STRUCTURE-ACTIVITY STUDIES ON OPEN-CHAIN ANALOGUES OF NUCLEOSIDES: INHIBITION OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE AND ANTIVIRAL ACTIVITY
1. NEUTRAL OPEN-CHAIN ANALOGUES*,**

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Over 70 alkyl derivatives of purine bases were examined for their inhibitory effects toward rat liver S-adenosyl-L-homocysteine hydrolase and their antiviral activity. The following structural features must be fulfilled by an inhibitor of SAH-hydrolase: an intact adenine moiety, an alkyl chain bound at the 9-position and bearing a vicinal diol at the 2′,3′-position, with 2*S* configuration. An additional substitution at the 3-position lowers or annihilates the inhibitory activity. The enzyme inhibition is reversible. Some of the compounds are substrates of adenosine aminohydrolase. All inhibitors of SAH-hydrolase exhibit antiviral activity, *e.g.* against vesicular stomatitis virus and vaccinia virus in cell culture, and this antiviral activity correlates with the inhibition of SAH-hydrolase.

Some time ago we described[1] the antiviral activity of an open-chain adenosine analogue, 9-(*S*)-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA; *Ib*). This compound is active against RNA viruses, particularly minus-stranded RNA viruses such as rhabdoviruses (*i.e.* vesicular stomatitis virus[1], rabies virus[2,3]) and double-stranded RNA viruses such as reoviruses (*i.e.* infectious pancreatic necrosis viruses of salmonid fishes[4]). (*S*)-DHPA is not metabolized in mammals[5,6] and acts as a potent inhibitor of S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase) from rat liver[7], mouse leukemic L-1210 cells[8] and *Nicotiana tabaccum* L. tissue culture[9]. Inhibition of SAH-hydrolase results in the accumulation of S-adenosyl-L-homocysteine in the cells; this effect has been demonstrated directly in rat liver hepatocytes[10]. Since S-adenosyl-L-homocysteine (SAH) is an inhibitor of methyltransferases[11], it affects cellular processes important for viral multiplication (*e.g.* capping of viral mRNA)

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(ref.[12]). SAH also affects the methylation and initiation of translation of viral RNA transcripts, as was shown for rhabdovirus[13]. Consequently, those nucleoside analogues with modified heterocyclic base or sugar component[14] that inhibit SAH-hydrolase (*e.g.* 3-deazaadenosine[15] or its carbocyclic analogue[16,17]), exhibit marked antiviral activity. In the course of our investigations we discovered another group of SAH-hydrolase inhibitors related to (*S*)-DHPA, the so-called eritadenines[18], which also show antiviral activity resembling that of DHPA (ref.[19]).

These findings stimulated us to synthetize a series of "aliphatic nucleoside analogues", *i.e.* substituted alkyl derivatives of heterocyclic bases, to examine their *in vitro* effect on SAH-hydrolases, and to evaluate the relationship between this effect and the antiviral activity of the compounds.

The enzyme inhibition studies were performed using the pure enzyme isolated from rat liver by affinity chromatography[20]. We systematically investigated the inhibitory effects of the compounds on the hydrolytic reaction, *i.e.* the cleavage of S-adenosyl-L-homocysteine to adenosine and L-homocysteine. Inhibition of SAH-hydrolase is expressed as the ratio of the initial rate of the reaction in the presence of the inhibitor to the reaction rate in the absence of the inhibitor, v_i/v_0. As the reference compound we chose adenine which is considered to be a weak inhibitor of SAH-hydrolases[21] ($v_i/v_0 = 0{\cdot}85$ under our experimental conditions). For stronger inhibitors, kinetic parameters of the inhibition were also determined. Since the reaction mixtures contained an excess of adenosine aminohydrolase to remove rapidly adenosine formed by the cleavage reaction, we cannot exclude the possibility that the adenine derivatives studied as inhibitors were (partially) deaminated during the incubation procedure. For this reason, all active compounds, their structurally close analogues and geometric isomers, were analyzed systematically for deamination during the course of SAH cleavage. In those cases where a detectable deamination occurred under the assay conditions, the compounds were also examined as inhibitors of S-adenosyl-L-homocysteine synthesis from adenosine and L-homocysteine, *i.e.* the reverse reaction which is catalyzed by the same enzyme but does not require the presence of adenosine aminohydrolase.

The antiviral effects of the compounds were evaluated in cell cultures infected with either vesicular stomatitis virus (VSV) as a representative of the RNA viruses (rhabdoviruses, minus stranded RNA viruses), or vaccinia virus (VV) as a representative of the DNA viruses (poxviruses). Some compounds were also tested for activity against herpesviruses (HSV-1). The antiviral activity is presented as the minimal inhibitory concentration required for 50% inhibition of viral cytopathogenicity (MIC_{50}) and is expressed in μg/ml. Compounds with $MIC_{50} > 400$ μg/ml (approximately $1{\cdot}5-2{\cdot}0 \cdot 10^{-3}$ mol l^{-1}) were considered as inactive. The antiviral activity of some derivatives has been described previously[22], but no attempts were made to correlate these antiviral effects with an inhibition of SAH-hydrolase.

The present study concerns our investigations on the structure–biological activity

relationship of various neutral derivatives of *Ib*, in which either the heterocyclic base or aliphatic side chain were modified.

EXPERIMENTAL

Materials and enzymes. [^{14}C]-S-Adenosyl-L-homocysteine was prepared according to ref.[7]. [^{14}C] Adenosine of specific activity 16 GBq mmol^{-1} was a product of Institute for Research, Production and Application of Radioisotopes (Prague, Czechoslovakia). Rat liver SAH-hydrolase was prepared as described[7], purified to homogeneity by affinity chromatography according to ref.[20] and stabilized by addition of serum albumin (50 μg ml^{-1}); SAH-hydrolase from mouse L-1210 leukemic cells was isolated according to ref.[8]. Both enzymes were dissolved in 20% aqueous glycerol. Adenosine aminohydrolase from calf intestine was from Boehringer (Mannheim. F.R.G.). All the inhibitors have been described in the original papers (see Tables I and II) and were homogeneous according to paper chromatography and HPLC under the conditions employed. The inhibitory effect, expressed as the ratio v_i/v_0, was determined in the direction of SAH-hydrolysis at an SAH concentration of $4 . 10^{-6}$ and an inhibitor concentration of $1 . 10^{-6}$ mol . $. l^{-1}$ in a standard[7] reaction mixture. The values of K_i were determined by Dixon plotting.

The substrate activity toward adenosine aminohydrolase was determined in a reaction mixture of total volume 0·25 ml, consisting of the corresponding inhibitor in the final concentration of 10^{-6} mol l^{-1} and 27 μkat ml^{-1} of adenosine aminohydrolase in 0·06 mol l^{-1} Sörensen

TABLE I

(*RS*)-(2,3-Dihydroxypropyl) derivatives of various purine bases[a]

Formula	Purine residue	v_i/v_0 (hydrolysis)[b]
1a	Adenin-9-yl[23]	0·63
2a	2-Methyladenin-9-yl[24]	0·87
2b	2-Aminoadenin-9-yl[24]	0·96
2c	2-Methylthioadenin-9-yl[25]	0·92
2d	6-Methylaminopurin-9-yl[25]	0·97
2e	6-Dimethylaminopurin-9-yl[25]	0·97
2f	6-Aminohexylaminopurin-9-yl	0·96
2g	6-Mercaptopurin-9-yl[25]	1·00
2h	8-Bromoadenin-9-yl[25]	0·88
2i	8-Hydroxyadenin-9-yl[25]	0·92
2j	8-Aminoadenin-9-yl[25]	0·82
2k	8-(3-Aminopropylamino)adenin-9-yl[26]	0·97
2l	8-Mercaptoadenin-9-yl[25]	0·98
3	Adenin-3-yl[25]	0·95

[a] The antiviral activity of the compounds against VSV and VV viruses did not show an MIC_{50} lower than 400 μg/ml except for compound *2j* which exhibited an MIC_{50} of 300 μg/ml against VV (ref.[22]); [b] Adenine: $v_i/v_0 = 0·85$ (hydrolysis).

TABLE II

N^9-Alkyl derivatives of adenine

Formula[a]	Substituent	Deamination[b]	v_i/v_0[c]		MIC_{50} (μg/ml)[d]	
			H	S	VSV	VV
		Monohydroxyalkyl derivatives				
4	2-Hydroxyethyl[27]	—	0·89	—	NA	NA
5a	(*RS*)-2-Hydroxypropyl[28]	—	0·96	—	NA	NA
5b	(2*S*)-2-Hydroxypropyl[28]	—	0·87	—	NA	NA
6	3-Hydroxypropyl[29]	—	0·95	—	NA	NA
7	(*RS*)-1-Hydroxy-2-octyl[30]	—	1·00	—	n.d.	n.d.
8	(*RS*)-*erythro*-2-Hydroxy-3-nonyl	—	1·08	—	100	15
		Dihydroxyalkyl derivatives				
1a	(*RS*)-2,3-Dihydroxypropyl[23]	—	0·63	—	20—40	40—70
1b	(2*S*)-2,3-Dihydroxypropyl[23]	—	0·54	0·67	7—20	10—40
1c	(2*R*)-2,3-Dihydroxypropyl[23]	—	1·00	—	NA	NA
9	1,3-Dihydroxy-2-propyl[29]	—	0·93	—	NA	NA
10a	(*RS*)-*threo*-2,3-Dihydroxybutyl[29]	—	0·89	0·80	200	200
10b	(2*S*,3*S*)-*threo*-2,3-Dihydroxybutyl[31]	+	0·54	0·67	70	150
10c	(2*R*,3*R*)-*threo*-2,3-Dihydroxybutyl[31]	—	0·82	—	NA	NA
10d	(2*S*,3*R*)-*erythro*-2,3-Dihydroxybutyl[31]	—	0·75	—	100	300
10e	(2*R*,3*S*)-*erythro*-2,3-Dihydroxybutyl[31]	—	0·80	—	150	70
11	(2*S*)-2,4-Dihydroxybutyl[24]	—	1·00	—	NA	NA
12a	(*RS*)-3,4-Dihydroxybutyl[29]	+	0·91	0·92	NA	NA
12b	(3*S*)-3,4-Dihydroxybutyl[29]	—	0·92	—	NA	NA
13	(2*S*)-1,2-Dihydroxy-3-butyl[24,e]	n.d.	0·68	—	NA	NA

14	(*RS*)-3,5-Dihydroxypentyl[29]	n.d.	1·00	—	NA	NA
15	(2*S*)-1,2-Dihydroxy-3-heptyl[30,f]	—	0·93	—	—	—
16	(*RS*)-2,3-Dihydroxynonyl[30]	—	1·00	—	—	—
17a	(2*S*)-1,2-Dihydroxy-3-nonyl[24,g]	—	0·92	—	—	—
17b	(2*R*)-1,2-Dihydroxy-3-nonyl[30,h]	—	0·87	—	—	—
18	(*RS*)-2-Hydroxy-2-hydroxymethyloctyl[30]	—	1·00	—	—	—
19	(2*S*)-1,2-Dihydroxy-3-undecyl[30,i]	—	0·89	—	—	—
	Trihydroxyalkyl derivatives					
20a	(2*S*,3*R*)-*erythro*-2,3,4-Trihydroxybutyl[31]	+	0·55	0·73	100	300
20b	(2*R*,3*S*)-*erythro*-2,3,4-Trihydroxybutyl[31]	+	0·84	0·83	300	NA
20c	(2*S*,3*S*)-*threo*-2,3,4-Trihydroxybutyl[32]	+	0·73	0·89	NA	NA
20d	(2*R*,3*R*)-*threo*-2,3,4-Trihydroxybutyl[32]	+	0·97	1·00	300	NA
21	(2*R*,3*R*,4*R*)-2,3,4-Trihydroxypentyl[29]	—	1·00	—	NA	NA
	1-(Adenin-9-yl)-1-deoxyalditols					
22a	D-Arabitol[13]	—	1·00	—	NA	NA
22b	L-Arabitol[33]	—	1·00	—	NA	NA
22c	DL-Ribitol[33]	+	1·00	0·96	NA	NA
22d	L-Ribitol[33]	—	1·00	—	NA	NA
22e	L-Lyxitol[33]	—	0·83	—	NA	NA
22f	D-Lyxitol[33]	—	0·79	—	NA	NA
22g	DL-Xylitol[33]	—	1·00	—	n.d.	n.d.
22h	L-Xylitol[33]	—	0·77	—	NA	NA
22i	L-Galactitol[35]	n.d.	0·93	—	NA	NA
22j	2-O-Methyl-L-ribitol[30]	—	0·76	—	n.d.	n.d.
22k	3-O-Methyl-D-lyxitol[30]	—	0·89	—	n.d.	n.d.

TABLE II
(*Continued*)

Formula[a]	Substituent	Deamination[b]	v_i/v_0		MIC_{50} (μg/ml)[d]	
			H	S	VSV	VV
	Other derivatives					
23	Allyl[34]	n.d.	1·00	—	NA	150
24	(*RS*)-2,3-Dichloropropyl[34]	—	1·00	—	NA	150
25	(*RS*)-2,3-Dibromopropyl[34]	—	0·94	—	NA	NA
26	2-Hydroxyethoxymethyl	—	1·00	—	n.d.	n.d.
27	(*RS*)-3-Hydroxy-2-methoxypropyl[29]	n.d.	0·69	—	300	NA
28	(*RS*)-2-Hydroxy-3-methoxypropyl[29]	n.d.	1·04		NA	NA
29	(*RS*)-2,3-Dimethoxypropyl[29]	n.d.	1·00	—	300	NA
30	(*RS*)-2-Amino-3-hydroxypropyl[29]	—	0·85	0·88	100—400	NA
31	(*RS*)-3-Amino-2-hydroxypropyl[29]	n.d.	0·95	—	200	400
32	(*RS*)-2,3-Diaminopropyl[29]	n.d.	1·01	—	NA	NA
33	(*RS*)-3-Aminopropylamino-2-hydroxypropyl[26]	n.d.	1·00	—	NA	NA
34	(*RS*)-2-Azido-3-hydroxypropyl[29]	n.d.	1·00	—	NA	NA
35	(*RS*)-3-Azido-2-hydroxypropyl[29]	n.d.	0·97	—	NA	NA
36	(*RS*)-2,3-Diazidopropyl[29]	n.d.	0·88	—	NA	70
37	(*RS*)-2,3-Dimercaptopropyl[34]	n.d.	0·34	—	NA	NA
38	(*RS*)-2-Hydroxy-3-isobutylthiopropyl[34]	n.d.	1·00	—	NA	NA
39a	(2*S*,3*S*)-*threo*-4-Amino-2,3-dihydroxybutyl[31]	—	0·94	—	NA	250
39b	(2*R*,3*R*)-*threo*-4-Amino-2,3-dihydroxybutyl[31]	n.d.	0·86	—	n.d.	n.d.
40	(2*S*,3*S*)-*threo*-2,3-Dihydroxy-4-isobutylthiobutyl[34]	n.d.	0·97	—	NA	300
41	1,4-Anhydro-5-deoxy-D-ribofuranos-5-yl[33]	+	0·81	0·93	NA	NA
42	6-Deoxy-6-galactosyl[35]	n.d.	1·02	—	n.d.	n.d.

[a] Numbers with small letters are for designation of stereoisomers; [b] enzymatic deamination under the assay conditions: + >5% conversion; [c] initial rates of v_i (inhibited reaction) to v_0 (control reaction) in the direction of H (hydrolysis) or S (synthesis); [d] NA (not active) refers to compounds with $IC_{50} > 400$ μg/ml, n.d. (not determined); [e] content 85% 3*R*-isomer; [f] content 70% 3*R*-isomer; [g] content 85% 3*R*-isomer; [h] content 84% 3*S*-isomer; [i] content 82% 3*R*-isomer.

phosphate buffer, pH 7·4, containing 0·1 mol l^{-1} EDTA. After incubation for 10 min at 37°C an aliquot (10 μl) of the incubation mixture was analyzed by HPLC on a glass column (3·3 × × 150 mm) with a Separon SIX C18 (5 μ) reversed phase (Laboratory Equipment, Prague), using a Constametric I pump; mobile phase: 10^{-2} mol l^{-1} potassium dihydrogen phosphate pH 3·1–methanol (9 : 1), flow rate 0·45 ml min^{-1}, detection at 254 nm, sensitivity 0·08 AUFS.

Identification of deamination products. The incubation mixture (final volume 0·25 ml), containing the inhibitor ($4 . 10^{-3}$ mol l^{-1}) and adenosine aminohydrolase (270 μkat ml^{-1}) in 0·04 mol l^{-1} Sörensen phosphate buffer, pH 7·4, was incubated for 180 min at 37°C. The mixture was analyzed by paper chromatography (Whatman No 3MM paper) in 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2) and the products were eluted with diluted (1 : 100) aqueous ammonia. UV-spectra of these eluates were measured in aqueous solutions (pH 2) on a Specord UV-VIS instrument and exhibited maxima characteristic for 9-substituted hypoxanthine derivatives (λ_{max} 249–251 nm).

Antiviral assays. Inhibition of virus-induced cytopathogenicity was evaluated in PRK (primary rabbit kidney), HSF (human skin fibroplast), HeLa and Vero cells. When grown to confluency in Sterilin microtiter trays, the cell cultures were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ corresponds to the virus dose required to infect 50% of the cell culture) of VSV (vesicular stomatitis virus), VV (vaccinia virus) or other viruses. After 1 h of virus adsorption, the residual virus was removed and the cell cultures were incubated with maintenance medium (Eagle's MEM supplemented with 3% calf serum) containing varying concentrations of the test compounds (400, 200, 100, ... μg/ml). Virus-induced cytopathogenicity was evaluated as soon as it reached completion in the control virus-infected cell cultures. The antiviral activity is expressed as the minimum inhibitory concentration of compound required to reduce virus cytopathogenicity by 50% (MIC_{50}) (Tables I, II, III).

TABLE III

Antiviral activity of 9-(2,3-dihydroxyalkyl)adenines against different viruses

Virus	Cell line	MIC_{50}, μg/ml[a]				
		1b	*10b*	*10c*	*10d*	*10e*
Vesicular stomatitis	HSF	7–20	70	NA	100	150
Vesicular stomatitis	HeLa	20	150	NA	300	NA
Reovirus 1	Vero	40	100	>300	NA	NA
Parainfluenza 3	Vero	40	100	NA	200	NA
Sindbis	Vero	NA	150	NA	NA	NA
Coxsackie B-4	Vero	400	NA	NA	NA	NA
Coxsackie B-4	HeLa	NA	NA	NA	NA	NA
Herpes simplex type 1 (strain KOS)	PRK	40-NA	NA	NA	NA	100
Vaccinia	PRK	10–40	150	NA	300	70

[a] NA not active (MIC_{50} > 400 μg/ml).

The inhibition of virus multiplication (Table V) was assessed in PRK or HSF cells inoculated with $10^{4.5}$ $CCID_{50}$ of VSV per Petri dish; the compounds (100 μg/ml) were added 1 h after virus absorption and the virus titer (plaque forming units per ml, PFU/ml) was determined by plaque formation in mouse L-929 fibroblasts at 1, 8, 24 and 38 h (see ref.[19]).

RESULTS AND DISCUSSION

Inhibition of SAH-hydrolase. The structure of the compounds varied so as to determine the effects of (*a*) substitution of the heterocyclic base while preserving the aliphatic side chain, and (*b*) substitution of the aliphatic side chain while preserving the heterocyclic base (adenine). Table I summarizes the data on SAH-hydrolase inhibition by (*RS*)-2,3-dihydroxypropyl derivatives with substitutions in the heterocyclic base moiety. It is evident that substitutions at positions 2, 6 or 8 of the adenine ring of the reference compound *1a*, invariably lead to a disappearance of the inhibitory effect on SAH-hydrolase. Also inactive were the N^3-isomer of compound *1a* (*3*) (Table I), the 2,3-dihydroxypropyl derivatives of uracil, thymine, cytosine (substituted in positions N^1 and N^3) as well as the 9-(*RS*)-2,3-dihydroxypropyl derivatives of hypoxanthine, guanine and xanthine (data not shown). These observations are in accord with the assumed function of the adenine derivatives *1a* and *1b* which should interact with the adenosine[4] or adenine binding site of the enzyme. Our findings are also in agreement with previous observations that adenosine analogues with modified heterocyclic bases do not inhibit SAH-hydrolases[36,37].

For this reason, the effect of substitutions of the aliphatic side chain was evaluated with the N^9-substituted adenine as the heterocyclic base for the whole series of compounds. The inhibitory effects of these derivatives on rat liver SAH-hydrolase are presented in Table II. The first part shows data for several monohydroxyalkyl derivatives of adenine. It is obvious that the presence of one hydroxyl in the alkyl chain (compounds 4–8) is not sufficient for imparting an enzyme-inhibitory effect. Only the dihydroxyalkyl derivatives show an inhibitory activity and the most inhibitory are the 2,3-dihydroxypropyl derivatives *1a,b*. A vicinal dihydroxyalkyl system seems to be necessary for the inhibitory activity. The 2,3-dihydroxypropyl derivative *1a*, 2,3-dihydroxybutyl derivatives *10b* and *10d* and 1,2-dihydroxy-3-butyl derivative *13* are all active inhibitors of SAH-hydrolase whereas compounds with separated hydroxy groups are inactive (compounds *9*, *11*, *14*). Also, the distance of the vicinal diol group from the adenine ring (or from the carbon atom linked to the adenine ring) is crucial: *e.g.* the 3,4-dihydroxybutyl derivatives *12* are inactive.

The adjoining center, *i.e.* $C_{(2)}$ in compounds *1* and *10*, must be in the (*S*)-configuration; the diastereoisomeric compounds with (*R*)-configuration at this center (*1c*, *10c*, *10e*) are inactive. An analogous situation is encountered with the 2,3,4-trihydroxybutyl derivatives *20*, of which only compounds with the (2*S*)-configuration are active. For the group of polyhydroxyalkyl derivatives the inhibitory effect disap-

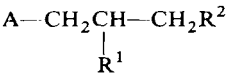
pears with increasing length of the side chain: 1-(adenin-9-yl)-1-deoxyalditols (*22*) are virtually inactive irrespective of the configuration in position 2. Also, compounds in which the vicinal diol is extended by an additional aminomethyl (*39*) or alkylthio group (*40*) show no inhibitory effect even if they have configuration 2*S* (compound *39a*). Replacement of one or both hydroxy groups of the vicinal diol by a halogen (*24*, *25*), azide (*34–36*) or amino group (*30–33*), or their alkylation (*27–29*) also results in a loss of activity. The only active non-hydroxylic compound of the whole series is the 2,3-dimercaptopropyl derivative *37*, which happens to be the only inhibitor more potent than DHPA (*1b*). However, this compound could very easily form the corresponding thioxirane derivative which might non-specifically inactivate the enzyme.

The structural features for SAH-hydrolase inhibitors in the series of compounds tested may be summarized as follows: The presence of an adenine base substituted at $N_{(9)}$ with an alkyl chain bearing in positions 2 and 3 (relative to the base) a vicinal diol with the (*S*)-configuration at the $C_{(2)}$ atom. These conclusions can be interpreted in terms of the interaction of the analogues with the adenosine binding site of SAH-hydrolases. The latter is determined not only by the presence of an intact adenine base but also by ability of the alkyl residue to simulate the configuration of the sugar moiety in adenosine. Although such configuration may not necessarily exist in aqueous solutions of the compound, it might arise during complex formation with the enzyme. Adenosine-like conformations would occur preferentially in those cases in which the vicinal diol is situated in positions 2 and 3 and the absolute configuration at $C_{(2)}$ corresponds to the absolute configuration at $C_{(2')}$ of adenosine, *i.e.* 2*S*. Other substituents such as methyl (*10b,d*) or hydroxymethyl (*20a,c*) group are ineffective, unless their additional intra- or intermolecular interactions change the adaptability of the chain. For the 3-aminomethyl (*39*) or 3-(1,2-dihydroxyethyl) derivatives (*22*) obviously other conformations are preferred. It is noteworthy that 9-(2-hydroxyethoxymethyl)adenine (*26*), which could also simulate part of the adenosine sugar ring, does not interact with the enzyme. This underlines the importance of the vicinal diol chirality for the interaction of the adenosine analogue with the enzyme.

The "adenosine-like conformation" of the analogue can be destabilized *e.g.* by the presence of a hydrophobic alkyl group in the immediate vicinity of the adenine ring. Such effect is clear when comparing the activity of compound *13* which has a methyl group adjacent to the adenine moiety, with its inactive homologues *15*, *17* and *19* bearing adjacent to the adenine ring an n-butyl, n-hexyl or n-octyl group, respectively. Similarly, the inhibitory effect of the adenosine analogue is negatively influenced by a hydrophobic group adjacent to the vicinal diol (compounds *16* and *18*).

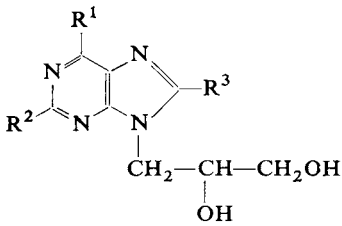
Under the conditions used, the results might be influenced by adenosine aminohydrolase whose presence is necessary for removal of the arising adenosine (*vide supra*). If this enzyme is inhibited by the test compound, adenosine will not be de-

aminated and since it acts itself as an SAH-hydrolase inhibitor, it may increase the apparent inhibitory effect of the compounds[38]. We used therefore an excess of the enzyme to ensure its proper task at the employed inhibitor concentration.

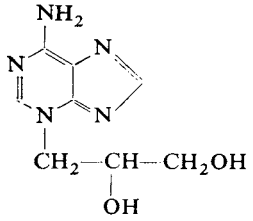


1, $R^1 = R^2 = OH$
5, $R^1 = OH$, $R^2 = H$
6, $R^1 = H$, $R^2 = OH$
24, $R^1 = R^2 = Cl$
25, $R^1 = R^2 = Br$
27, $R^1 = OCH_3$, $R^2 = OH$
28, $R^1 = OH$, $R^2 = OCH_3$
29, $R^1 = R^2 = OCH_3$

30, $R^1 = NH_2$, $R^2 = OH$
31, $R^1 = OH$, $R^2 = NH_2$
32, $R^1 = R^2 = NH_2$
33, $R^1 = OH$, $R^2 = NH(CH_2)_3NH_2$
34, $R^1 = N_3$, $R^2 = OH$
35, $R^1 = OH$, $R^2 = N_3$
36, $R^1 = R^2 = N_3$
37, $R^1 = R^2 = SH$
38, $R^1 = OH$, $R^2 = SCH_2CH(CH_3)_2$



	R^1	R^2	R^3		R^1	R^2	R^3
1,	NH_2	H	H	*2f*,	$NH(CH_2)_6NH_2$	H	H
2a,	NH_2	CH_3	H	*2g*,	SH	H	H
2b,	NH_2	NH_2	H	*2h*,	NH_2	H	Br
2c,	NH_2	SCH_3	H	*2i*,	NH_2	H	OH
2d,	$NHCH_3$	H	H	*2j*,	NH_2	H	NH_2
2e,	$N(CH_3)_2$	H	H	*2k*,	NH_2	H	$NH(CH_2)_3NH_2$
				2l,	NH_2	H	SH



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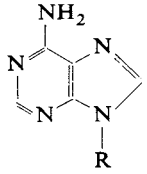
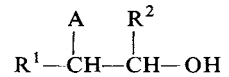
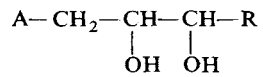


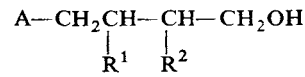
4, $R = CH_2CH_2OH$
23, $R = CH_2CH{=}CH_2$
26, $R = CH_2OCH_2CH_2OH$



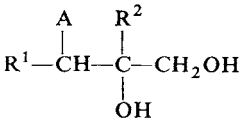
7, $R^1 = C_6H_{13}$, $R^2 = H$
8, $R^1 = C_6H_{13}$, $R^2 = CH_3$
9, $R^1 = CH_2OH$, $R^2 = H$



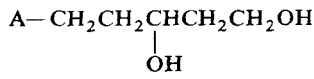
10, $R = CH_3$
16, $R = C_6H_{13}$
21, $R = CH(OH)CH_3$
39, $R = CH_2NH_2$
40, $R = SCH_2CH(CH_3)_2$



11, $R^1 = OH$, $R^2 = H$
12, $R^1 = H$, $R^2 = OH$
20, $R^1 = R^2 = OH$



13, $R^1 = CH_3$, $R^2 = H$
15, $R^1 = C_4H_9$, $R^2 = H$
17, $R^1 = C_6H_{13}$, $R^2 = H$
18, $R^1 = H$, $R^2 = C_6H_{13}$
19, $R^1 = C_8H_{17}$, $R^2 = H$



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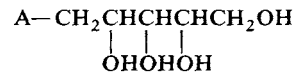

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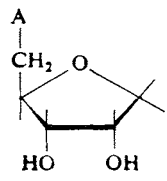

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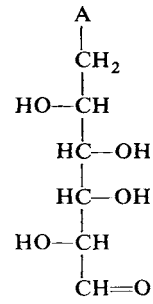

42

In formulae *1*–*42*, A represents the adenin-9-yl residue

Under such conditions, however, the adenine derivatives tested might be deaminated to the corresponding hypoxanthine derivatives; such degradation would decrease the actual concentration of the inhibitor. Therefore, we investigated for most compounds their behavior toward adenosine aminohydrolase under conditions similar to those used in the hydrolytic assay of SAH-hydrolase. Many of the aliphatic analogues in this series, particularly the dihydroxybutyl and trihydroxybutyl derivatives of adenine, are good substrates of adenosine aminohydrolase (Table II). The deamination products were identified by their characteristic UV spectra. In the course of this study we did not investigate in detail the structure–activity relationship for adenosine aminohydrolase. A possible effect of this enzyme on the inhibitor concentration in the mixture was eliminated by evaluating the inhibition of SAH-hydrolase

in the synthetic reaction. In this case, the deamination cannot take place (Table II). However, it must be emphasized that SAH synthesis is generally less sensitive toward inhibition by adenosine analogues than is SAH hydrolysis.

For a selected group of compounds which, according to determination of v_i/v_0, significantly inhibited SAH-hydrolase in one or both directions we have estimated the kinetic parameters of the inhibitory effect (Table IV). For the neutral analogues the inhibition is reversible. The K_i/K_m values show that the most potent enzyme inhibitors are (*S*)-2,3-dihydroxypropyl (*1b*) and (*RS*)-2,3-dimercaptopropyl (*37*) derivatives of adenine whereas the (2*S*,3*R*)-*erythro* derivatives *10d* and *20a* and the L-xylitol derivative *22h* have a weaker effect.

The existing data indicate that SAH-hydrolases from different biological sources do not differ in their behaviour toward inhibitors[5,6,36]. This fact, which reflects an analogous topochemistry of their adenosine-binding sites, is confirmed also by the data in Table V which demonstrate the inhibitory effect of some compounds (which are active toward the enzyme from rat liver) (Table II) on the hydrolytic reaction catalyzed by SAH-hydrolase from L-1210 murine leukemia cells. The inhibitory effects of the adenosine analogues on both enzymes are matched. The latter enzyme seems to be even more sensitive toward the inhibitors than the enzyme from rat liver. We, therefore, assume that the conclusions drawn from the structure–activity studies performed with rat liver SAH-hydrolase are valid for SAH-hydrolases in general.

Antiviral activity. The data on the antiviral activity of the compounds have in part been published previously[22]. Comparing the antiviral activity against vesi-

TABLE IV

Inhibition constants (K_i) of competitive inhibitors of rat liver SAH-hydrolase

Formula	Substituent[a]	Reaction[b]	K_i, μmol	K_i/K_m
1b	(2*S*)-2,3-Dihydroxypropyl	H	0·46	0·056
10b	(2*S*,3*S*)-*threo*-2,3-Dihydroxybutyl	S	24·0	0·89
10d	(2*S*, 3*R*)-*erythro*-2,3-Dihydroxybutyl	H	1·5	0·18
10e	(2*R*,3*S*)-*erythro*-2,3-Dihydroxybutyl	H	5·2	0·62
20a	(2*S*,3*R*)-*erythro*-2,3,4-Trihydroxybutyl	H	1·5	0·18
		S	6·0	0·22
20c	(2*S*,3*S*)-*threo*-2,3,4-Trihydroxybutyl	H	5·2	0·62
22f	1-Deoxy-D-lyxitol	H	2·0	0·24
22h	1-Deoxy-L-xylitol	H	1·2	0·14
37	(*RS*)-2,3-Dimercaptopropyl	H	0·4	0·048

[a] At position 9 of adenine; [b] H hydrolysis of SAH; $K_m^{SAH} = 8{\cdot}33 \cdot 10^{-6}$ mol; S synthesis of SAH; $K_m^{Ado} = 2{\cdot}7 \cdot 10^{-5}$ mol.

cular stomatitis and vaccinia viruses with the results on SAH-hydrolase inhibition, we may infer that all SAH-hydrolase inhibiting compounds are active against both viruses, the effect being greater for VSV than for VV. The relation between *in vitro* inhibition of the enzyme and inhibition of the cytopathogenic effect of the virus in cell culture is shown in Fig. 1. We must take into consideration that the antiviral activity of a compound is influenced by a variety of factors, not at least by its actual intracellular concentration, which depends on its penetration through the cell membrane, its degradation within and outside the cell, *etc.*. Fig. 1 indicates that there is an apparent correlation between SAH-hydrolase inhibition and antiviral activity.

TABLE V

Inhibition of SAH-hydrolase from L-1210 murine leukemia cells by 9-substituted adenine derivatives

Formula	Substituent	v_i/v_0[a]
1b	(2*S*)-2,3-Dihydroxypropyl	0·26
10b	(2*S*,3*S*)-*threo*-2,3-Dihydroxybutyl	0·52
10c	(2*R*,3*R*)-*threo*-2,3-Dihydroxybutyl	0·89
20a	(2*S*,3*R*)-*erythro*-2,3,4-Trihydroxybutyl	0·62
20c	(2*S*,3*S*)-*threo*-2,3,4-Trihydroxybutyl	0·96
13	(*RS*)-1,2-Dihydroxy-3-butyl	0·87

[a] $v_0 = 0{\cdot}84 \cdot 10^6$ mol l^{-1} min^{-1}.

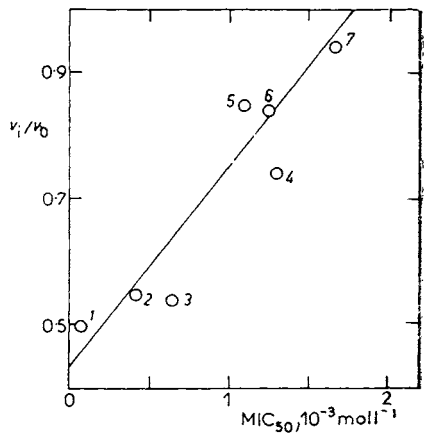

FIG. 1

Relation between the antiviral (VSV) activity (MIC_{50}) and inhibition of rat liver SAH-hydrolase (v_i/v_0) (v_i and v_0 denote the initial reaction rate of inhibited and non-inhibited reaction, respectively). *1 1b, 2 20a, 3 10b, 4 10d, 5 30, 6 20b, 7 20c*

These parameters may be interrelated either directly, *e.g.* the higher SAH concentration resulting from SAH hydrolase inhibition prevents the initiation of viral mRNA translation, or indirectly, *via* methyltransferases which are inhibited by SAH. Inhibition of methyltransferases can indeed affect virus multiplication: *e.g.* NDV mRNA (guanine-7-)-methyltransferase (which ensures the capping of NDV mRNA), is strongly inhibited by SAH-hydrolase inhibitors[39]. Also, the higher relative content of SAH in interferon-treated HeLa cells infected with VSV, is associated with a deficient cap formation and reduced synthesis of VSV mRNA (ref.[40]).

Of all test compounds the most potent inhibitor of both VSV and VV is 9-(*S*)-(2,3-dihydroxypropyl)adenine (*1b*); other active compounds are the isomeric 2,3-dihydroxybutyl derivatives *10b,d,e*. These compounds were investigated in more detail (Table VI) for their inhibitory effects on the growth of vesicular stomatitis virus in both human skin fibroblasts and primary rabbit kidney cells. These data not only confirm the results obtained by the MIC_{50} method (Table II) but also demonstrate the effectiveness of the (2*S*)-isomers *1b*, *10b* and *10d* as virus inhibitors. The relative activity of the compounds was *1b* : *10b* : *10d* = 16 : 2 : 1, with some variation depending on the cell type. The cell-dependent antiviral activity of compound *1b* is further documented in ref.[19,41].

The relative order of activity of the compounds *1b*, *10b* and *10d* also extends to viruses other than VSV (Table III). All three compounds *1b*, *10b* and *10d* inhibit vesicular stomatitis virus, parainfluenza virus, reovirus and vaccinia virus, but they

TABLE VI

Effect of 9-(2,3-dihydroxyalkyl)adenines (at $5 \cdot 10^{-4}$ mol l^{-1}) on the growth of vesicular stomatitis virus in primary rabbit kidney (PRK) and human skin fibroblast (HSF) cells

Compound	VSV yield (log PFU[a])					
	8 h		24 h		48 h	
	PRK	HSF	PRK	HSF	PRK	HSF
None	4·0	3·3	4·7	5·8	4·3	5·8
1a	2·3	1·3	2·4	3·8	3·0	3·8
1b	2·2	1·3	2·8	3·5	2·6	3·6
10b	2·3	1·3	3·6	4·8	3·5	4·6
10c	4·2	3·2	4·6	5·7	4·3	5·7
10d	3·1	2·0	4·1	5·0	3·8	4·8
10e	4·2	2·9	4·6	5·7	4·3	5·3

[a] PFU plaque forming units.

do not affect coxsakie, polio or sindbis viruses. This spectrum of antiviral activity is typical for SAH-hydrolase inhibitors of the above-mentioned type (an analogous specificity is apparent for the eritadenines[19]). The quantitative differences in activity against different viruses are in agreement with the known facts concerning the importance of specific methylation processes. Thus, in vaccinia and reovirus-infected cells, an increase in SAH concentration does not preferentially affect the capping enzyme, (guanine-7-)methyltransferase, as is the case for VSV-infected cells (*vide supra*), but rather the nucleoside 2'-O-methyltransferase[42,43]. The latter enzyme is of lesser importance for the activity of viral mRNA than the capping enzyme[44].

Most of the compounds were also tested for activity against herpes simplex virus type 1. In contrast to the corresponding open-chain derivatives of guanine of which *e.g.* 4-hydroxybutyl and 3,4-dihydroxybutyl compounds show a specific activity against HSV-1 (ref.[45-47]), none of the adenine derivatives is markedly active against HSV-1. However, the guanine derivatives act by a mechanism that is completely different from that of the adenine derivatives. Upon phosphorylation within the cell to their triphosphate they interfere with the synthesis of viral DNA (ref.[45,47]).

Inhibition of viral cytopathogenicity was also observed with adenosine analogues which do not inhibit SAH-hydrolase, *e.g.* compound *8* ("EHNA"). EHNA is a strong adenosine aminohydrolase inhibitor, and causes a general perturbation of purine metabolism by diminishing the degradation of intracellular adenosine[48]. Also the weak antiviral activity of compound *40* can be rationalized in view of the antiviral effects of its predecessor[49] SIBA. The amino alcohols *30*, *31* and *39a* may owe their antiviral activity to the fact that within the cell they are transformed to the corresponding di- or trihydroxyalkyl derivatives which then inhibit SAH-hydrolase. Of course, these compounds, as well as compounds *23*, *24* and *36*, can act also by mechanism other than inhibition of SAH-hydrolase. Their activity is not extraordinarily high and the presently available data do not suffice to propose a hypothesis for their mechanism of action.

Our assumption of a direct correlation between antiviral activity and SAH-hydrolase inhibition must be corroborated by direct measurements of SAH-hydrolase activity within the virus-infected cell. Several factors may influence the interaction of the compounds with SAH-hydrolase within the cell: *e.g.* their penetration into, and fate inside, the cell. We must also consider the possibility that the inhibitory effects of the compounds on native enzyme or enzyme occupied by substrate or product may substantially differ from their effects upon the isolated enzyme. In spite of these considerations, we postulate that compounds showing an inhibitory activity on the cell-free enzyme may be biologically active in cell culture systems. This can most likely be expected from those compounds (*e.g.* DHPA) that are not rapidly catabolized. Since SAH-hydrolase inhibitors, in addition to their antiviral effects, also show other specific biological activities[50,51], the data obtained here represent a lead for further biological investigation of these compounds.

We are indebted to Dr K. Jošt for the preparation of L-*homocystine and Dr A. Merta for the kind gift of SAH-hydrolase from* L-1210 *murine leukemia cells. We thank Dr R. Zhuk, Institute of Organic Synthesis, Riga (USSR) for a sample of* 9-(2-*hydroxyethoxymethyl)adenine and Burroughs Wellcome Co (U.S.A.) for a gift of* EHNA *hydrochloride. The excellent technical assistance of Dr I. Rosenberg, Mrs B. Nováková and Mrs H. Miklová is gratefully acknowledged.*

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