Biochemical Pharmacology, Vol. 44, No. 9, pp. 1697–1700, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

RAPID COMMUNICATION

INHIBITION OF ADENOSINE DEAMINASE BY AZAPURINE RIBONUCLEOSIDES

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(Accepted 10 September 1992)

ABSTRACT-- We have synthesized several 8-azapurine nucleosides as inhibitors of adenosine deaminase. The presence of a nitrogen on the imidazole ring decreased the K_i value for nebularine by 100-fold but did not lower the K_i value for coformycin. Evaluation of these compounds in a MOLT-4 growth assay revealed that 2-azacoformycin was as effective as 2'-deoxycoformycin in potentiating growth inhibition by 2'-deoxyadenosine. The azapurine nucleosides merit further study as antitumor agents.

INTRODUCTION

The importance of adenosine deaminase (ADA; EC 3.5.4.4) in maintaining purine nucleotide pools in cells of the immune system has been emphasized by two hereditary conditions in which the amount of ADA is altered. Overproduction of ADA has been associated with hemolytic anemia [1], while a deficiency of ADA results in a severe immunodeficiency disease characterized by a complete lack of T lymphoblasts [2]. This latter observation has led to the treatment of patients with acute T lymphoblastic leukemia using an inhibitor of ADA, which has met with limited success [3,4]. Knowledge of the exact mechanism of deamination of adenosine by ADA may lead to the rational synthesis of inhibitors of ADA which will be more effective antileukemic agents.

One proposed mechanism for the ADA-catalyzed deamination invokes the addition of water across the purine 1,6-bond to yield the tetrahedral intermediate **1** [5-7], as illustrated in Fig. 1. This proposed mechanism has also been invoked in the inhibition of ADA by nebularine through the formation of the intermediate structure **2**. If this supposition is correct, then analogs of nebularine with an increased propensity towards addition of water to the pyrimidine ring should exhibit greater affinities towards ADA than the parent compound.

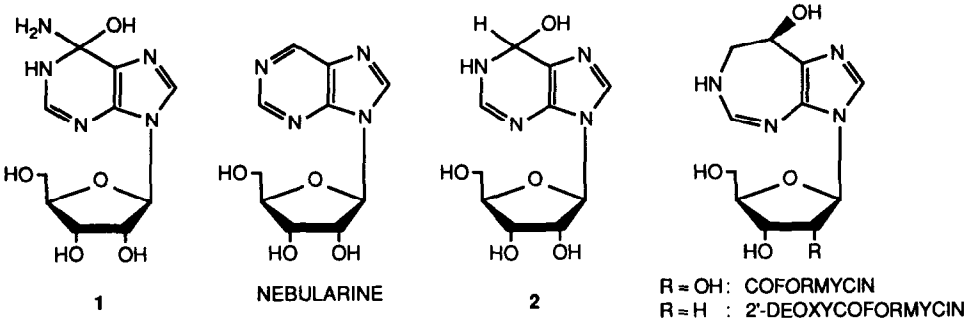

Fig. 1. Structures of intermediates and inhibitors of the adenosine deaminase reaction.

Thus, we chose as our synthetic targets several azapurine derivatives based on previous reports [8,9] of the stability of the 1,6-dihydro analog of 9-benzyl-8-azapurine, as well as the documented propensity of the 8-azapurine

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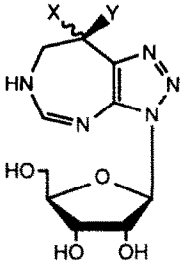
ring system to hydrate covalently [10-12]. The synthesis of these analogs was especially compelling since we had previously synthesized the corresponding azacoformycin analog [13].

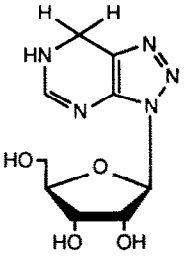
MATERIALS AND METHODS

The compounds illustrated in Fig. 2 were synthesized and evaluated for inhibitory activity with ADA. The synthesis of both 8-R and 8-S epimers of 2-azacoformycin has been reported previously [13] and a preliminary account of their inhibition of ADA has been presented [14]. The 8-azanebularine analogs were synthesized by a novel procedure resulting from the synthesis of 2-azacoformycin and will be reported in detail separately. A preliminary account of the synthesis of **5** and **6** has been presented [15].

The ability of the azanebularine analogs to inhibit calf spleen ADA (Sigma Chemical Co., St. Louis, MO) was analyzed using a standard radiochemical assay which quantitated the conversion of [^{14}C]adenosine to [^{14}C]inosine [16]. Deamination of adenosine appeared linear with time in the presence of all inhibitors except epi- and 2-azacoformycin, which required a 15 min preincubation period for maximal ADA inhibitory activity. Kinetic data for these two compounds were obtained after preincubation of inhibitor with ADA before the addition of substrate.



X = H, Y = OH: **3**
X = OH, Y = H: **4**



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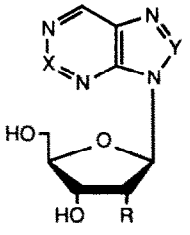

X = C, Y = N, R = OH: **6**
X = C, Y = N, R = H: **7**

Fig. 2. Structures of azapurine nucleosides.

RESULTS AND DISCUSSION

Table 1 lists the K_i values for the inhibition of ADA by the compounds illustrated in Figs. 1 and 2. Of these compounds, 2-azacoformycin was the most potent inhibitor of ADA with a K_i value of 1.2×10^{-9} M. The S-isomer (**4**, epi-2-azacoformycin) was 1000-fold weaker as an inhibitor of ADA, illustrating the stereospecificity of the ADA molecule. These results are in accord with the previously reported difference in inhibitory potencies between the epimers of the naturally-occurring 2'-deoxycoformycin [17]. Relative to the reported literature K_i value of 1.0×10^{-10} M for the inhibition of ADA by coformycin [18], 2-azacoformycin was approximately 10-fold weaker as an inhibitor. However, this difference may be explained by differences in assay conditions since, in our hands, 2'-deoxycoformycin was 6-fold weaker as an inhibitor of ADA compared to the literature value [19]. Thus, it is likely that 2-azacoformycin is similar to coformycin in its ability to inhibit ADA.

Nebularine and its analogs exhibited competitive inhibition kinetics with ADA. 8-Azanebularine (**6**) with a K_i value of 4.0×10^{-8} M was a more potent inhibitor of ADA than either nebularine or 1,6-dihydro-8-azanebularine (**5**). These results are consistent with the hypothesis that hydration of these compounds across the 1,6-bond is necessary to mimic the structure of the transition state for adenosine as it is deaminated by ADA. Since 1,6-dihydro-8-azanebularine is unable to add a water across the 1,6-bond, it would be expected to be a much weaker inhibitor than the fully aromatic 8-azanebularine. The more potent inhibition of ADA by 8-azanebularine compared to nebularine could be explained by the presence of the 8-nitrogen, which may facilitate the formation of the covalent hydrate. Although the corresponding 9-methyl analog does not hydrate [10-12], the substitution of the methyl group by the more electron-withdrawing ribofuranosyl moiety may enhance the electrophilicity of the 6-position.

As illustrated in Table 1, 2'-deoxy-8-azanebularine and its ribosyl derivative exhibited similar K_i values. This is in direct contrast to the 10-fold difference in K_i values of 2'-deoxycoformycin and its ribosyl congener. Thus, the substitution of deoxyribose for ribose does not always increase the potency of nucleoside ADA inhibitors.

We have evaluated the biologic activity of these inhibitors by determining their effects at a concentration of 5 μM on the 2'-deoxyadenosine-mediated growth inhibition of MOLT-4 T lymphoblasts. Of the compounds tested, only 8-azanebularine, 2-azacoformycin and 2'-deoxycoformycin decreased the IC_{50} value of 2'-deoxyadenosine in this assay (Table 2). Although the IC_{50} value for 2'-deoxyadenosine was lowest in the presence of 8-azanebularine, this synergy may not be due solely to the inhibition of ADA activity by 8-azanebularine since this drug by itself inhibited MOLT-4 cell growth with an IC_{50} value of 7 μM. 2'-Deoxy-8-azanebularine was less toxic in this system, exhibiting an IC_{50} value of approximately 600 μM.

Table 1. Kinetic constants for inhibition of adenosine deaminase by purine analogs

Compound	K_i
Nebularine	1.6×10^{-5} M
1,6-Dihydro-8-azanebularine	1.0×10^{-5} M
Epi-2-azacoformycin	1.6×10^{-6} M
8-Azanebularine	4.0×10^{-8} M
2'-Deoxy-8-azanebularine	3.4×10^{-8} M
2-Azacoformycin*	1.2×10^{-9} M
Coformycin†	1.0×10^{-10}M
2'-Deoxycoformycin*	9.0×10^{-11}M

*K_i value was determined by the I_{50} method [20].
†K_i value (I_{50} method) was obtained from Ref. 18.

Table 2. Effects of ADA inhibitors on MOLT-4 cell growth inhibition by 2'-deoxyadenosine

Drug Addition (5 μM)	IC_{50} for 2'-Deoxyadenosine* (μM)
No addition	200
1,6-Dihydro-8-azanebularine	>100
Epi-2-azacoformycin	>100
8-Azanebularine	1.5
2-Azacoformycin	5.3
2'-Deoxycoformycin	5.6

*IC_{50} values were determined in a standard 3-day assay system [21].

2-Azacoformycin and 2'-deoxycoformycin did not inhibit MOLT-4 cell growth and, at a concentration of 5 μM, were equally efficient at decreasing the IC_{50} value for 2'-deoxyadenosine. In an effort to discriminate between the two ADA inhibitors, MOLT-4 cells were incubated for 3 days with 5 μM 2'-deoxyadenosine and either 2-azacoformycin or 2'-deoxycoformycin at concentrations ranging from 0.05 to 5 μM. The effects of the ADA inhibitors appeared indistinguishable in this system as well. Only at the highest concentration of 5 μM were the inhibitors able to decrease cell growth (to 70% of the control value). The inability of these potent, tight-binding inhibitors at concentrations less than 5 μM to enhance growth inhibition by 5 μM 2'-deoxyadenosine most likely reflects their poor transport into the cell, consistent with the reported K_m value of 10 mM for transport of 2'-deoxycoformycin across the erythrocytic cell membrane [22].

Thus, we have reported the enzymatic and biologic evaluation of several new inhibitors of ADA. 8-Azanebularine was 100-fold more potent as an inhibitor of ADA than nebularine, possibly due to the fact that 8-azanebularine should form a transition state analog more easily than nebularine. In contrast, the addition of a nitrogen to the imidazole ring of coformycin did not increase its K_i value for ADA. Compared to 2'-deoxycoformycin, 2-azacoformycin was less potent as an inhibitor of ADA but was indistinguishable biologically in its ability to increase the inhibition of MOLT-4 cell growth by 2'-deoxyadenosine. This compound merits further study as a potential antitumor agent.

ACKNOWLEDGEMENTS--We thank Drs. Linda Wotring and Orna Resnikov for preliminary evaluation of compounds 5 and 6. We are grateful to Denise Gribbin and Amanda Speedy for excellent technical assistance. This research was supported by the American Foundation for Pharmaceutical Education (S.H.K.) and Grants CA 46452 and 5-T32-GM-07767 from the NIH.

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