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ANALOGS OF 9-DEAZAADENOSINE: POTENT INHIBITORS OF METHYLTHIOADENOSINE PHOSPHORYLASE

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Methylthioadenosine (5'-deoxy-5'-methylthioadenosine, MTA), produced as a naturally occurring by-product during the synthesis of spermidine and spermine via the propylamine transferases spermidine synthase and spermine synthase, respectively, has been shown to exert profound antiproliferative effects in a number of biological systems [1,2]. While its mechanism of action has not been proven, it is known to be a potent inhibitor of the propylamine transferases [3], S-adenosylmethionine-dependent methyltransferases [4], and S-adenosylhomocysteine hydrolase [5]. In mammalian cells, however, its accumulation is prevented by rapid phosphorolytic cleavage by MTA phosphorylase into adenine and methylthioribose-1-phosphate, which in turn are converted into adenine nucleotides and methionine respectively [6]. This latter pathway may be a critical salvage mechanism by which cells are able to conserve purine nucleosides/nucleotides and the essential amino acid methionine during periods of rapid cell growth [7], and inhibition of MTA phosphorylase may be an effective target in the design of potential antiproliferative agents. Towards this end, we have focused on derivatives of 9-deazaadenosine, in which a carbon-carbon bond replaces the normal glycosidic bond of adenosine, thus reducing or eliminating its susceptibility to phosphorolytic degradation. This report describes two such analogs, 5'-chloro-5'-deoxy-9-deazaadenosine (Clc^9Ado) and 5'-methylthio-5'-deoxy-9-deazaadenosine (MTc^9Ado) and their effects on mammalian MTA phosphorylase *in vitro*.

MATERIALS AND METHODS

Methylthioadenosine phosphorylase (EC 2.4.2.1) (sp. act. 10.5 nmol/min/mg protein) was isolated from rat liver and assayed using 5'-[CH_3-^{14}C]methylthioadenosine (1.3×10^7 cpm/μmol) as previously described [8]. Labeled MTA was prepared by the acid hydrolysis of S-adenosyl-L-[CH_3-^{14}C]methionine (pH 4, 30 min at 100°) [9] and purified by high performance liquid chromatography (μBondapak C_{18}, 8% MeOH in 50 mM ammonium acetate, pH 6, UV detection at 254 nm, 1.0 ml/min, r = 8.7 min). The synthesis of Clc^9Ado and MTc^9Ado from 9-deazaadenosine [10] and their chemical characterization will be described elsewhere. Briefly, 9-deazaadenosine was converted to Clc^9Ado using thionyl chloride in hexamethylphosphoramide, followed by displacement of the chloro group with methyl mercaptan in 2N NaOH to yield MTc^9Ado.

RESULTS AND DISCUSSION

Both Clc^9Ado and MTc^9Ado were found to be potent, competitive inhibitors of rat liver MTA phosphorylase with K_i values of 5×10^{-7} and 2×10^{-7} M respectively (Table I) (K_m for

MTA = 6 x 10^{-6} M). When assayed by HPLC methods [11], neither compound was found to act as an alternate substrate for the enzyme, and enzyme activity could be recovered after the inhibitor was removed by dialysis. Compared to 5'-chloro-5'-deoxyformycin and 5'-chloro-5'-deoxytubercidin, which were found previously to be potent inhibitors of MTA phosphorylase isolated from Sarcoma 180 cells and rat ventral prostate respectively [12,13], both CDAdo and MDAdo were significantly more potent, supporting not only the importance of the N7 position but also the relative contribution of the C8 position to enzyme affinity. This observed inhibition is in contrast to that observed for the purine salvage enzyme S-adenosylhomocysteine hydrolase, which displays almost no affinity for 9-deaza and 7-deaza nucleosides and only moderate affinity for formycin [14]. Thus, analogs such as $Clc^{9}Ado$ and $MTc^{9}Ado$ may be useful in studying the cellular regulatory role(s) of methylthioadenosine and the consequences of its accumulation.

Table I. Inhibition of methylthioadenosine phosphorylase by 5'-modified analogs of 9-deazaadenosine in vitro

Target compound	K_i (μM)
5'-Chloro-9-deazaadenosine	0.5
5'-Methylthio-9-deazaadenosine	0.2
5'-Chloroformycin	3.6
5'-Chlorotubercidin	34.2

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