Antiproliferative effects of 4′, 5′-unsaturated adenine nucleosides on leukemia L 1210 cells in vitro

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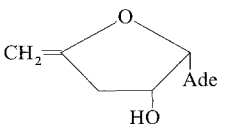
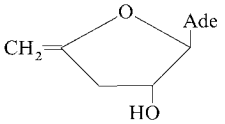
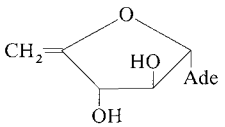
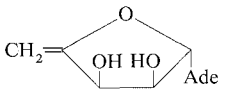
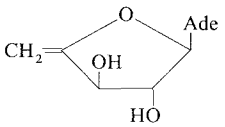
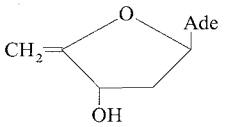
Received 16 December 1988; accepted 20 April 1989

Summary. Several 4′, 5′-unsaturated adenine nucleosides were shown to have antiproliferative activity against L1210 leukemia cells in vitro. The active nucleosides were cytotoxic to the L1210 cells as demonstrated by Trypan Blue uptake. The cytotoxicity was not induced by alterations in the ribonucleoside and deoxyribonucleoside triphosphate levels of the L1210 cells.
Key words. 4′, 5′-unsaturated adenine nucleosides; L1210 leukemia cells; cytotoxicity.

In a previous publication the synthesis of the two anomeric forms of 9-(3,5-dideoxy-D-glycero-pent-4-eno-furanosyl) adenine was reported. These nucleosides were shown to have antiproliferative activity against L1210 leukemia cells in vitro[1]. It seemed surprising that these compounds had any activity because neither one can be enzymatically phosphorylated in the 5′ position of the sugar moiety. Phosphorylation of a nucleoside to the 5′ nucleotide is the mechanism by which clinically useful anticancer agents become active[2]. Based upon this observation, a study was undertaken to determine the antiproliferative effects of a group of closely related enol ether containing adenine nucleosides. The structures of the nucleosides used in the present study are illustrated in table 1.

Table 1. Chemical structures of 4′, 5′-unsaturated adenine nucleosides.

Compound No.	Structure[a]	Reference
1		1
2		1
3		13
4		14
5		13
6		15

[a] The method of synthesis and proof of structure is given in the reference.

Material and methods

Antiproliferative agents. The 4′, 5′-unsaturated adenine nucleosides were previously reported from one of our laboratories (see references in table 1). 6-Mercaptopurine was purchased from Aldrich (Milwaukee, WI).

Antileukemic assay. The antiproliferative effects of the nucleosides were determined by a previously described procedure from our laboratory[3]. All experimental compounds were dissolved in distilled water (heating in a boiling water bath when necessary) at a concentration of 10^{-3} M and sterilized by filtration. Aliquots from these solutions were transfered to 25-cm^2 culture flasks containing 4×10^5 L1210 cells in 2 ml of medium and then incubated for 48 h. Cell counts and viability were assessed, before and after incubation with the drugs by Trypan Blue exclusion, using a hemocytometer.

Ribonucleoside and deoxyribonucleoside triphosphate pools. The triphosphate pools were determined by a high-pressure-liquid-chromatography (HPLC) assay. A Beckman Model 334 HPLC was employed. The apparatus was equipped with three pumps, Model 110 A; a computerized controller, Model 421; and an Altex integrator and recorder. HPLC grade methanol and acetonitrile were purchased from Aldrich. Buffer solutions were filtered through 0.45-μm filters (Millipore Corp., Bedford, MA), and deaerated before use. The ribonucleotide and deoxyribonucleotide standards were purchased from Sigma (St. Louis, MO) and Boehringer Mannheim (Indianapolis, IN). The L1210 cells were washed twice in Ringer's solution. The final pellet was homogenized in 6% tricholoroacetic acid, and the nucleotides extracted using a modification of the Freon-amine method[4, 5]. The ribonucleotides in the cell extract were degraded by periodate oxidation in order to determine the deoxyribonucleoside triphosphate pools[6, 7]. Aliquots (20 μl) of cell samples, standards, and cell standards mixed with stan-

dard solutions were first neutralized with NaOH, then injected onto a 4 mm × 25 cm column packed with Partisil SAX (Whatman, Hillsboro, OR), and eluted with 0.3 M KH_2PO_4, pH 3.0. The separations were completed within 60 min, and the retention times were compared with the retention times of the standards [8].

Results and discussion

Microscopic examination of the L1210 cells revealed that the cells had become markedly enlarged and stained positive with Trypan Blue after exposure to compounds **1**–**4** (See table 1). In addition, there was abundant cellular debris accompanying the swollen L1210 cells, thus indicating that the active nucleosides were cytotoxic to the L1210 cells, and prevented cellular division before complete cessation of macromolecular synthesis. The ID_{50} (50% inhibition of proliferation after 48-h incubation) of compound **1** was 6×10^{-5} M; **2**, 1×10^{-4} M and **3**, 1×10^{-4} M. Compound **4** resulted in cell death of 30% of the L1210 cells at 1×10^{-4} M, while compounds **5** and **6** had no detectable antiproliferative activity. Utilizing the same assay system, 6-mercaptopurine, which is widely employed to treat human leukemia, had an ID_{50} of 6×10^{-6} M. Therefore, compound **1** is only one order of magnitude from being as active as 6-mercaptopurine.

By HPLC it was determined that there were no alterations in the ribonucleoside- and deoxyribonucleoside triphosphate levels in the L1210 cells which were exposed to compound **1** as compared to untreated cells (table 2).

Table 2. Ribonucleoside and deoxyribonucleoside triphosphate pools in L1210 leukemia cells before and after incubation with nucleoside 1.

Nucleoside	Concentration (p moles/10^6 cells)[a]	
	Untreated L1210 Cells	Inhibited L1210 Cells
UTP	895 + 95	818 + 42
CTP	159 + 25	192 + 21
ATP	2147 + 310	2308 + 230
GTP	320 + 28	302 + 41
dTTP	61 + 14	71 + 16
dCTP	24 + 4	22 + 5
dATP	44 + 10	51 + 15
dGTP	12 + 4	11 + 3

[a] The standard deviations were calculated from results from two separate samples, each done in duplicate.

This data suggests that the nucleoside-induced cytotoxicity of L1210 cells is not due to the inhibition of a metabolic pathway essential for the synthesis of any of the purine or pyrimidine precursors of RNA or DNA [9, 10]. However, it is possible that these cytotoxic compounds may be active via the purine salvage pathway through the inhibition of purine ribonucleoside phosphorylase. This would culminate in an accumulation of dATP which in turn has been demonstrated to be toxic to lymphoid tissue by inhibiting ribonucleotide reductase [11, 12].

Although the antiproliferative effects of compounds **1**, **2**, and **3** are not very pronounced, they do offer an advantage over existing antineoplastic purine and pyrimidine analogues, in that they do not require a lethal enzymatic phosphorylation in the C-5′ position of the sugar. Therefore, the 4′, 5′-unsaturated adenine nucleosides are not susceptible to drug resistance via the inability of resistant cells to synthesize the required corresponding nucleotides necessary for activity [2]. Based on these results, it will be of interest to determine the exact mechanism of action of the 4′, 5′-unsaturated adenine nucleosides, and to synthesize other purine and pyrimidine enol ether nucelosides in anticipation of obtaining more efficacious antiproliferative agents.

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0014-4754/89/080729-02$1.50 + 0.20/0