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

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A series of new nucleoside analogues based on a C-3 branched ethynyl sugar derivative as present in 3'-C-ethynlycytidine (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 cytotstatic activity of EAdo was confirmed. EAdo and its 2,6-diaminopurine analogue showed inhibitory activity against vaccinia virus (EC50: 0.31 and 51 μM, respectively). Derivative 10 on the other hand was found active against varicella zoster virus (EC50: 4.68 μM).

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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 (IC50: 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 than EAdo. All nucleosides were found to be inactive in the antiviral assays up to a concentration of 100 μM.

**Scheme 1.** Reagents and conditions: (a) 1-N-benzoyladenine, HMDS, cat. (NH4)2SO4, reflux, (2) TMSOTf, MeCN, reflux; (b) 7 N NH3 in MeOH (40% over 2 steps (a + b)); (c) 1,2-dichloropurine, HMDS, cat. (NH4)2SO4, reflux, (2) TMSOTf, 1,2-dichloroethane, reflux (72%); (d) 7 N NH3 in MeOH for 7 (31%), NaOMe in MeOH for 8 (72%), for 9: (1) c-pentylamine, EtOH, reflux, (2) 7 N NH3 in MeOH (98%); for 10: (1) 3-chlorobenzylamine, EtOH, reflux, (2) 7 N NH3 in MeOH (77%); (e) 1,2-dimethyl-6-chloropurine, HMDS, cat. (NH4)2SO4, reflux, (2) TMSOTf, 1,2-dichloroethane, reflux (43%); (f) 7 N NH3 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-dimethyl-6-chloropurine (18) or 2,6-dimethoxypurine (19), HMDS, cat. (NH4)2SO4, reflux, (2) TMSOTf, 1,2-dichloroethane, reflux; 29% for 20; 36% for 21; (c) NaOMe in MeOH; 62% for 14; 66% for 15.
except for three. EAdo 1 showed potent anti-vaccinia virus activity at subtoxic concentrations [EC\(_{50}\): 0.35 ± 0.05 \(\mu\)M; MCC (minimal cytotoxic concentration): 20 \(\mu\)M]. Diaminopurine derivative 13, showed weak activity against vaccinia virus (EC\(_{50}\): 51 ± 6 \(\mu\)M; MCC: >100 \(\mu\)M). Finally, m-chlorobenzylamino derivative 10 showed moderate activity against VZV (EC\(_{50}\): 4.68 \(\mu\)M; MCC: 100 \(\mu\)M; CC\(_{50}\): 34.46 \(\mu\)M), 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 \(\mu\)M for 9 and 10, respectively), which is in line with previous observations.\(^9\) 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 synthesized 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 synthesized 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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