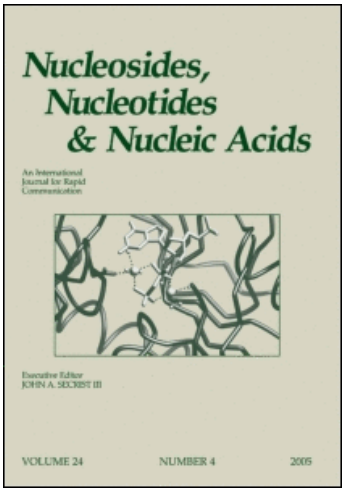

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5'-SUBSTITUTED 2'-DEOXYCYTIDINES AS NON-SUBSTRATE INHIBITORS OF HUMAN DEOXYCYTIDINE KINASE

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Abstract: Several 5'-substituted analogues of 2'-deoxycytidine (dC), including 5'-amino-2',5'-dideoxycytidine (5'-amino-ddC), 5'-azido-2',5'-dideoxycytidine (5'-azido-ddC) and 5'-O-ethyl-2'-deoxycytidine (5'-O-ethyl-dC), are non-substrate inhibitors of human dC kinase. Whereas 5'-amino-ddC inhibits phosphorylation of deoxyadenosine (dA) with $K_i \sim 1$ μM, inhibition of phosphorylation of dC is bimodal and more effective at low inhibitor concentrations. In particular 5'-O-ethyl-dC inhibits phosphorylation of dA 10-fold more effectively ($K_i \sim 3$ μM) than that of dC ($K_i \sim 30$ μM). For 5'-azido-ddC inhibition was shown directly to be competitive with respect to substrate.

Although considerable effort has been devoted to development of potent selective inhibitors of herpesvirus thymidine kinase (TK),[1] relatively little attention has been paid to inhibitors of cellular nucleoside kinases. One notable exception is adenosine kinase, for which 5'-amino-5'-deoxyadenosine has been reported as a good inhibitor.[2] It was also long ago shown that 5'-amino-5'-deoxythymidine is a reasonably good inhibitor of mouse ascites carcinoma 180 thymidine kinase.[3]

During the course of a study on the specificity of human deoxycytidine kinase (dCK), to be elsewhere reported, the foregoing suggested that 5'-amino-2',5'-dideoxycytidine (5'-amino-ddC) might serve as an inhibitor of dCK, the natural substrates of which include also 2'-deoxyadenosine (dA) and 2'-deoxyguanosine. This enzyme is also responsible for "activaction" (by phosphorylation) of a number of chemotherapeutically active nucleoside analogues.[1]

5'-amino-ddC was prepared by electrochemical reduction, as elsewhere described, of 5'-azido-ddC.[4] The latter was synthetised according to standard procedures by Dr. K. Felczak, and purified on a silica gel column. The product of reduction, 5'-amino-ddC

TABLE 1

Inhibition of activity of dCK *vs* dA and dC by 5'-substituted analogues of dC

Inhibitor	pH	IC_{50} (μM)		K_i (μM)	
		30 μM dA	1 μM dC	dA	dC
5'-amino-ddC	7.2	2.3 ± 0.2	18 ± 2 [a]	1.3 ± 0.1	3.6 ± 0.6 / 32 ± 3 [b]
	8.6	1.7 ± 0.2	10 ± 1 [a]	1.2 ± 0.2	1.7 ± 0.3 / 32 ± 6 [b]
5'-azido-ddC	7.2	14.0 ± 0.6	104 ± 4	8.0 ± 0.4	60 ± 2
5'-O-methyl-dC	7.2	100 ± 20	500 ± 20	60 ± 15	230 ± 8
5'-O-ethyl-dC	7.2	5.4 ± 0.2	68 ± 2	3.1 ± 0.1	32 ± 1

[a] IC_{50} values measured at 0.4 μM dC.
[b] The two K_i values were calculated from extrapolated IC_{50} values for low concentrations of inhibitor (< 2.5 μM) and for the higher concentration range: 5.3 μM and 46 μM at pH 7.2 and 2.3 μM and 41 μM at pH 8.6.

was, in turn, purified by TLC on a cellulose F plate (Merck, Darmstadt) with 1 M ammonium acetate / 96% ethanol (2:5, v/v) (R_f = 0.57).

Purified human dCK [5] was kindly made available by Dr. S. Eriksson. Potential substrate activity was followed according to standard procedures [6] with the use of [γ-^{32}P] ATP (0.7 mCi/mmol) as phosphate donor. Inhibitory activity was measured with use of cold Mg·ATP as phosphate donor and ^{3}H-labeled dC and dA (15-45 Ci/mmol) as acceptors at concentrations of 1 μM and 30 μM, respectively (hence close to their measured K_m values of 0.9 μM and 40 μM at pH 7.2, and 1.5 μM and 70 μM at pH 8.6).

Bearing in mind that 5'-amino-5'-deoxythymidine is a substrate for the TK of herpes simplex virus type 1,[7] it was first established that 5'-amino-ddC is not a substrate for dCK. Following the observation that 5'-azido-ddC exhibits inhibitory properties (see below), this compound was also shown not to be a substrate and, in turn, prompted us to examine 5'-O-methyl-dC and 5'-O-ethyl-dC, kindly prepared by Dr. Z. Kazimierczuk, and the syntheses of which will be described elsewhere.

The inhibitory properties of the foregoing compounds were initially expressed in terms of their IC_{50} values, the concentration giving half-maximal inhibition.

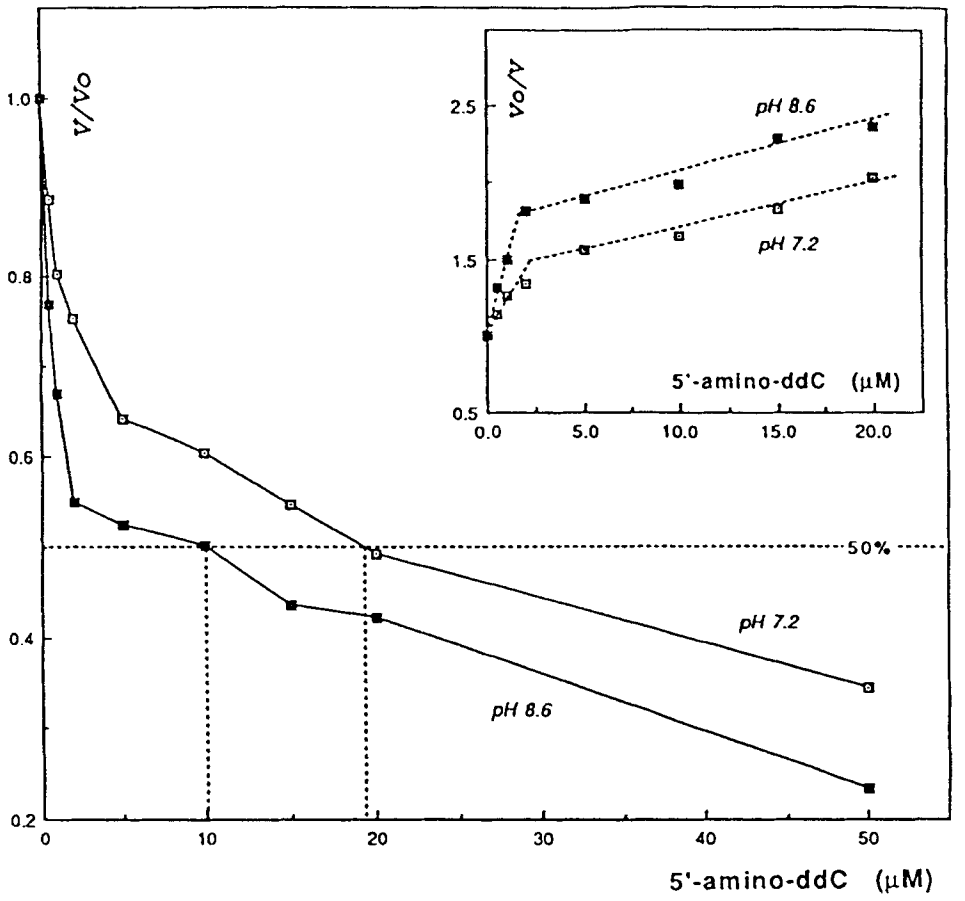

FIG. 1

Inhibition of activity of dCK with dC as substrate by 5'-amino-ddC. Rates are normalised to the uninhibited rate, taken as 1.0. **Insert**: Same data in the form of a Dixon plot.

Subsequently, the type of inhibition exhibited by 5'-azido-ddC was directly established as competitive with respect to dC. Assuming that the other inhibitors were also competitive, their K_i values were calculated from the relationship

$$K_i = IC_{50} \times K_m / ([S]_0 + K_m)$$

where $[S]_0$ is the substrate concentration at the measured value of IC_{50}, and the K_m values are Michaelis constants (listed above).

The IC_{50} values, and the measured or calculated K_is, with dC or dA as substrates, are listed in Table 1.

It will be noted that, with dA as a substrate, the lead compound 5'-amino-ddC is an effective inhibitor, with Ki ~ 1 μM. By contrast, the same compound inhibited phosphorylation of dC effectively only at low inhibitor concentrations (< 2.5 μM); at higher concentrations it was 10-fold less effective. This bimodal inhibitory activity (see Fig. 1) was exhibited only by 5'-amino-ddC with dC as a substrate, and the resulting calculated K_i values, based on extrapolated values of IC_{50} at low (< 2.5 μM) and high (> 2.5 μM) inhibitor concentrations, must be considered as only rough approximations.

Inhibition by 5'-amino-ddC was measured at both pH 7.2 and 8.6 because of the previous demonstration that 5'-amino-5'-deoxyadenosine at neutral pH is protonated at the 5'-amino group (pKa ~ 8.5), and is a much better inhibitor of adenosine kinase at more alkaline pH, where there is a preponderance of the neutral form. However, at pH 8.6, 5'-amino-ddC was only 2-fold more active *vs* dC, but not dA, and only in the low inhibitor concentration range (Fig. 1 and Table 1). This complex behaviour may be related to the known high negative cooperativity of the enzyme,[5,8] and is being further investigated. It remains to note that the specificity of 5'-amino-ddC as an inhibitor of dCK is further underlined by the fact that the corresponidng 5'-amino-ddU was found not to be a significant inhibitor.

From Table 1 it will be seen that 5'-azido-ddC is a reasonably good and better inhibitor of phosphorylation of dA than of dC. In this case the K_i value *vs* dC was obtained directly with the aid of Dixon plots, demonstrating that inhibition was competitive. By contrast, 5'-O-methyl-dC is a much poorer inhibitor. Replacement of the methyl group by an ethyl strikingly enhanced inhibitory activity, leading to a K_i of 3.1 μM with dA as a substrate and 32 μM with dC. The 10-fold difference in K_i values points to the utility of 5'-O-ethyl-dC as a tool for following the intracellular effects of phosphorylation of dC with concominant marked inhibition of phosphorylation of dA.

The foregoing results suggest that larger and/or bulkier hydrophobic 5'-substituents may lead to more specific and potent inhibitors (*cf.* [9]). It is also worth noting that 5'-azido-ddC, with a K_i of 8 μM for phosphorylation of dA, may prove to be a useful photoaffinity label for dCK.

Finally, in the light of reports on elevated dCK activity in tumor cells, the above non-substrate inhibitors may prove useful in combination chemotherapy.[10]

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