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SHORT COMMUNICATIONS

Antiviral, antimetabolic and antineoplastic activities of 2′- or 3′-amino or -azido-substituted deoxyribonucleosides

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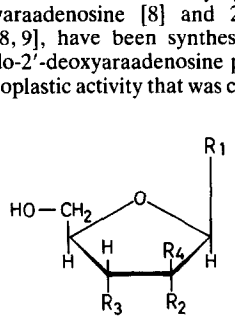
Several nucleoside analogues are endowed with either antiviral activity [e.g. 5-iodo-2′-deoxyuridine (IDU)], antitumor activity, or both [e.g. 9-β-D-arabinofuranosyladenine, araadenosine (ara-A) and 1-β-D-arabinofuranosylcytosine, aracytidine (ara-C]. In attempts to increase the selectivity and/or potency of these antiviral and antitumor agents, various modifications have been carried out at either the heterocyclic or sugar moiety. In some cases, this approach yielded quite valuable compounds, i.e. (*E*)-5-(2-bromovinyl)-2′-deoxyuridine [1] and 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine [2], which were more active and more selective in their antiherpes virus activity than the compounds they were derived from (IDU and ara-C, respectively). Similarly, the 5′-aminoanalogue of IDU proved more selective, albeit less active, as an antiherpes agent than its parent compound [3, 4]. Yet, the 5′-amino and 5′-azido analogues of ara-C had no antiviral activity [5]. Neither did they inhibit tumor cells [5].

Here we report on the antiviral and antitumor properties of a new series of nucleoside analogues, namely 2′-deoxy- and 2′,3′-dideoxyribonucleosides, in which an amino or azido group was substituted at the 2- and/or 3-position of the sugar moiety. Some 2′- or 3′-amino (or azido) nucleoside analogues, viz. 3′-amino-3′-deoxythymidine [6, 7], 2′-amino-2′-deoxyaraadenosine [8] and 2′-azido-2′-deoxyaraadenosine [8, 9], have been synthesized in the past: whereas 2′-azido-2′-deoxyaraadenosine possessed an antiviral and antineoplastic activity that was comparable to that of ara-A, 2′-amino-2′-deoxyaraadenosine was relatively inert as an antiviral or antineoplastic agent [8, 9]. Also, 3′-amino-3′-deoxythymidine potently inhibited tumor cell growth, but was not good as an antiviral agent [6, 7].



		R_1	R_2	R_3	R_4
2′-amino-2′-deoxyuridine	(14)	uracil	NH_2	OH	H
2′-amino-2′-deoxycytidine	(14)	cytosine	NH_2	OH	H
2′-amino-2′-deoxyadenosine	(12)	adenine	NH_2	OH	H
2′-amino-2′-deoxyguanosine	(12)	guanine	NH_2	OH	H
2′-azido-2′-deoxycytidine	(15)	cytosine	N_3	OH	H
2′-azido-2′-deoxyadenosine	(10)	adenine	N_3	OH	H
2′-azido-2′-deoxyguanosine	(10)	guanine	N_3	OH	H
3′-amino-2′,3′-dideoxyadenosine	(11)	adenine	H	NH_2	H
3′-amino-2′,3′-dideoxyguanosine	(11)	guanine	H	NH_2	H
3′-azido-3′-deoxythymidine	(11)	thymine	H	N_3	H
3′-azido-3′-deoxyadenosine	(13)	adenine	OH	N_3	H
3′-azido-3′-deoxyaraadenosine	(13)	adenine	H	N_3	OH
3′-azido-2′,3′-dideoxyadenosine	(11)	adenine	H	N_3	H
3′-azido-2′,3′-dideoxyguanosine	(11)	guanine	H	N_3	H

Fig. 1. Formulae of 2′- or 3′-amino- or -azido-substituted deoxyribonucleosides. References to the synthesis of the compounds are indicated in parentheses.

The 2′- and 3′-amino (or azido) 2′-deoxyribonucleosides that were evaluated for antiviral and antitumor activity are depicted in Fig. 1. Their synthesis has been described elsewhere [10–15]. One of these nucleoside analogues, 2′-azido-2′-deoxycytidine [15], has already been the subject of some previous studies: the compound was shown to inhibit mammalian cell DNA replication [16], polyoma virus DNA replication [17, 18], and, in its 5′-triphosphate form, it also inhibited the action of primase in a reconstructed *E.coli* enzyme system [19].

The antiviral properties of the compounds were assessed in primary rabbit kidney (PRK) cell cultures challenged with either vaccinia, herpes simplex or vesicular stomatitis virus. The methodology for measuring antiviral activity has been described previously [1]. The ID_{50} for antiviral activity was defined as the concentration of compound required to reduce viral cytopathogenicity by 50 per cent, when it had reached completion in the control virus-infected cell cultures. The antitumor potentials of the compounds were explored with L1210 mouse leukemic cells as the indicator system [20]. These assays were performed in Linbro microplates; L1210 cells were seeded at 50,000 cells per well, in the presence of various concentrations of test compound, and allowed to proliferate for 48 hr at 37° in a humidified CO_2-controlled atmosphere. At the end of this incubation period, the cells were counted in a Coulter counter. The number of dead cells was evaluated by staining with trypan blue. The ID_{50} was defined as the concentration of compound required to reduce the number of living cells by 50 per cent. In both PRK and L1210 cells DNA synthesis was measured by monitoring [^{3}H-methyl]deoxythymidine (dThd) incorporation into acid-insoluble material, as has also been described previously [1, 20]. The ID_{50} for antimetabolic activity was defined as the concentration of compound required to reduce [^{3}H-methyl] dThd incorporation by 50 per cent.

None of the compounds tested appeared to exert an appreciable inhibitory effect on either herpes simplex virus or vesicular stomatitis virus replication (Table 1). Several compounds, i.e. 2′-amino-2′-deoxyadenosine and 3′-azido-3′-deoxyadenosine, inhibited vaccinia virus replication at a fairly low concentration (4 μg/ml). However, these compounds were cytotoxic at 40 μg/ml, thus affording a safety margin of only 10-fold. Various other compounds, i.e. 2′-azido-2-deoxyxytidine, 3′-amino-2′,3′-dideoxyguanosine and 3′-azido-2′,3′-dideoxyadenosine, were active against vaccinia virus, but, again, at concentrations that were only slightly lower than those afflicting normal cell morphology. Some selectivity was displayed by 3′-azido-3′-deoxyaraadenosine, which inhibited vaccinia virus replication at 20 μg/ml, while not being cytotoxic at 200 μg/ml. However, in terms of potency, the 3′-azido analogue of ara-A compared unfavorably to its parent compound, which inhibited

Table 1. Antiviral, antimetabolic and antineoplastic effects of 2′- or 3′-amino- or -azido-substituted deoxyribonucleosides in primary rabbit kidney (PRK) cell cultures and mouse leukemia L1210 cells

	ID_{50}* (μg/ml)						
Compound	Vaccinia virus (PRK)	Herpes simplex 1 (KOS) virus (PRK)	Vesicular stomatitis virus (PRK)	[^{3}H-methyl]dThd incorporation into host cell DNA (PRK)	Normal cell morphology (PRK)	Cell proliferation (L1210)	[^{3}H-methyl]dThd incorporation into host cell DNA (L1210)
2′-amino-2′-deoxyuridine	150	>200	>200	>200	>200	243	>1000
2′-amino-2′-deoxycytidine	≥200	>200	150	>200	>200		>1000
2′-amino-2′-deoxyadenosine	4	≥40	≥40	10	40	20.6	324
2′-amino-2′-deoxyguanosine	>200	>200	>200	>200	>200	3.9	>1000
2′-azido-2′-deoxycytidine	40–100	≥200	>200	20	200	20.3	147
2′-azido-2′-deoxyadenosine	40–100	>200	>200		>200		
2′-azido-2′-deoxyguanosine	>200	>200	>200	100	>200	313	378
3′-amino-2′,3′-dideoxyadenosine	>200	>200	>200	100	>200	37.2	>1000
3′-amino-2′,3′-dideoxyguanosine	40–100	100	>200	10	100		
3′-azido-3′-deoxythymidine	40–100	>200	>200	7	>200	280	9.6
3′-azido-3′-deoxyadenosine	4	≥40	≥40	10	40	39.0	310
3′-azido-3′-deoxyaraadenosine	20	>200	>200	50	>200	498	>1000
3′-azido-2′,3′-dideoxyadenosine	4–10	>100	>200	4	100	1.88	45
3′-azido-2′,3′-dideoxyguanosine	>200	>200	>200	100	>200	53.0	>1000
Aracytidine (ara-C)	0.04	0.04	10	0.1	0.4	0.007	0.03
Araadenosine (ara-A)	0.4	4	30	10	100	26	39

* Concentration of compound to reduce virus-induced cytopathogenicity (in PRK cells) or the number of exponentially growing (L1210) cells or [^{3}H-methyl]dThd incorporation (in PRK or L1210 cells) by 50 per cent, or the minimum concentration of compound required to cause a microscopically detectable alteration of normal cell morphology (in PRK cells).

the replication of vaccinia virus at a concentration as low as 0.4 μg/ml (Table 1).

There appeared to be a rather strong correlation (r = 0.827) between the anti-vaccinia activity of the compounds and their inhibitory effects on host cell DNA synthesis (as monitored by [^{3}H-methyl] dThd incorporation) (Table 1). (3′-Azido-3′-deoxythymidine was excluded from this correlation analysis, as its inhibitory effect on [^{3}H-methyl]dThd incorporation may result from a direct interference with the dThd salvage pathway and not necessarily reflect an overall inhibition of cellular DNA synthesis.) Thus, those compounds that were most effective against vaccinia virus, viz. 2′-amino-2′-deoxyadenosine, 3′-azido-3′-deoxyadenosine and 3′-azido-2′,3′-dideoxyadenosine, also ranked among the most powerful inhibitors of DNA synthesis. These compounds also inhibited viral replication and cellular DNA synthesis at approximately the same concentration. One may assume, therefore, that their antiviral activity was achieved through an inhibition of host cell DNA synthesis.

Various compounds, including 2′-amino-2′-deoxyadenosine, 2′-azido-2′-deoxycytidine, 3′-amino-2′,3′-dideoxyadenosine, 3′-azido-3′-deoxyadenosine and 3′-azido-2′,3′-dideoxyguanosine, inhibited the proliferation of L1210 cells at an ID_{50} that, like that of ara-A, fell within the 10–100 μg/ml range (Table 1). 3′-Amino-2′,3′-dideoxyadenosine and 3′-azido-2′,3′-dideoxyguanosine may be considered as rather selective in their antineoplastic action, as neither compound was toxic for normal (primary rabbit kidney) cells, even at 200 μg/ml. The highest potency as an antineoplastic agent was demonstrated by 3′-azido-2′,3′-dideoxyadenosine, which suppressed L1210 cell growth at an ID_{50} of about 2 μg/ml. However, the highest selectivity as an antineoplastic agent was demonstrated by 2′-amino-2′-deoxyguanosine: this compound was not active as an antiviral agent and not toxic for rabbit kidney cells, but inhibited L1210 cell proliferation at an ID_{50} of 3.9 μg/ml (Table 1). 2′-Amino-2′deoxyguanosine has also been evaluated for its inhibitory effects on the growth of Namalva and TK^- (dThd kinase deficient) Raji cells, two human lymphoblastoid B cell lines derived from Burkitt's lymphoma. The ID_{50} of this compound for TK^- Raji cells was 29.9 μg/ml and the ID_{50} for Namalva cells was 233 μg/ml, and was thus considerably higher than the ID_{50} for L1210 cells. For ara-A, the ID_{50} values for TK^- Raji and Namalva were 4.3 and 17.2 μg/ml, respectively.

2′-Amino-2′deoxyguanosine (which has recently been isolated from *Enterobacter cloacae* [21]) was not inhibitory to [^{3}H-methyl]dThd incorporation in either primary rabbit kidney cells or L1210 cells (Table 1), which contrasts with its marked inhibitory effect on L1210 cell growth. Except for 2′-amino-2′-deoxyguanosine and 3′-azido-3′-deoxythymidine, there appeared to be a relatively strong correlation ($r = 0.781$) between the antineoplastic activity of the compounds and their inhibitory effects on L1210 DNA synthesis (as monitored by [^{3}H-methyl]dThd incorporation). Thus, as postulated previously for 3′-amino-3′-deoxythymidine [7], inhibition of DNA synthesis may account for the tumor cell toxicity of most of the 2′- or 3′-amino (or azido) nucleoside analogues listed in Table 1. Only 2′-amino-2′-deoxyguanosine does not seem to follow the rule. The anti-tumor activity of this compound, or at least its inhibitory effect on L1210 cell proliferation, may be mediated through a mechanism other than inhibition of DNA biosynthesis.

In summary, various 2′- and/or 3′-amino- and -azido-deoxyribonucleoside analogues were evaluated for their antiviral and antitumor potentials, in primary rabbit kidney and mouse leukemia L1210 cell cultures, respectively. Some compounds, viz. 2′-amino-2′-deoxyadenosine, 3′-azido-3′-deoxyadenosine and 3′-azido-2′,3-dideoxyadenosine, proved quite effective in inhibiting vaccinia virus replication and L1210 cell growth. Their antiviral and antitumor properties correlated well with an inhibitory effect on host cell DNA synthesis. However, one particular compound, 2′-amino-2′-deoxyguanosine, strongly inhibited L1210 cell proliferation without a concomitant inhibitory effect on DNA metabolism.

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