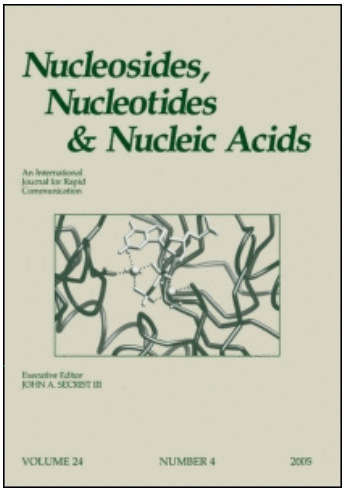

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Targets for the Antiviral and Antitumor Activities of Nucleoside, Nucleotide and Oligonucleotide Analogues

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TARGETS FOR THE ANTIVIRAL AND ANTITUMOR ACTIVITIES OF NUCLEOSIDE, NUCLEOTIDE AND OLIGONUCLEOTIDE ANALOGUES

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Summary. The following targets can be considered in the development of antiviral agents : (i) DNA polymerase via dThd kinase, (ii) S-adenosylhomocysteine hydrolase; and in the development of antitumor agents : (iii) dTMP synthetase and (iv) protein synthesis via the 2-5A pathway.

Various enzymatic targets can be envisaged in the development of nucleoside, nucleotide or oligonucleotide analogues as antiviral or antitumor agents : I. the axis deoxythymidine (dThd) kinase-DNA polymerase for nucleoside analogues active against herpesviruses; II. S-adenosyl-L-homocysteine (SAH) hydrolase for nucleoside analogues active against a broad range of (-)RNA viruses; III. deoxythymidylate (dTMP) synthetase for nucleotide analogues with cytostatic activity; and IV. the 2-5A [oligo(2'-5')adenylate] pathway for analogues of 2-5A, a mediator of interferon action.

I. The recently developed antiherpes agents, 9-(2-hydroxyethoxymethyl)guanine [acyclovir (ACV)], 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), 9-(3,4-dihydroxybutyl)guanine (DHBG), (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), and other (E)-5-(2-bromovinyl)pyrimidine derivatives such as BVaraU, BVamU, BVFaraU, and several 5-substituted 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracils and -cytosines such as FIAU, FIAC, FMAU, FMAC, FEAU and FEAC (Fig. 1) owe at least part of their selective activity against herpes simplex virus (HSV) and varicella-zoster virus (VZV) to a specific phosphorylation by the virus-encoded dThd kinase. In their 5'-triphosphate form the compounds may act as competitive inhibitors of the viral DNA polymerase and/or serve as substrates for the enzyme and be incorporated into viral DNA. BVDU may be incorporated

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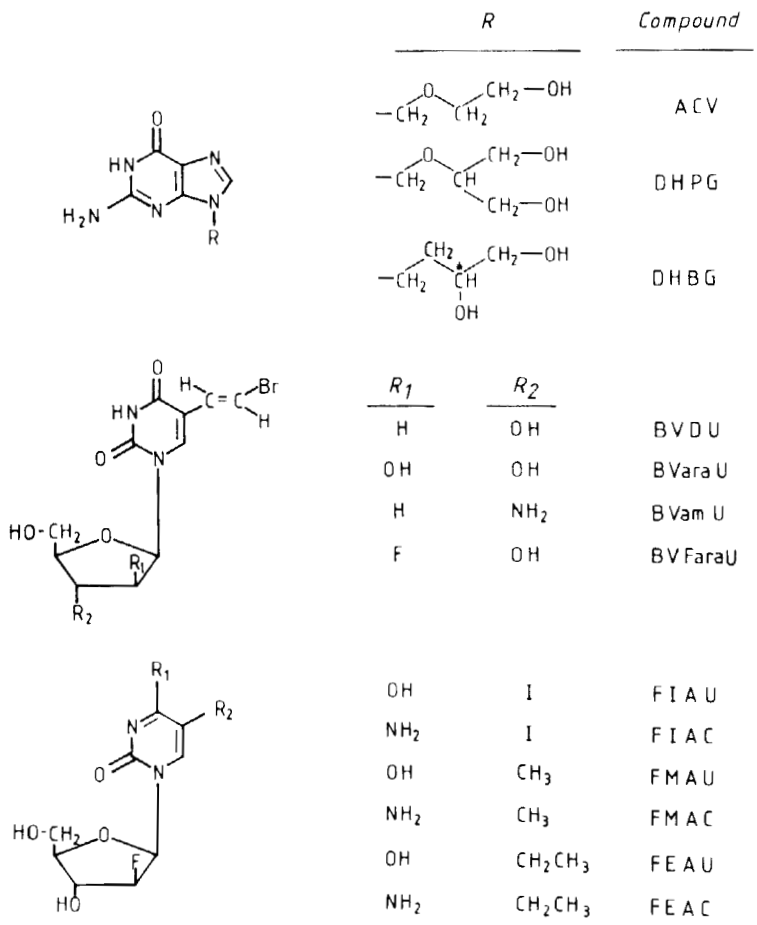


FIG. 1. Acyclovir (ACV), bromovinyldeoxyuridine (BVDU), fluoroiodoarauracil (FIAU) and their derivatives.

internally; ACV and BVaraU may be incorporated terminally, and FIAC, FMAU and DHPG may be incorporated either internally or terminally; DHBG, however, would not be incorporated at all. Table 1 summarizes the data on the inhibitory effects of the compounds on HSV-1 replication as well as their inhibition constants for HSV-1 dThd kinase and HSV-1 DNA polymerase. Although the data do not point to a direct correlation between antiviral potency and affinity for either HSV-1 dThd kinase or DNA polymerase, it is evident that all compounds are potent inhibitors of HSV-1 replication, efficient substrates of HSV-1 dThd kinase and strong inhibitors of HSV-1 DNA polymerase.

TABLE 1. Minimum inhibitory concentration (MIC) of ACV, BVDU, FIAU and their congeners for HSV-1 and their inhibition constants (Ki) for HSV-1 dThd kinase and HSV-1 DNA polymerase[a]

Compound	MIC	Ki	Ki (triphosphate)	References
	HSV-1 replication	HSV-1 dThd kinase	HSV-1 DNA polymerase	
ACV	0.3	200	0.003-0.006	1,2,3,4
DHPG	0.2	66 (Km)	0.03-0.08	1,4,5,6
DHBG	4	1.5	ND	7
BVDU	0.01	0.24	0.068-0.75	8,9,10,11,12
BVaraU	0.1	0.94	0.013-0.14	12,13,14,15
BVamU	0.3	1.9	0.13	16,17
BVFaraU	0.4	0.67	ND	14,18
FIAU	0.025	0.68	ND	19
FIAC	0.01	1.09	0.028	12,14,19
FMAU	0.013	0.59	0.048	12,14,19
FMAC	0.64	15.88	0.044	12,14,19
FEAU	0.024	ND	ND	20
FEAC	0.035	ND	ND	20

[a]All concentrations are expressed in μM. ND, not determined.

II. Several acyclic adenosine (Ado) analogues such as (S)-9-(2,3-dihydroxypropyl)adenine [(S)-DHPA] and (S)-3-(adenin-9-yl)-2-hydroxypropanoic acid [(S)-AHPA], and carbocyclic Ado analogues such as carbocyclic 3-deazaadenosine (C-c^{3}Ado) and neplanocin A (Fig. 2) have been found effective against a broad variety of viruses, i.e. poxviruses (vaccinia), (-)RNA viruses [vesicular stomatitis virus (VSV), rabies, measles, parainfluenza] and (+)RNA viruses (reo, rota). A common characteristic of these compounds is that they are strong inhibitors of SAH hydrolase, itself a regulatory enzyme in transmethylation reactions. Table 2 presents the minimal inhibitory concentrations of these compounds for VSV as well as their inhibition constants for SAH hydrolase. The source and purity of the SAH hydrolase preparations varied widely, which may at least partially explain why there is no close correlation between the Ki for SAH hydrolase and antiviral potency. Furthermore, it is not clear how an inhibitory effect on SAH hydrolase could impart selective antiviral activity. To obtain further insight in this matter, the compounds should be evaluated for their effects on the SAH hydrolase levels in both virus-infected and uninfected cells.

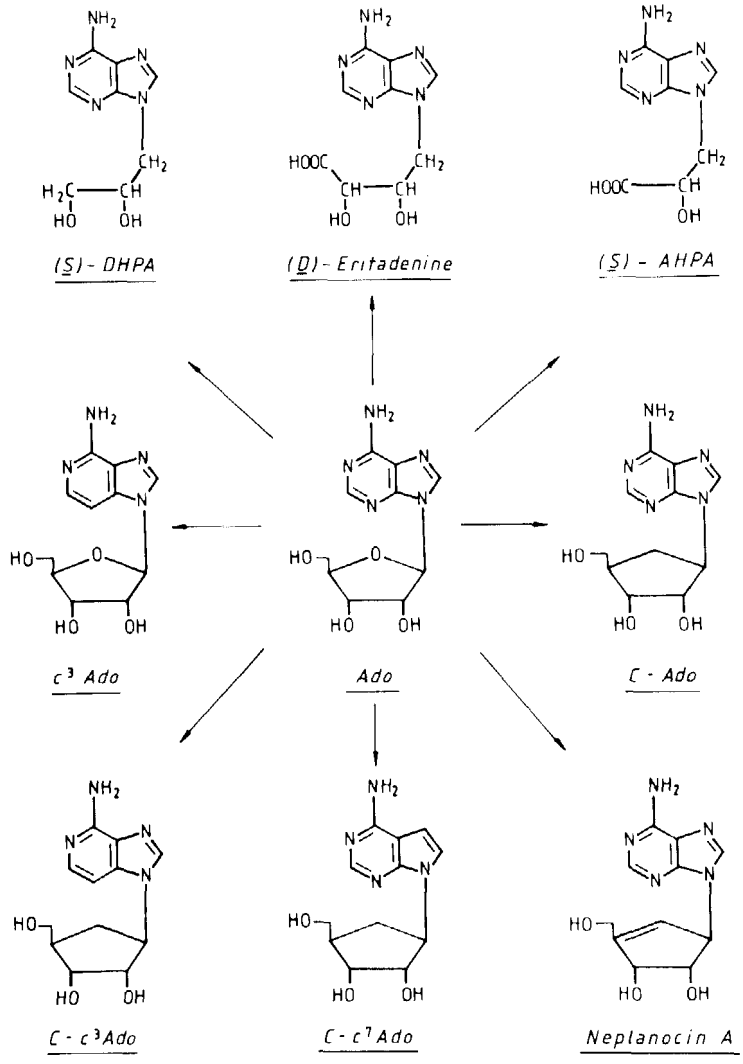


FIG. 2. Acyclic and carbocyclic analogues of adenosine.

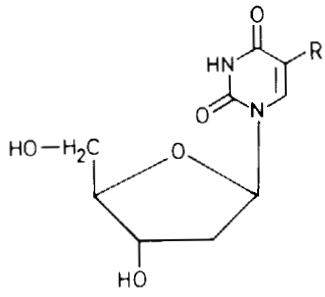
TABLE 2. Minimum inhibitory concentration (MIC) of the acyclic and carbocyclic analogues of adenosine for (-)RNA viruses (i.e. vesicular stomatitis virus) and their inhibition constants (Ki) for SAH hydrolase[a]

Compound	MIC VSV replication	Ki SAH hydrolase[b]	References
(S)-DHPA	10-50	0.9-3.5	21,22,23,24
(D)-Eritadenine	100	0.003	24,25,26
(S)-AHPA	5[c]	0.04	24,27
c^{3}Ado	30-100	4	22,26,28
C-Ado	15	0.005	26,28
C-c^{3}Ado	0.7	0.001-3	22,26,29,30
C-c^{7}Ado	10-50	44	26,30
Neplanocin A	0.1	0.008	31,32

[a]All concentrations are expressed in μM.
[b]Of different origin (rat liver, beef liver, hamster liver or murine L1210 leukemia cells).
[c]As the alkylester of the RS-racemate.

III. Being a crucial enzyme in the de novo biosynthesis of dTTP, dTMP synthetase could be considered as an attractive target for antitumor chemotherapy. Among the most potent cytostatic agents are 2'-deoxyuridine derivatives (Fig. 3) with a small and electron-withdrawing C-5 substituent, i.e. 5-fluoro-dUrd, 5-trifluoromethyl-dUrd, 5-nitro-dUrd, and 5-formyl-dUrd. The inhibitory effects of these compounds on tumor cell proliferation correlate closely with the inhibitory effects of their 5'-monophosphates on dTMP synthetase, as based upon a number of criteria : (a) the differential inhibitory effects of the nucleoside analogues on the incorporation of dUrd and dThd into host cell DNA, (b) the differential reversing effects of dUrd and dThd on the inhibition of cell proliferation by the nucleoside analogues, and (c) the Ki values of the nucleoside 5'-monophosphates for the cell-free dTMP synthetase. Fig. 4 illustrates the close correlation between the cell growth-inhibiting effects of a set of fifteen 5-substituted 2'-deoxyuridines and the Ki/Km of dTMP synthetase for the corresponding 5'-monophosphates. This close correlation points to dTMP synthetase as the target for the cytostatic action of the dUrd derivatives.

IV. Based on the premise that the various biologic effects of interferon, i.e. its antimitogenic, antiviral, cytostatic and immunomodulatory activities, are mediated, at least partially, by the so-called



R	Compound	Symbol
$-CH_3$	dThd	a
$-F$	5 - fluoro - dUrd	b
$-Br$	5 - bromo - dUrd	c
$-I$	5 - iodo - dUrd	d
$-NO_2$	5 - nitro - dUrd	e
$-CHO$	5 - formyl - dUrd	f
$-CHNOH$	5 - oxime of 5-formyl-dUrd	g
$-CH_2N_3$	5 - azidomethyl - dUrd	h
$-CH=CHBr(E)$	5 - (2-bromovinyl) - dUrd	i
$-CH_2CH_3$	5 - ethyl - dUrd	j
$-CH_2CH_2CH_3$	5 - propyl - dUrd	k
$-C-CH(SCH_2)_2$	5 - (1,3-dithiolan-2-yl) - dUrd	l
$-CH_2SCH_3$	5 - methylthiomethyl - dUrd	m
$-CH_2SOCH_3$	5 - methylsulfinylmethyl - dUrd	n
$-CH_2SO_2CH_3$	5 - methylsulfonylmethyl - dUrd	o

FIG. 3. 5-Substituted 2'-deoxyuridines.

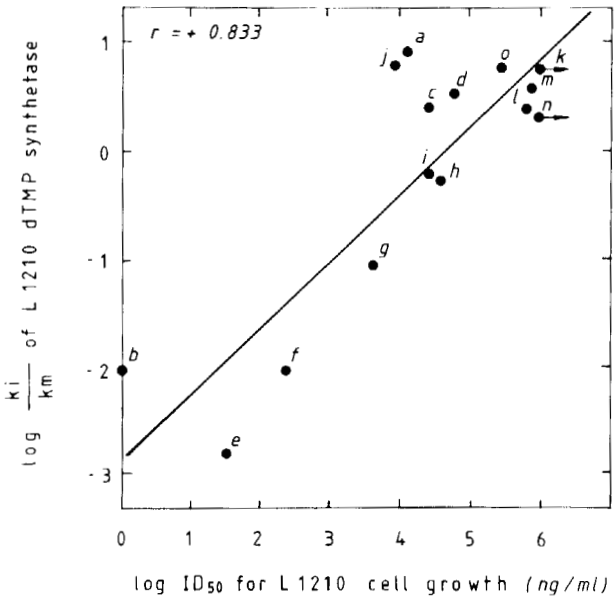


FIG. 4. Correlation between the 50 % inhibitory dose (ID_{50}) of various 5-substituted 2'-deoxyuridines for the growth of L1210 cells and the Ki/Km of L1210 dTMP synthetase for the corresponding 5'-monophosphates. Symbols for the 5-substituted 2'-deoxyuridines are as indicated in Fig. 3. The data are taken from references 33 and 34.

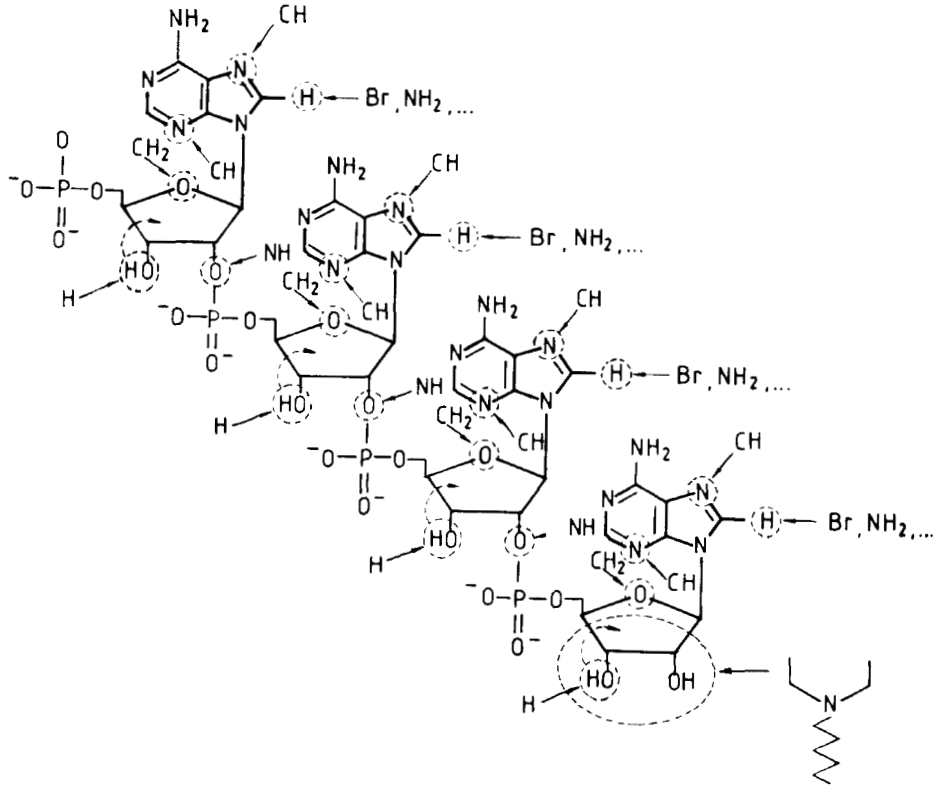


FIG. 5. Modifications of p5'A2'p5'A2'p5'A2'p5'A that have been carried out or could be envisaged.

2-5A pathway, analogues of 2-5A (ppp5'A2'p5'A2'p5'A) may in their own right be pursued as antiviral and/or antitumor agents. Various 2-5A analogues (Fig. 5) have been synthesized. The cordycepin, tubercidin, 8-aminoadenosine and 8-bromoadenosine tetranucleotides were found to exert a much stronger antimitogenic and cytostatic activity than 2-5A itself, whether the compounds were used as core (5'-dephosphorylated), 5'-monophosphate or 5'-triphosphate. The exact mode of action of the 2-5A analogues remains to be determined. On the one hand, they may act via the classical 2-5A pathway, thus activate a specific endoribonuclease and thereby digest mRNA and shut off protein synthesis. On the other hand, the 2-5A analogues may be degraded, either intra- or extracellularly, to the nucleoside (nucleotide), and thus function as prodrugs. The close parallelism in the cell growth-inhibiting effects of

TABLE 3. Inhibitory effects of various 2-5A analogues and their corresponding nucleosides on the growth of Balb/c 3T3 cells[a]

Compound	50 % inhibitory dose (μM)	
	as such	as the nucleoside
p5'A2'p5'A2'p5'A2'p5'A	23	> 300
p5'(3'dA)2'p5'(3'dA)2'p5'(3'dA)2'p5'(3'dA)	0.57	3
p5'(am^8A)2'p5'(am^8A)2'p5'(am^8A)2'p5'(am^8A)	0.22	0.5[b]
p5'(c^7A)2'p5'(c^7A)2'p5'(c^7A)	0.005	0.04

[a]Data taken from references 35 and 36.
[b]As the nucleoside 5'-monophosphate.

a number of 2-5A analogues and their corresponding nucleosides (Table 3) suggests that these 2-5A analogues indeed act by release of the free nucleoside (or nucleotide). Measures should be undertaken to prevent this premature degradation if the compounds are to be targeted at the 2-5A-dependent endoribonuclease.

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