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Adenosylhomocysteine hydrolase inhibitors: Synthesis of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine and its effect on Rous sarcoma virus and Gross murine leukemia virus

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SUMMARY. The synthesis of a new analog of adenosylhomocysteine (AdoHcy), 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine, is described. The compound is a non-competitive inhibitor of AdoHcy hydrolase, with a K_i of 0.4 mM, as compared to a K_i of 3 μM for 3-deazaadenosine, a competitive inhibitor of the enzyme. 5'-Deoxy-5'-(isobutylthio)-3-deazaadenosine is not hydrolyzed by AdoHcy hydrolase. 5'-Deoxy-5'-(isobutylthio)-3-deazaadenosine when tested has a selective anti-viral activity against Rous sarcoma virus in chick embryo cells, and against Gross murine leukemia virus in mouse embryo cells. Possible mechanisms of this anti-viral activity are discussed.

INTRODUCTION

Adenosylmethionine (AdoMet) is known to participate as a methyl donor in many reactions of physiological importance (1, 2). Adenosylhomocysteine (AdoHcy), the product of these methyl transfer reactions, is known to be a competitive inhibitor of these reactions, and analogs of AdoHcy with varying degrees of specificity as inhibitors of methylases have been reported (3-6). A wide variety of analogs of AdoHcy or adenosine have been surveyed by Chiang et al. (7) as potential inhibitors of AdoHcy hydrolase. Among the analogs surveyed by Chiang et al. (7), 3-deazaadenosine was found to be the most potent inhibitor of AdoHcy hydrolase, and is nearly 100-fold more potent than 5'-deoxy-

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Abbrevations: AdoHcy, adenosylhomocysteine; 3-deaza-AdoHcy, 3-deazaadenosylhomocysteine; DMSO, dimethylsulfoxide; RSV-BH, Bryan "high titer" strain of Rous sarcoma virus; Gross MLV, Gross murine leukemia virus.

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5'-S-isobutyl-adenosine as an inhibitor of this enzyme. It was also shown that in isolated rat hepatocytes, the levels of AdoMet and AdoHcy were greatly increased upon incubation with 3-deazaadenosine with the simultaneous formation of 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) (7). In a detailed investigation by some of us, it was found that 3-deazaadenosine (0.1 mM) will prevent replication and growth of Rous sarcoma virus and other viruses in chick embryo cells, and that under certain conditions it will reverse the malignant transformation induced by oncogenic virus (submitted for publication).

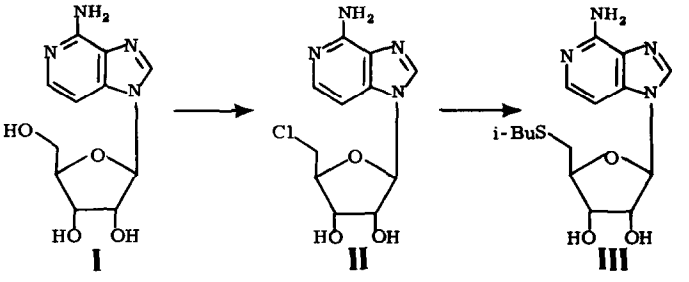
5'-Deoxy-5'-S-isobutyl-adenosine, a compound that is an inhibitor of AdoHcy hydrolase (7, 8), has been found to have anti-viral activity in vitro (8-10). We report here some biological properties of 5'-deoxy-5'-(isobutylthio)-3-deaza-adenosine, a compound that incorporates 3-deazaadenosine into a bioisostere of 5'-deoxy-5'-S-isobutyl-adenosine, the last two compounds being potent inhibitors of AdoHcy hydrolase, and also anti-viral, anti-tumor agents well tolerated by cells

MATERIALS AND METHODS

Synthesis of Compounds: 5'-Deoxy-5'-(isobutylthio)-3-deazaadenosine was synthesized as follows:

3-Deazaadenosine (I), prepared by an improved procedure (11), was chlorinated with thionyl chloride in hexamethylphosphoramide to give 5'-chloro-5'-deoxy-3-deazaadenosine, which on reaction with isobutyl mercaptan in ethanol containing sodium methoxide gave 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine (Scheme 1).

Scheme 1



5'-Chloro-5'-deoxy-3-deazaadenosine (II). A solution of 3-deazaadenosine (1.89 mmol) in hexamethylphosphoramide (5 ml) containing thionyl chloride (0.75 ml) was kept for 20 h at ambient temperature and then poured into 2 ml of cold chloroform. The pH of the resulting solution was raised to 9 with concentrated ammonium hydroxide with cooling. Water (25 ml) was added with shaking and the aqueous layer removed and lyophilized to give a fluffy, yellow solid. This material was used without further purification.

5'-Deoxy-5'-(isobutylthio)-3-deazaadenosine (III). A solution of 1.89 mmol of crude 5'-chloro-5'-deoxy-3-deazaadenosine in 189 ml of 1 N sodium methoxide in absolute ethanol containing 2.71 ml (25 mmol) of isobutyl mercaptan was refluxed for 30 min, neutralized with glacial acetic acid, and evaporated to dryness in vacuo. The filtered chloroform extract was evaporated to dryness in vacuo. An aqueous solution of the residue was treated with 1.89 mmol of solid picric acid. Stirring and gentle heating produced a heavy crystalline precipitate. After refrigeration of the mixture for several hours, the solid was collected by filtration, washed with a small amount of cold water, and dissolved in methanol. The methanol solution was stirred with Dowex 1-X8 (carbonate) ion-exchange resin until it became colorless. The resin was filtered and washed several times with methanol and then water. The combined filtrate and washings was evaporated to dryness in vacuo. The residue crystallized from water and on drying in vacuo at 78° melted to a glass, yield 134 mg (21%); uv max in nm ($\varepsilon \times 10^{-3}$): (0.1 N HCl) 263 (11.90), (pH 7) 264 (10.44), (0.1 N NaOH) 265 (10.86). TLC homogeneous (silica gel, 3 $CHCl_3$:1 MeOH). Anal. Calculated for $C_{15}H_{21}N_4O_3S$: C, 53.39; H, 6.27; N, 16.61. Found: C, 53.33; H, 6.50; N, 16.96.

9-[5'-(Methylthio)-β-D-arabinofuranosyl]adenine was synthesized as described (12). 3-Deaza-AdoHcy was synthesized by allowing L-homocysteine to react to completion with 3-deazaadenosine in the presence of excess AdoHcy hydrolase crystallized from cow liver (13). The reaction was stopped by adding sulfosalicylic acid to a final concentration of 5%. The reaction mixture was then purified by a column of VYDAC cation exchanger (14). The peak substance of 3-deaza-AdoHcy, collected from fractions, were lyophilized and resuspended in a small volume of H_2O. Ethanol (95%) was added to reprecipitate the 3-deaza-AdoHcy. The precipitate after centrifugation was then dissolved in H_2O. N^{γ}-Adenosyl-α,γ-diaminobutyric acid was a gift of James K. Coward of Yale University.

Assay of AdoHcy hydrolase: AdoHcy hydrolase, from cow liver, was assayed as described previously (7), with 0.1 mM [^{14}C]AdoHcy in the presence and absence of AdoHcy analogs.

Cells and Viruses: Infection of sparsely distributed chick embryo cells by Rous sarcoma virus (RSV-BH), and development of infected cells into foci of transformed cells, proceeded as described previously (15). AdoHcy analogs were dissolved in dimethylsulfoxide (DMSO), and were added to infected cells 18 h after infection; a final concentration of 0.4% DMSO was maintained in the treated cell cultures.

The compound 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine was evaluated for selective anti-viral activity against Gross murine leukemia virus (MLV) in vitro using the UV-XC plaque assay of Rowe et al. (16) in a quantitative plaque-reduction test conducted as previously described (17).

RESULTS AND DISCUSSION

Fig. 1 shows the comparison of the inhibition of AdoHcy hydrolase by 5'-deoxy-5'-S-isobutyl-adenosine, 9-[5'-deoxy-5'-(methylthio)-β-D-arabinofuranosyl]-adenine, 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine, and N^{γ}-adenosyl-α,γ-diaminobutyric acid, with I_{50}'s of >1.0, 0.43, 0.28, and 0.14 mM respectively. 3-Deazaadenosine with an I_{50} of 8 μM (7) thus remains the most potent inhibitor of AdoHcy hydrolase. 3-Deazaadenosine was found to be the only competitive inhibitor,

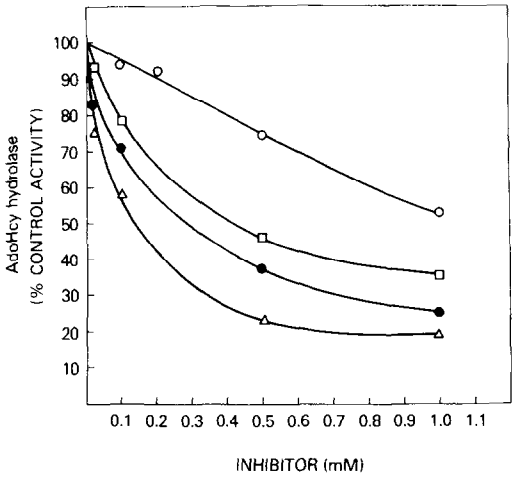


Fig. 1. Inhibition of AdoHcy hydrolase from cow liver by: (○) 5'-deoxy-5'-S-isobutyl-adenosine, (□) 9-[5'-deoxy-5'-(methylthio)-β-D-arabinofuranosyl]adenine, (●) 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine, (△) N^{γ}-adenosyl-α-γ-diaminobutyric acid.

with a K_i of 3 μM. The inhibitions caused by N^{γ}-adenosyl-α,γ-diaminobutyric acid with a K_i of 0.3 mM, 5'-deoxy-(isobutylthio)-3-deazaadenosine with a K_i of 0.4 mM and 9-[5'-deoxy-5'-(methylthio)-β-D-arabinofuranosyl]adenine with a K_i of 0.5 mM were non-competitive in nature (not shown).

Next we examined the ability of these compounds to inhibit the oncogenic transformation of chick embryo cells induced by RSV-BH. As shown in Table I, 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine was just as effective as 3-deaza-adenosine itself in inhibiting focus formation in chick embryo cells infected with RSV-BH. At 0.1 mM of both of these compounds, focus formation in RSV-BH infected cells was reduced by 95%, without noticeable effects on the host cells. It is noteworthy that, like other AdoHcy analogs having modifications in the amino acid moiety, it was found not to be hydrolyzed by AdoHcy hydrolase (7). Neither 3-deaza-AdoHcy nor 9-[5'-deoxy-5'-(methylthio)-β-D-arabinofuranosyl]adenine had any significant effect on focus formation. To what extent the last 2 compounds are taken up into cells has not been determined.

The ability of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine to inhibit

Table I

Effects of AdoHcy analogs on focus formation by RSV-BH in chick embryo cells

Treatment		No. of Foci*	% inhibition
None		328	—
3-Deazaadenosine	0.1 mM	17	95
	0.03 mM	106	68
5'-Deoxy-5'-(isobutylthio)-3-deazaadenosine	0.1 mM	3	99
	0.03 mM	93	72
3-Deazaadenosylhomocysteine	0.1 mM	276	16
	0.03	330	0
9-[5'-Deoxy-5'-(methylthio) β-D-arabinofuranosyl]-adenine	0.1 mM	298	9
	0.03 mM	274	17

*Compounds were added 18 h after infection, and foci were counted 7 days later.

replication of Gross MLV in mouse embryo cells was tested next. As can be seen in Figure 2A, 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine inhibited the replication of Gross MLV in mouse embryo cells with an 85% inhibition of plaque formation in the indicator XC cells at 3 μM. Complete inhibition of virus replication was obtained at dose levels of 30 to 100 μM, but at these levels cytotoxicity was observed (Figure 2B). The selectivity ratio (i.e., the highest noncytotoxic concentration of drug over the lowest effective concentration of drug) was about 10. This compound therefore appears to be a promising candidate for evaluation against RNA tumor viruses in vivo.

Whereas the K_i of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine is about 100 times higher than the K_i of 3-deazaadenosine as inhibitors of AdoHcy hydrolase, the ability of these 2 compounds to inhibit viral replication is almost equal. 3-Deazaadenosine was found by us to have a powerful, although reversible,

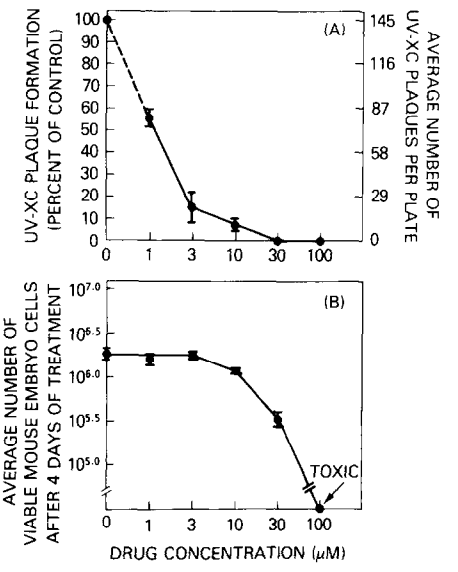


Fig. 2. Anti-viral activity of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine against Gross MLV in vitro: (A) Inhibition of virus replication in Swiss mouse embryo cells. Each point represents the mean number of UV-XC plaques per culture (triplicate assays) ± the standard deviation. The drug was dissolved in DMSO at the time of virus inoculation and was present throughout the incubation period. (B) Effect of treatment on the multiplication of host cells after 4 days exposure to drug. Each point represents the mean number of viable mouse embryo cells per culture ± the standard deviation. The initial number of cells plated was 3.5×10^5 cells.

effect on the oncogenic transformation induced by Rous sarcoma virus, and on its infectivity. Sensitivity to 3-deazaadenosine is maximal during the phase of virus replication which requires the synthesis of viral mRNA and protein, and is minimal during the early period when DNA synthesis is required. The inhibition of virus growth and replication effected by 5'-deoxy-5'-(isobutylthio) 3-deazaadenosine can be ascribed as a working hypothesis to 2 mechanisms: 1) Its ability to inhibit AdoHcy hydrolase, inhibition of which would result in a change in a ratio of AdoMet/AdoHcy, which could affect methylation reactions required for viral growth and replication. 2) Its possible direct inhibition of methylases which utilize AdoMet, by its intrinsic similarity to AdoHcy. Whether one or both these postulated mechanisms are responsible for its anti-viral activity remains to be demonstrated.

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