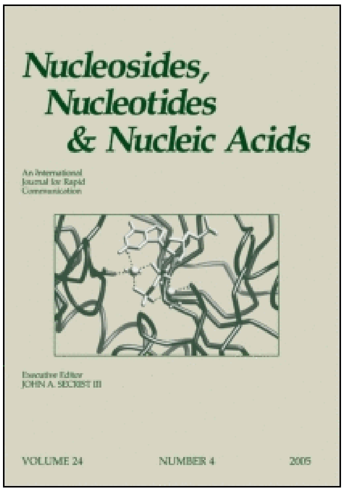

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Inhibition and Substrate Specificity of Adenosine Deaminase. Interaction with 2'-, 3'- and/or 5'-Substituted Adenine Nucleoside Derivatives

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INHIBITION AND SUBSTRATE SPECIFICITY OF ADENOSINE DEAMINASE. INTERACTION WITH 2'-, 3'- AND/OR 5'- SUBSTITUTED ADENINE NUCLEOSIDE DERIVATIVES.

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Abstract. A series of adenine nucleoside derivatives, most of them prepared for the first time, have been evaluated as substrates or inhibitors of adenosine deaminase. The best inhibitory results were obtained with the 3',5'-di-O-benzoyl esters of 9-β-D-pentofuranosyladenines.

INTRODUCTION

Most of the adenine nucleoside analogues which have potential interest in chemotherapy suffer from the main shortcoming that they are rapidly deaminated and thus generally inactivated by the ubiquitous enzyme adenosine deaminase (ADA). For example, 9-β-D-arabinofuranosyladenine (AraA, vidarabine, VIRA-A), one of the best known drugs with a rather selective antiherpetic activity which has been licensed for clinical use,[1,2] is susceptible to deamination by ADA, resulting in the formation of the less potent 9-β-D-arabinofuranosylhypoxanthine.[3,4] To overcome this problem, the use of powerful ADA inhibitors such as coformycin and 2'-deoxycoformycin has been suggested. However the latter compounds have adverse effects *in vivo* and are toxic by inducing immunosuppression.[5] Another approach is to increase the resistance of the adenosine analogues to deamination by modifying their sugar moiety.[3,6,7]

In the present work, we have examined the substrate and/or inhibitor properties of new sugar-modified adenosine analogues, with the aim to provide further examples about the relationship between the structural parameters and the susceptibility to deamination by ADA.

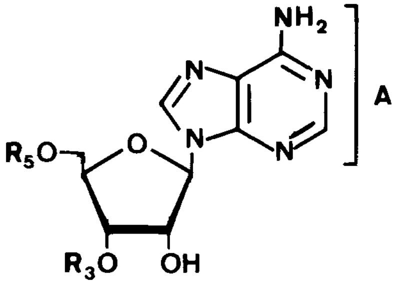
RESULTS AND DISCUSSION

For the sake of comparison with the already published data, we chose to carry out our experiments with calf intestinal mucosal type I ADA which was, together with the ADA of human erythrocytes,[8,9] one of the most studied enzymes.[10,11] The compounds evaluated (*Figure 1*) were synthesized in our laboratory and consist of various O-acyl and O-nitro esters of D-pentofuranosyladenines and some acyclic nucleosides. Their enzymatic deamination to hypoxanthine nucleoside derivatives was followed by determining the variation of the optical density at 265 nm. The kinetic parameters have been obtained according to the method of Lineweaver and Burk[12] or Dixon[13] and are presented in Table 1. For comparison purposes, the already known data[11] of the parent compounds 1, 4, 17 and 23 have also been included.

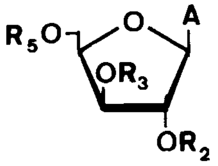
First, we examined the susceptibility of the compounds to deamination. Ten of them, all having the 5'-position free, were partially deaminated and none of the 5'-substituted derivatives was deaminated. This result is in agreement with the notion that an unsubstituted 5'-hydroxyl group, by interacting with the enzyme by means of a hydrogen bond, is a prerequisite for substrate activity.[11,14] Except for the 9-β-D-xylofuranosyladenine (4) and its 2'-O-nitro derivative 9, the other eight compounds are markedly poorer substrates than adenosine (1). For the parent compounds in the β-D-pentofuranosyl series, the decreasing order of ADA catalytic efficiency was riboA (1) > xyloA (4) > araA (17) > lyxoA (23). Although these results agree with those already reported,[10,11] we found xyloA (4) to be a better substrate for ADA as compared to the published data.

In the β-D-xylofuranosyl series, two derivatives 9 and 10 with a free 5'-hydroxyl function and a O-nitro group in the 2' or 3' position, respectively, have been examined. We found that a compound with a 2'-O-nitro group is a better substrate than the one with a 3'-O-nitro group. This parallels the behaviour of 2' and 3'-azido (or bromo) xylofuranosyladenines,[11] and indicates that, in the D-xylose series, substitution at the 3'-position inhibits more deamination than the substitution at the 2'-position. The situation is the opposite to that already observed in the β-D-arabinofuranosyl series where it was found that 3'-O-acyl derivatives were good substrates while 2'-O-acyl isomers were resistant to ADA.[15,16] In the latter series, we found that, contrary to the published data,[11] 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (18) is a substrate of the enzyme though a very poor one.

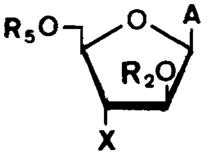
Also, of interest are the observations that the only α-D-nucleoside analogue which was tested [namely, α-lyxoA



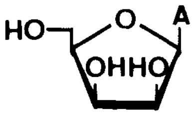
1 $R_3 = R_5 = H$
2 $R_3 = C_6H_5CO$, $R_5 = H$
3 $R_3 = H$, $R_5 = C_6H_5CO$



4 $R_2 = R_3 = R_5 = H$
5 $R_5 = CH_3CO$, $R_3 = R_2 = H$
6 $R_2 = R_5 = CH_3CO$, $R_3 = H$
7 $R_3 = R_5 = CH_3CO$, $R_2 = H$
8 $R_2 = R_3 = R_5 = CH_3CO$
9 $R_2 = NO_2$, $R_3 = R_5 = H$
10 $R_3 = NO_2$, $R_2 = R_5 = H$
11 $R_5 = NO_2$, $R_2 = R_3 = H$
12 $R_3 = R_5 = NO_2$, $R_2 = H$
13 $R_2 = R_3 = R_5 = NO_2$
14 $R_5 = C_6H_5CO$, $R_2 = R_3 = H$
15 $R_3 = R_5 = C_6H_5CO$, $R_2 = H$
16 $R_3 = R_5 = C_6H_5CO$, $R_2 = CH_3CO$



17 $R_2 = R_5 = H$, $X = OH$
18 $R_2 = R_5 = X = H$
19 $R_5 = C_6H_5CO$, $R_2 = H$, $X = OH$
20 $R_5 = C_6H_5CO$, $R_2 = H$, $X = C_6H_5COO$
21 $R_5 = P(O)CH_3(OCH_3)$, $R_2 = H$, $X = OH$
22 $R_5 = P(O)(OH)_2$, $R_2 = H$, $X = OH$,



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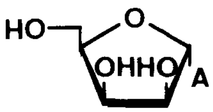

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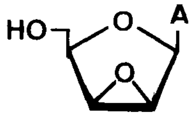

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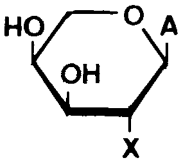
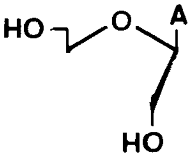


26 $X = OH$
27 $X = H$



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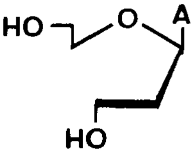

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Table 1. Kinetic parameters of adenine nucleoside derivatives as substrates or as inhibitors of calf intestinal adenosine deaminase.

Compounds		K_m (μM)	V_{max} (Relative to adenosine)	K_i (μM)
Ribose series :				
1	Adenosine (riboA)	33 (31)^{d}	100	*b*
2	3'-O-Benzoyladenosine	12	4	26
3	5'-O-Benzoyladenosine	*a*		210
Xylose series :				
4	9-β-D-Xylofuranosyl adenine (xyloA)	30 (73)^{d}	34 (55)^{d}	*b*
5	5'-O-AcetylxyloA	*a*		140
6	2',5'-Di-O-acetylxyloA	*a*		*b*
7	3',5'-Di-O-acetylxyloA	*a*		100
8	2',3',5'-Tri-O-acetyl xyloA	*a*		*b*
9	2'-O-NitroxyloA	24	53	*b*
10	3'-O-NitroxyloA	220	14	90
11	5'-O-NitroxyloA	*a*		*b*
12	3',5'-Di-O-nitroxyloA	*a*		75
13	2',3',5'-Tri-O-nitroxyloA	*a*		45
14	5'-O-BenzoylxyloA	*a*		36
15	3',5'-Di-O-benzoylxyloA	*a*		0.14 [0.08]^{c}
16	2'-O-Acetyl-3',5'-di-O-benzoylxyloA	*a*		0.8 [0.3]^{c}

.../...

(continued)

Table I continued

Arabinose series :				
17	9-β-D-Arabinofuranosyl adenine (araA)	89 (142)[d]	23 (53)[d]	*b*
18	3'-DeoxyaraA	330	3	*b*
19	5'-O-BenzoylaraA	*a*		13
20	3',5'-Di-O-benzoylaraA	*a*		3.2
21	5'-O-Methylmethylphosphonyl araA	*a*		*b*
22	5'-O-PhosphorylaraA	*a*		*b*
Lyxose series :				
23	9-β-D-Lyxofuranosyl adenine (lyxoA)	164 (171)[d]	2 (2)[d]	140
24	9-α-D-Lyxofuranosyl adenine (α-lyxoA)	*a*		*b*
25	2',3'-AnhydrolyxoA	190	69	*b*
Miscellaneous :				
26	9-α-L-Arabinopyranosyl adenine	*a*		*b*
27	9-α-L-(2-deoxyarabinopyranosyl)adenine	250	1	*b*
28	9-[1(R)-(2-hydroxyethoxy)-2-hydroxyethyl]adenine	*a*		16 (12)[d]
29	9-[1(R)-(2-hydroxyethoxy)-3-hydroxypropyl]adenine	*a*		3.2

[a] K_m *not measurable.*

[b] K_i *not measurable.*

[c] K_i *determined after incubation of the compound with ADA prior to adding adenosine.*

[d] *Already published data :* 1,[11] 4,[11] 17,[11] 23[10] and 28.[17]

(24)], as well as various pyranosyl 26, 27, and open chain analogues 28, 29 of adenine nucleosides were completely resistant to deamination under the conditions of the assay. These observations are not surprising since it is known that, besides the requirement of a free 5'-hydroxy function, a proper configuration (β) and conformation (anti) of the molecules are crucial for the enzyme activity.[17,18]

Subsequently, we examined the adenosine analogues for their inhibitory properties on ADA and determined the inhibition constants (K_i) which are presented in Table 1. Approximately half of the compounds studied were inhibitors of ADA and all yielded patterns of competitive inhibition when adenosine was used as a substrate. Most of the active compounds were weak inhibitors with the K_i values in the range of 30 to 210 µM. Others showed a much greater affinity for the enzyme, suggesting that they might belong to a different class of inhibitors.[8]

Among the weak inhibitors of ADA were, with approximately the same potency, the 5'-O-acetyl and 3',5'-di-O-acetyl derivatives of xyloA, 5 and 7, respectively. While acetylation of the 2'-hydroxyl function of these two compounds suppressed the inhibitory properties, an opposite effect was observed in the case of O-nitro esters of xyloA for which the more active inhibitor was the 2',3',5'-tri-O-nitro derivative 13.

The compound with greatest inhibition potency was the 3',5'-di-O-benzoyl derivative of xyloA 15 (K_i = 0.14 µM). Acetylation at the 2'-position of 15 lowered only slightly the affinity for the enzyme (K_i = 0.8 µM), while 3'-debenzoylation resulted in a much lesser inhibition activity (K_i = 36 µM). The same effect was observed in the β-D-arabinofuranosyl series since the 5'-O-benzoyl derivative 19 (K_i = 13 µM) was a weaker inhibitor than the 3',5'-di-O-benzoyl derivative 20 (K_i = 3.2 µM).

The relatively high inhibitory potency of the 3',5'-di-O-benzoyl esters 15 and 16 of xyloA suggests that their mechanism of interaction with ADA differs from that of the other compounds. It has been established that the inhibitors of ADA generally belong to three classes:[8,19] i) those which inhibit the enzyme instantaneously without any variation in function with time (this is a classical competitive inhibition shown by substrate analogues with a relatively high K_i); ii) the inhibitors which appear to inhibit the enzyme slowly and are very tightly bound at the equilibrium (the best studied compounds in this class mimic the transition state structures of the reaction and include coformycin and 2'-deoxycoformycin); iii) the inhibitors which have a fairly high initial affinity for the enzyme resulting in rapid inhibition, but they also presumably slowly induce a conformational change of the enzyme, thus delaying the establishment of steady state conditions. In this case, the K_i determined in the early stage of the reaction is higher than the constant measured after incubating the inhibitor and the enzyme prior to adding the substrate. Among these so-called semi-tight binding inhibitors, the most important compound is 9-(*erythro*-2-hydroxy-3-nonyl)adenine (EHNA).[19] Studies with EHNA and analogues have led to a suggestion that the active site of the enzyme contains a large hydrophobic region which interacts favorably with the alkyl substituents of these molecules.[19-22] We, therefore, repeated the kinetic experiments for all compounds and found a lower K_i, when ADA and the inhibitor were incubated for 3 min prior to adding adenosine, only for the 3',5'-di-O-benzoyl derivatives 15 and 16 (Table 1). It is likely that the increased affinity of these two xylose compounds results from a correct stereochemical *cis*-orientation of the two phenyl rings which allows in an aqueous environment a better hydrophobic interaction between them than in the arabinose series. Analogous effects have been postulated in the conformational studies of adenosine derivatives bearing aromatic substituents.[23] The two interacting phenyl rings in 15 and 16 may in turn interact favorably with a hydrophobic pocket on the enzyme surface thus increasing the binding energy.

Among the other active inhibitors found in this study were the two acyclic analogues 28 and 29. They both displayed a classical competitive inhibition kinetics, but the hitherto unknown 9-[1(R)-(2-hydroxyethoxy)-3-hydroxypropyl]adenine (29) was a better inhibitor than the already reported[17] 9-[1(R)-(2-hydroxyethoxy)-2-hydroxyethyl]adenine (28).

CONCLUSIONS

In the present work we describe the properties of a series of ester derivatives related to D-pentofuranosyl adenines as substrates or inhibitors of adenosine deaminase. The best results were obtained with the 3',5'-di-O-benzoyl derivatives in the xylose series. These compounds which lacked substrate properties and behaved like semi-tight binding inhibitors could act as prodrugs of the antiviral xyloA,[24] even though their solubility in water is quite low. In keeping with this suggestion, it has been reported that 9-(5-O-valeryl-β-D-arabinofuranosyl)adenine which is an average inhibitor of calf mucosal ADA,[25] shows a higher antiviral activity than araA itself.[3]

EXPERIMENTAL

Chemical Synthesis.

General procedures and the instrumentation used were described in ref. 26.

Compounds 4,[24,27] 15,[27] 16,[24,27] 20,[28] 21,[29] 23[30] and 24[30] were synthesized as previously described. The synthesis of compounds 5-13, 18,[31] 25,[32] 26,[33] 27, 28[17] and 29 will be published elsewhere.

Synthesis of the monobenzoyl β-D-pentofuranonucleosides 2, 3, 14 and 19.

Adenosine (1), 9-β-D-xylofuranosyladenine (4) or 9-β-D-arabinofuranosyladenine (17) (0.5 g, 1.87 mmol) was first coevaporated three-times with anhydrous pyridine and then dissolved in a mixture of pyridine and *N*,*N*-dimethylformamide (1:1, v/v; 19 mL), and benzoyl chloride (0.26 mL, 2.24 mmol, 1.2 eq.) was added dropwise to the stirred solution cooled to 0°C in an ice bath. Stirring of the reaction mixture was continued for 18 h, at which time water (7 mL) was added. The residue obtained after evaporation to dryness, was first coevaporated three times with toluene and then chromatographed on a silica gel column using as eluent a stepwise gradient of methanol (0-10%) in methylene chloride. Pooling and evaporation of the appropriate fractions as indicated by TLC gave pure 2 (0.13 g, 19%) and 3 (0.07 g, 10%) from adenosine, 14 (0.15 g, 22%) from xyloA and 19 (0.32 g, 46%) from araA. Purity and structural assignments for these compounds are based on their physical properties reported below:

9-(3-O-Benzoyl-β-D-ribofuranosyl)adenine (2).
Mp 206-207°C (from ethanol) [lit.[34] mp 206-207°C (from ethanol); lit.[35] mp 205-206°C (from aqueous ethanol)]; UV (ethanol) λ_{max} 259 nm (ϵ, 14,200), 231 nm (ϵ, 13,600), λ_{min} 245 nm (ϵ, 10,600), 222 nm (ϵ, 11,300); ^{1}H NMR (Me_2SO-d_6) δ ppm 8.40 and 8.16 (2s, 2x1H, H-2 and H-8), 8.1-7.5 (m, 5H C_6H_5-CO), 7.40 (s, 2H, NH_2), 6.02 (d, 1H, H-1'; $J_{1',2'}$= 7.2 Hz), 5.90 (d, 1H, OH-2'; J = 6.2 Hz), 5.72 (dd, 1H, OH-5'; J = 4.6 and 7.4 Hz), 5.54 (dd, 1H, H-3'; J = 1.8 and 5.34 Hz), 5.02 (m, 1H, H-2'), 4.3 (m, 1H, H-4'), 3.6-3.8 (m, 2H, H-5',5'') in close agreement with lit.[35]; mass spectra (matrix glycerol-thioglycerol, 1:1, v/v), FAB > 0: 372 $[M+H]^+$, 136 $[BH_2]^+$; FAB < 0: 370 $[M-H]^-$, 134 $[B]^-$; $[\alpha]_D^{20}$ -107.3° (c 0.96, Me_2SO).

Anal. Calcd. for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.62; N, 18.86. Found: C, 54.98; H, 4.66 N, 18.83.

9-(5-O-Benzoyl-β-D-ribofuranosyl)adenine (3). Mp 162-164°C (from ethanol) [lit.[36] 166-170°C (from aqueous ethanol); lit.[37] 106-108°C (from ethanol)]; lit.[38] 126-129°C (from methanol); lit.[39] 128-129°C (from methanol)]; UV (ethanol) λ_{max} 259 nm (ϵ, 14,500), 231 nm (ϵ, 14,900), λ_{min} 245 nm (ϵ, 10,600), 223 nm (ϵ, 12,800); ^1H NMR (Me_2SO-d_6) δ ppm 8.29 and 8.10 (2s, 2x1H, H-2 and H-8), 8.0-7.4 (m, 5H, C_6H_5-CO), 7.30 (s, 2H, NH_2), 5.92 (d, 1H, H-1'; $J_{1',2'}$ = 4.8 Hz), 5.60 (d, 1H, OH-2'; J = 5.7 Hz), 5.42 (d, 1H, OH-3'; J = 5.7 Hz), 4,76 (m, 1H, H-2'; $J_{2',3'}$ = 4.8 Hz after D_2O exchange), 4.60 (m, 1H, H-4'), 4.4 (m, 2H, H-3' and 5'), 4.2 (m, 1H, H-5''), in quite close agreement with lit.[38]; mass spectrum (matrix glycerol-thioglycerol, 1:1, v/v), FAB > 0: 372 $[M+H]^+$, 136 $[BH_2]^+$; $[\alpha_D{}^{20}]$ -44.7° (c 0.94, [lit.[38] $[\alpha_D{}^{18}]$ -35.6° (c 1.5,DMF)].

9-(5-O-Benzoyl-β-D-xylofuranosyl)adenine (<u>14</u>). Mp 98-103°C (lyophilized from dioxane); UV (ethanol)λ_{max} 259 nm (ϵ, 14,200), 231 nm (ϵ, 14,200), λ_{min} 245 nm (ϵ, 10,200), 223 nm (ϵ, 13,200); ^1H NMR (Me_2SO-d_6) δ ppm 8.29 and 8.15 (2s, 2x1H, H-2 and H-8), 8.1-7.5 (m, 5H, C_6H_5CO), 7.36 (s, 2H, NH_2), 6.21 (d, 1H, OH-3'; J = 5.5 Hz), 6.03 (d, 1H, OH-2'; J = 4.1 Hz), 5.93 (d, 1H, H-1'; $J_{1',2'}$ = 1.6 Hz), 4.7-4.5 (m, 3H, H-4',5' and 5"), 4.4 (m, 1H, H-2'), 4.2 (m, 1H, H-3'); mass spectra (matrix glycerol-thioglycerol, 1:1, v/v), FAB > 0: 372 $[M+H]^+$, 136 $[BH_2]^+$; FAB < 0: 370 $[M-H]^-$,134 $[B]^-$; $[\alpha_D{}^{20}]$ +1.1° (c 0.87, Me_2SO).

Anal. Calcd. for $C_{17}H_{17}N_5O_5 \cdot \frac{1}{2} C_4H_8O_2$: C, 54.93; H, 5.10; N, 16.86. Found: C, 54.75; H, 5.05; N, 16.65.

9-(5-O-Benzoyl-β-D-arabinofuranosyl)adenine (<u>19</u>). Mp 226-228°C (from methanol) [lit.[40] 222-223°C (from aqueous ethanol); lit.[3] 222-224°C]; UV (ethanol) λ_{max} 259 nm (ϵ, 17,800), 231 nm (ϵ, 16,700), λ_{min} 245 nm (ϵ, 12,800), 223 nm (ϵ, 14,700); ^1H NMR (Me_2SO-d_6) δ ppm 8.13

and 8.12 (2s, 2x1H, H-2 and H-8), 8.0-7.5 (m, 5H, C_6H_5CO), 7.19 (s, 2H, NH_2), 6.33 (d, 1H, H-1'; $J_{1',2'}$ = 4.8 Hz), 5.80 (d, 1H, OH-2'; J = 3.6 Hz), 5.75 (d, 1H, OH-3'; J = 3.8 Hz), 4.7-4.5 (m, 2H, H-5',5''), 4.3 (m, 1H, H-3'), 4.2 (m, 1H, H-2'), 4.1 (m, 1H, H-4'); mass spectra (matrix glycerol-thioglycerol, 1:1, v/v), FAB > 0: 372 $[M+H]^+$, 136 $[BH_2]^+$; FAB < 0: 370 $[M-H]^-$, 134 $[B]^-$; $[\alpha_D{}^{20}]$ = +26.4° (c 1.1, Me_2SO) [lit.[3] $[\alpha_D{}^{23}]$ +42° (c 1.0, methanol)].

Anal. Calcd. for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.62; N, 18.86. Found: C, 54.91, H, 4.39; N, 18.68.

Kinetic Studies.

The enzyme used was adenosine deaminase (E.C. 3.5.4.4) from calf intestinal mucosa (Sigma, type VIII) with a specific activity of 190 units/mg. Rates of deamination were determined at 25°C ± 1°C by following the decrease of absorbance at 265 nm with a digital Philips 7800 UV spectrometer. Except for the benzoate derivatives, the molar extinction coefficients of substrates and products were assumed to be identical to those of adenosine and inosine respectively. The concentrations of the poorly soluble benzoate derivatives were obtained from their predetermined UV spectra.

For the substrate specificity studies, assays were carried out at 25°C in 0.05 M phosphate buffer at pH 7.5 with an initial substrate concentration in the range of 10 to 70 µM and with 10^{-2} to 1 unit of ADA per mL to start the reaction. The K_m values were obtained from Lineweaver and Burk double reciprocal plots.

To verify that the deamination compound is the sole product of the reaction, analogous experiments were carried out starting with 100 µM solutions of the substrates. In each case, HPLC analysis of the reaction medium after 1 h at 25°C yielded only one product with a UV spectrum similar to that of inosine (λ_{max} : 248-249 nm). HPLC analyses were carried out on a Waters Associates unit equipped with a U6K injector, 510 pumps and a 680 solvent programmer. The samples were injected onto a C_{18} µBondapak column (Waters Associates) and the elution was carried out for 20 min using a linear gradient of acetonitrile-10% ammonium acetate (2-98, adjusted to pH 5.9). The flow rate was 3 mL/min. The products were monitored by UV detection at 254 nm. Appropriate eluted peaks were isolated and used for UV spectrum determination.

The procedure used to determine inhibition kinetics against adenosine was essentially similar to that used during the substrate specificity studies. The concentration range of adenosine was 10-70 µM whereas the concentration range of the inhibitor was generally included between zero and about four times the estimated K_i value. The concentration of the enzyme added to start the reaction was 10^{-2} unit per mL. According to Lineweaver and Burk, double reciprocal plots yielded a straight line for each of the four constant inhibitor concentrations used, and the overall pattern was characteristic of competitive inhibition.[41] In some cases (benzoate derivatives) the Dixon method was also used and gave similar results.

To test how fast the steady-state conditions of the reaction were reached, a second procedure was also used (especially for compounds 15 and 16), in which the enzyme and the potential inhibitor were incubated at 25°C for 3 min prior to the addition of adenosine to start the reaction. Longer periods of incubation did not result in any appreciable change.

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REFERENCES

1. *Adenine Arabinoside: An Antiviral Agent;* Pavan-Langston, D., Buchanan, R.A., Alford, C.A., Jr., Eds.; Raven: New York, 1975.
2. For a review, see: Galasso, G.J. In *The Antimicrobial Agents, Annual 2;* Peterson, P.K., Verhoef, J., Eds; Elsevier: Amsterdam, 1987; pp 363-370.
3. Baker, D.C.; Haskell, T.H.; Putt, S.R. *J. Med. Chem.* 1978, **21**, 1218.
4. Fox, J.L.; Yu, C.D.; Higuchi, W.I.; Ho, N.F.H. *Int. J. Pharm.* 1979, **2**, 41.
5. Agarwal, R.P. *Pharmacol. Therap.* 1982, **17**, 399.
6. Stoeckler, J.D.; Bell, C.A.; Parks, R.E.; Chu, C.K.; Fox, J.J.; Ikehara, M. *Biochem. Pharmacol.* 1982, **31**, 1723.
7. Nair, V.; Purdy, D.F.; Sells, T.B. *J. Chem. Soc. Chem. Commun.* 1989, 878.
8. Agarwal, R.P.; Spector, T.; Parks, R.E. *Biochem. Pharmacol.* 1977, **26**, 359.
9. Agarwal, R.P.; Parks, R.E. *Methods in Enzymology,* 1978, **51**, 502.
10. Zielke, C.L.; Suelter, C.H. In *The Enzymes*, Boyer, P.D. Eds., Academic Press: New York, 1971, Vol. 4, 3rd ed., p. 47.
11. Mikhailopulo, I.A.; Wiedner, H.; Cramer, F. *Biochem. Pharmacol.* 1981, **30**, 1001.
12. Lineweaver, L.; Burk, D. *J. Am. Chem. Soc.* 1934, **56**, 658.
13. Dixon, M. *Biochem. J.* 1953, **55**, 170.
14. Bloch, A.; Robins, M.J.; Mc Carthy, J.R. *J. Med. Chem.* 1967, **10**, 908 and references cited therein.

15. Baker, D.C.; Haskell, T.H.; Putt, S.R.; Sloan, B.J. *J. Med. Chem.* 1979, **22**, 273.

16. Baker, D.C.; Kumar, S.D.; Waites, W.J.; Arnett, G.; Shannon, W.M.; Higuchi, W.I.; Lambert, W.J. *J. Med. Chem.* 1984, **27**, 270.

17. Lerner, L.M.; Rossi, R.R. *Biochemistry*, 1972, **11**, 2772.

18. Zemlicka, J. *J. Am. Chem.* Soc. 1975, **97**, 5896.

19. Frieden, C.; Kurz, L.C.; Gilbert, H.R. *Biochemistry*, 1980, **19**, 5303.

20. Schaeffer, H.J.; Schwender, C.F. *J. Med. Chem.* 1974, **17**, 6.

21. North, T.W.; O'Connor, L.; Abushanab, E.; Panzica, R.P. *Biochem. Pharmacol.* 1983, **32**, 3541.

22. Antonini, I.; Cristalli, G.; Franchetti, P.; Grifantini, M.; Martelli, S.; Lupidi, G.; Riva, F. *J. Med. Chem.* 1984, **27**, 274.

23. Skorka, G.; Shuker, P.; Gill, D.; Zabicky, J.; Parola, A.H. *Biochemistry*, 1981, **20**, 3103.

24. Gosselin, G.; Bergogne, M.-C.; De Rudder, J.; De Clercq, E.; Imbach, J.-L. *J. Med. Chem.* 1986, **29**, 203.

25. Lipper, R.A.; Machkovech, S.M.; Drach, J.C.; Higuchi, W.I. *Mol. Pharmacol.* 1978, **14**, 366.

26. Huss, S.; Gosselin, G.; Stawinski, J.; Stromberg, R.; Imbach, J.-L. *Nucleosides Nucleotides*, 1988, **7**, 321.

27. Gosselin, G.; Imbach, J.-L. *J. Heterocycl. Chem.* 1982. **19**, 597.

28. Gosselin G.; Bergogne, M.-C.; Imbach, J.-L. *Nucleosides Nucleotides*, 1984, **3**, 265.

29. Puech, F.; Gosselin, G.; Balzarini J.; De Clercq, E.; Imbach, J.-L. *J. Med. Chem.* 1988, **31**, 1897.

30. Gosselin G.; Bergogne, M.-C.; De Rudder, J.; De Clercq, E.; Imbach, J.-L. *J. Med. Chem.* 1987, **30**, 982.

31. Kawana, M.; Nishikawa, M.; Yamasaki, N.; Kuzuhara, H. *J. Chem. Soc, Perkin Trans.* 1, 1989, 1593, and references cited therein.

32. **a.** Robins, M.J.; Fouron, Y.; Mengel, R. *J. Org. Chem.* 1974, **39**, 1564; **b.** Robins, M.J.; Hansske, F.; Low, N.H.; Park, J.I. *Tetrahedron Lett.* 1984, **25**, 367; **c.** Bazin, H.; Chattopadhyaya, J. *Synthesis*, 1985, 1108.

33. Lerner, L.M.; Sheid, B.; Gaetjens, E. *J. Med. Chem.* 1987, **30**, 1521.

34. Kamaike, K.; Uemura, F.; Yamakage, S.I.; Nishino, S.; Ishido, Y. *Nucleosides Nucleotides*, 1987, **6**, 699.
35. Wagner, D.; Verheyden, J.P.H.; Moffat, J.G. *J. Org. Chem*. 1974, **39**, 24.
36. Gerzon, K.; Kau, D. *J. Med. Chem*. 1967, **10**, 189.
37. Kolodkina, I.I.; Guseva, A.S.; Ivanova, E.A.; Varshavskaya, L.S.; Yurkevich, A.M. *Zhur. Obshchei Khim*. 1970, **40**, 2489.
38. Ishido, Y.; Nakazaki, N.; Sakairi, N. *J. Chem. Soc., Perkin Trans* 1, 1979, 2088.
39. Ishido, Y.; Sakairi, N.; Okazaki, K.; Nakazaki, N. *J. Chem. Soc., Perkin Trans*. 1, 1980, 563.
40. Renis, H.E.; Gish, D.T.; Court, B.A.; Eidson, E.E.; Wechter, W.J. *J. Med. Chem*. 1973, **16**, 754.
41. *The Enzyme Molecule*; Ferdinand, W., Ed.; J. Wiley and Sons: Bristol (G.B.), 1978, Chapter 5: pp. 148-185.

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