30 times less potent then EAdo. All nucleosides were found to be inactive in the antiviral assays up to a concentration of 100 μM,

Table 1
Inhibition of proliferation

	L1210 IC_{50} (μM)	CEM IC_{50} (μM)	HeLa IC_{50} (μM)
1	0.73 ± 0.14	0.61 ± 0.08	0.29 ± 0.11
7	167 ± 37	>250	>250
8	>250	210 ± 56	175 ± 106
9	170 ± 28	104 ± 33	150 ± 1
10	50 ± 14	22 ± 10	43 ± 20
12	193 ± 80	⩾250	123 ± 7
13	>250	>250	>250
14	>250	>250	>250
15	>250	>250	>250

except for three. EAdo **1** showed potent anti-vaccinia virus activity at subtoxic concentrations [EC_{50}: 0.35 ± 0.05 μM; MCC (minimal cytotoxic concentration): 20 μM]. Diaminopurine derivative **13**, showed weak activity against vaccinia virus (EC_{50}: 51 ± 6 μM; MCC: >100 μM). Finally, *m*-chlorobenzylamino derivative **10** showed moderate activity against VZV (EC_{50}: 4.68 μM; MCC: 100 μM; CC_{50}: 34.46 μM), with no markedly different results obtained in either TK^+ or TK^- strains.

Compounds **9** and **10** were also evaluated for their agonistic behaviour at the adenosine A_3-receptor. Both were found to bind only weakly (70 ± 9% and 48 ± 6% inhibition at 10 μM for **9** and **10**, respectively), which is in line with previous observations.[13]

Interestingly, compound **7** did not show any cytostatic activity, even though the C-2 chloro substituent should make it more resistant towards adenosine deaminase,[14] the enzyme responsible for the breakdown of EAdo.[9] Furthermore, lack of phosphorylation by cellular kinase(s) could also be a contributor to the observed results, and further investigation on a prodrug approach that allows intracellular release of the monophosphate form might be more promising.

In conclusion, a subset of purine-modified nucleosides based on C-3 branched chain sugar matched with different purines was synthetized and evaluated against a broad array of viruses and tumour cell lines. The potent cytostatic activity of EAdo was confirmed. This compound was found inhibitory to vaccinia virus at subtoxic concentrations. Two of the newly synthetized compounds were found active antivirally. While their activity is only moderate, they could serve as a starting point for further structural elaboration to improve antiviral activity. Furthermore, these results indicate the usefulness of the 'mix-and match' approach in finding novel biologically active nucleosides.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.005.

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