ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1991, p. 1437–1443
0066-4804/91/071437-07$02.00/0

Vol. 35, No. 7

6-*N*-Substituted Derivatives of Adenine Arabinoside as Selective Inhibitors of Varicella-Zoster Virus

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Received 7 August 1990/Accepted 16 April 1991

A series of 6-alkylaminopurine arabinosides were synthesized and found to inhibit varicella-zoster virus (VZV). The antiviral activities of these nucleosides were limited to VZV. None of the other viruses tested in the herpesvirus family were affected. The in vitro antiviral potencies of the 18 arabinosides correlated with their efficiencies as substrates of the VZV-encoded thymidine kinase in all but one case. The arabinosides of 6-methylaminopurine and 6-dimethylaminopurine were the most potent analogs, with 50% inhibitory concentrations against VZV of 3 and 1 μM, respectively. They were not cytotoxic to uninfected MRC-5 cells, human Detroit 98 cells, or mouse L cells (50% inhibitory concentration, >100 μM). Neither 6-methylaminopurine arabinoside nor 6-dimethylaminopurine arabinoside was detectably phosphorylated by either adenosine kinase or 2′-deoxycytidine kinase. These two alkylaminopurine arabinosides were also resistant to deamination catalyzed by adenosine deaminase. The VZV-dependent phosphorylation of these nucleosides offers the possibility of a potent and highly selective therapy for VZV infection.

Acyclovir [ACV; 9-(2-hydroxyethoxymethyl)guanine; Zovirax) is the drug of choice in the treatment of a variety of herpesvirus infections. This nucleoside analog inhibits varicella-zoster virus (VZV) at concentrations greater than those required for the treatment of herpes simplex virus (HSV) infections both in vitro (16) and in vivo (21). In VZV- and HSV-infected cells, the initial activation step of ACV is conversion to the monophosphate by virally encoded thymidine kinases (6, 10). However, the HSV thymidine kinase is more efficient than the VZV enzyme in phosphorylating ACV. This relative inefficiency of ACV phosphorylation by the VZV-encoded thymidine kinase correlates with its diminished potency.

Adenine arabinoside (ara-A) is efficacious in the treatment of human VZV infections (31). However, the toxicity of this agent precludes its general use (12, 17, 24). Previously, the in vitro therapeutic index of 6-methoxypurine arabinoside for VZV was reported to be superior to that of ara-A (1). This report describes a series of *N*-substituted derivatives of ara-A as anti-VZV agents.

MATERIALS AND METHODS

Biology. The inhibition of the replication of VZV and of other viruses; the kinetic constants for adenosine kinase, 2′-deoxycytidine kinase, and the various thymidine kinases; the growth inhibition of MRC-5 cells; and the immobilization of purine nucleoside phosphorylase and uridine phosphorylase were determined as described previously (1). Growth inhibition of D-98 cells and mouse L cells was determined by the vital dye neutral red assay described previously (29).

ADA assay. Calf intestinal adenosine deaminase (ADA; 200 U/mg) was purchased from Boehringer Mannheim (Indianapolis, Ind.) and dialyzed to remove the ammonium sulfate. Adenosine deamination was assayed spectrophotometrically at 270 nm (extinction change [$\Delta\varepsilon$] = -5.85 mM^{-1} cm^{-1}), 25°C, and pH 6.8 by progress curve analysis as described previously (28). The conversion of 6-methylaminopurine arabinoside to hypoxanthine arabinoside (ara H) was similarly assayed ($\Delta\varepsilon = -12$ mM^{-1} cm^{-1} at 270 nm). Conversion of ara-A to ara-H was monitored at 265 nm ($\Delta\varepsilon = -8.2$ mM^{-1} cm^{-1}), and the kinetic constants were determined from initial velocity analysis. Inhibition assays were also analyzed by the progress curve method (28).

Physical characterization of compounds. Elemental analyses of all the compounds listed in Table 1 were within ±0.4% of calculated values. Analyses were performed by Atlantic Microlabs, Atlanta, Ga. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. UV spectra were recorded with a scanning Gilford 250 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on either a Varian XL-200 or a Varian XL-300 instrument in $(CD_3)_2SO$. Mass spectra were obtained from Oneida Research Service, Whitesboro, N.Y., with a Finnegan 45 TFQ mass spectrometer. Optical rotations were obtained with a Perkin-Elmer model 241 polarimeter. Solvents were removed with a rotary evaporator at temperatures not exceeding 40°C.

Materials. Polyacrylamide gel (P-2; 200-400 mesh) and anion-exchange resin (AG1-X2; OH^-) were from Bio-Rad Laboratories (Richmond, Calif.). Preparative flash chromatography was performed on silica gel 60 (40 to 63 μm; no. 9385; E. Merck; EM Science, Cherry Hill, N.J.) (30). Thin-layer chromatography (TLC) was performed on cellulose (no. 13254) and silica gel (no. 13181) from Eastman-Kodak Co. (Rochester, N.Y.) and E. Merck (EM Science), respectively.

Syntheses. 6-Chloropurine, 2,6-dichloropurine, 2-amino-6-chloropurine, and various substituted amines were from Aldrich Chemical Co. (Milwaukee, Wis.) and Sigma Chemical Co. (St. Louis, Mo.). 6-Methylamino-, 6-dimethylamino-, and 6-anilinopurine were from Sigma. 2-Fluoroadenine and 6-cyclohexylaminopurine were prepared by previously published procedures (9, 11). Except for 6-cyclohexylaminopurine, the aglycones listed in Table 1 that were not commercially available (compounds 1d, 1e, 1f, 1g, 1h, 5, and 6) were prepared by displacement reactions involving 6-chloropurine or 2-amino-6-chloropurine and the appropri-

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ate amine. A typical amination procedure is described in detail below.

6-Propylaminopurine. 6-Chloropurine (3.85 g, 0.025 mol) and propylamine (2.3 g, 0.039 mol) were added to 25 ml of acetonitrile and heated at reflux for 18 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography with CH_3CN-H_2O (95:5). Product-containing fractions were combined, and the solvent was removed to yield 2.66 g (60%) of propylaminopurine: melting point, 242 to 244°C; TLC silica gel R_f, 0.45 (CH_3CN-H_2O [95:5]); UV λ_{max} (ε m^{-1} cm^{-1} [10^3]) at pH 13, 275 nm (16.4); at pH 7.0, 269 nm (17.0); ^1H NMR (200 MHz, dimethyl sulfoxide [DMSO]-d_6) δ 12.8 (brs, 1 H, H_9), 8.14 and 8.04 (brs, 2 H, H_8 and H_2), 7.55 (brs, 1 H, NH), 3.44 (brs, 2 H, CH_2), 1.59 (m, 2 H, CH_2), 0.88 (t, 3 H, J = 7.38 Hz, CH_3); mass spectra (MS), ci (CH_4) 178 (M + 1) 297, [(M + 1)-pentose] 165; analytical $C_8H_{11}N_5 \cdot 0.05\ H_2O$.

6-Methylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1a). 6-Methylaminopurine (6.7 mmol, 1 g) and uracil arabinoside (7.04 mmol, 1.79 g) were combined with 50 ml of 10 mM potassium phosphate (pH 7.4). Uridine phosphorylase (16,000 IU) and purine nucleoside phosphorylase (14,400 IU) were added, and the reaction was stirred at 35°C overnight. After the addition of CH_3OH (150 ml), the reaction was filtered, and the filtrate was chromatographed twice in CH_3OH-H_2O (9:1) on a column (2.5 by 9 cm) of Dowex-1–hydroxide (AG1-X2). Fractions containing product were combined, the solvent was removed, and the residue was dissolved in C_2H_5OH-H_2O (1:1). The solvent was removed by lyophilization, resulting in 1.09 g of compound 1a: TLC R_f, 0.38 (silica gel, $CHCl_3$-CH_3OH [4:1]); MS, ci (CH_4) 282 (M + 1), 150 [(M + 1)-pentose].

6-Dimethylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1b). 6-Dimethylaminopurine (0.288 mol, 47 g) and uracil arabinoside (0.346 mol, 84.3 g) were suspended in 4 liters of 10 mM potassium phosphate (pH 7.4). Uridine phosphorylase (250,000 IU) and purine nucleoside phosphorylase (2,500,000 IU) adsorbed onto 1.2 liters of DEAE cellulose were added, and the suspension was stirred at 50°C. Five days later, the reaction mixture was filtered, and the filtrate was stored at 3°C overnight. The resulting precipitate was collected, washed with cold water, and dried under vacuum to yield 51.8 g of compound 1b: TLC R_f, 0.4 (silica gel, CH_3CN–15 N NH_4OH–H_2O [85:5:10]); MS, ci (CH_4) 296 (M + 1), 164 [(M + 1)-pentose].

The recovered immobilized enzyme was washed with 1.2 liters of 10 mM potassium phosphate (pH 7), and the filtrate was combined with the filtrate described above. This solution was adjusted to pH 10.5 with ammonium hydroxide and then chromatographed on a column containing 1 liter of Dowex-1–formate (5 by 45 cm) resin. The column was eluted with CH_3OH. Fractions containing product were combined, and the solvent was removed. The residue was recrystallized from CH_3OH-H_2O (4:1) to give 19.1 g of compound 1b: melting point, 199°C. This sample of compound 1b had NMR and MS identical to those described above.

6-Dimethylamino-8-bromo-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1c). Bromine (2.27 g, 0.78 ml) and sodium acetate (1 M, 16 ml) (pH 4) were combined and mixed for 2 min, after which compound 1b (0.5 g, 1.7 mmol) was added. After 15 min at 25°C, the solvent was removed and the residue was purified by flash chromatography with $CHCl_3$-CH_3OH (9:1) as the eluant. Fractions containing only product were combined. Removal of the solvent gave 0.105 g of compound 1c: TLC R_f, 0.4 (silica gel, $CHCl_3$-CH_3OH [9:1]); MS, ci (CH_4) 376 (M + 1), 242 [(M + 1)-pentose], 164 [(M + 1)-pentose and Br].

6-Ethylamino-9-(β-D-arabinofuranosyl)-9*H*-purine(compound 1d). 6-Ethylaminopurine (0.5 g, 3 mmol) and uracil arabinoside (1.49 g, 6 mmol) were combined in 25 ml of 10 mM potassium phosphate (pH 7.4). Uridine phosphorylase (28,500 IU) and purine nucleoside phosphorylase (228,000 IU) adsorbed onto DEAE cellulose were added, and the suspension was stirred at 60°C. Three days later, the reaction was filtered, the pH of the filtrate was adjusted to 10.6, and the filtrate was chromatographed on a column containing Dowex-1–formate resin (2.5 by 18 cm) in CH_3OH-H_2O (9:1). The solvent was removed, and the product was further purified by flash chromatography with $CHCl_3$-CH_3OH (9:1) as the eluant. Product-containing fractions were combined, and the solvent was removed to give 0.482 g of compound 1d: TLC R_f, 0.19 (silica gel, $CHCl_3$-CH_3OH [9:1]); MS, ci (CH_4) 296 (M + 1), 164 [(M + 1)-pentose].

6-*N*-Ethylmethylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1e). 6-*N*-Ethylmethylaminopurine was prepared by nucleophilic displacement of the chlorine group on 6-chloropurine by *N*-ethylmethylamine in CH_3CN. 6-*N*-Ethylmethylaminopurine (0.5 g, 2.8 mmol) and uracil arabinoside (1.38 g, 5.6 mmol) were suspended in 575 ml of a solution of 10 mM potassium phosphate containing 0.04% potassium azide (pH 7.4) and 10% (vol/vol) *n*-propanol. Uridine phosphorylase (6,000 IU) and purine nucleoside phosphorylase (8,400 IU) were added, and the solution was stirred at 37°C. Nineteen days later the reaction was filtered; the filtrate was chromatographed on a column (2.5 by 13 cm) containing Dowex-1–hydroxide resin in CH_3OH-H_2O (9:1). Fractions containing product were combined, and the solvent was removed. The residue was dissolved in 30% *n*-propanol–water and purified by chromatography on Bio-Rad P-2 resin (7.5 by 90 cm). Product-containing fractions were combined. Lyophilization of the solvent yielded 0.680 g of compound 1e: TLC R_f, 0.89 (cellulose, H_2O); MS, ci (CH_4) 310 (M + 1), 178 [(M + 1)-pentose].

6-*N*,*N*-Diethylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1f). 6-*N*,*N*-Diethylaminopurine (0.5 g, 2.6 mmol) and uracil arabinoside (1.27 g, 5.2 mmol) were combined in 25 ml of 10 mM potassium phosphate (pH 7.4). Reaction conditions and product isolation were identical to those described above for compound 1d, except that the mobile phase for the flash chromatography was $CHCl_3$-CH_3OH (9:1). This procedure resulted in 0.566 g of compound 1f: TLC R_f, 0.38 (silica gel, $CHCl_3$-CH_3OH [9:1]); MS, ci (CH_4) 324 (M + 1), 192 [(M + 1)-pentose].

6-*n*-Propylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1g). 6-*n*-Propylaminopurine (0.5 g, 2.8 mmol) and uracil arabinoside (1.37 g, 5.6 mmol) were combined in 25 ml of 10 mM potassium phosphate (pH 7.4). Reaction conditions and product isolation were identical to those described above for compound 1f. This procedure resulted in 0.621 g of compound 1g: TLC R_f, 0.56 (silica gel, $CHCl_3$-CH_3OH 8:2); MS, ci (CH_4) 310 (M + 1), 178 [(M + 1)-pentose].

6-Isopropylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1h). 6-Isopropylaminopurine (0.5 g, 2.8 mmol) and uracil arabinoside (1.38 g, 5.6 mmol) were combined in 100 ml of 10 mM potassium phosphate (pH 7.4). Reaction conditions and product isolation were identical to those described above for compound 1d. This procedure resulted in 0.622 g of compound 1h: TLC R_f, 0.8 (cellulose, H_2O); MS, ci (CH_4) 310 (M + 1), 178 [(M + 1)-pentose].

6-*n*-Hexylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1i). 6-*n*-Hexylaminopurine (0.96 g, 4.4 mmol; Sigma)

and uracil arabinoside (1.6 g, 6.6 mmol) were combined in 310 ml of 10 mM potassium phosphate (pH 7.4) containing 12% *n*-propanol. Reaction conditions were identical to those described above for compound 1d. After 112 days, the reaction mixture was filtered and the precipitate that formed overnight at room temperature was discarded. The resulting filtrate was cooled to 3°C, and after 18 h it was filtered and dried under vacuum to yield 0.326 g of crystalline compound 1i: TLC R_f, 0.63 (cellulose, H_2O); MS, ci (CH_4) 352 (M + 1), 220 [(M + 1)-pentose].

6-Cyclohexylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1j). 6-Cyclohexylaminopurine (0.45 g, 1.99 mmol), prepared by the procedure of Girgis and Pedersen (11), was combined with uracil arabinoside (0.97 g, 3.9 mmol) and 100 ml of 10 mM potassium phosphate (pH 7.4). Reaction conditions and product isolation were identical to those described above for compound 1d, except that the mobile phase for flash chromatography was $CHCl_3$-CH_3OH (85:15). This procedure resulted in 0.304 g of compound 1j that contained 1.0 H_2O: TLC R_f, 0.42 (silica gel, $CHCl_3$-CH_3OH [85:15]); MS, ci (CH_4) 350 (M + 1), 218 [(M + 1)-pentose].

6-Anilino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 1k). 6-Anilinopurine (1.3 g, 6.16 mmol; Sigma) and cytosine arabinoside (2 g, 5.35 mmol) were combined in 505 ml of 10 mM potassium phosphate (pH 7.3). Uridine phosphorylase (400 IU) and purine nucleoside phosphorylase (190 IU) containing cytidine deaminase (11 IU) were added, and the suspension was stirred at 35°C. Twenty-one days later, the reaction was filtered and the filtrate was adjusted to pH 10.5. This was chromatographed first on a column of Dowex-1–formate resin (2.5 by 10 cm) and then on P-2 gel (5 by 60 cm). The mobile phase for both columns was 30% *n*-propanol–water. Fractions containing product were combined and, after lyophilization of the solvent, yielded 0.13 g of compound 1k: MS, ci (CH_4) 344 (M + 1), 212 [(M + 1)-pentose].

2-Chloro-6-methylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 3). A solution of 2,6-dichloro-2′,3′,5′-tri-*O*-benzyl-9-(β-D-arabinofuranosyl)purine (19) (5.92 g, 10 mmol) in benzene was combined with 35 ml of benzene containing 2.1 g of methylamine at room temperature in a sealed bomb for 4 days. The bomb was cooled thoroughly in ice, and the contents were filtered to remove the methylamine hydrochloride. The solvent was removed to give an oil that was combined with an oil from a reaction of the same scale that was conducted at 125°C. The total weight was 11.4 g. TLC indicated that this material was a mixture of starting material and mono- and dimethylamino compounds. The oil was purified by flash chromatography with acetone-cyclohexane (3:7) as the eluant. The component running below the starting material (TLC, silica gel acetone-cyclohexane [3:7]) was collected, and the solvent was removed to yield 4.8 g of 2-chloro-6-methylamino-2′,3′,5′-tri-*O*-benzyl-9-(β-D-arabinofuranosyl)purine as an oil. A 1.1-g portion of this product in 40 ml of 2-methoxyethanol was added to a pressure bottle containing palladium chloride (0.87 g) that was prereduced in a Parr apparatus. This was hydrogenated at 50 lb/in^2 for 30 min, with the hydrogen atmosphere changed after 15 min. The catalyst was removed by filtration through a bed of Celite and washed with methanol. The filtrate was neutralized by the addition of Dowex-1–HCO_3^-. The resin was removed by filtration and washed with methanol. The solvent was removed and the residue was triturated first with chloroform and then with hot water. The crude product was dissolved in hot methanol, filtered, and cooled; and the solid was collected. Crystallization from boiling water gave 44.5 mg of the product: MS, ci (CH_4) 316 (M + 1), 184 [(M + 1)-pentose].

2-Fluoro-6-amino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 4). 2-Fluoro-6-aminopurine (1.3 g, 8.49 mmol) and cytosine arabinoside (2.85 g, 11.72 mmol) were combined in 703 ml of 4 mM potassium phosphate (pH 7.0). Uridine phosphorylase (102 IU) and purine nucleoside phosphorylase (792 IU) containing cytidine deaminase (10 IU) were added, and the suspension was stirred at 35°C. Thirty-six days later, the reaction was filtered and the pH of the filtrate was adjusted to 10.5. The product was purified by chromatography of the filtrate on a column containing Dowex-1–formate resin (2.5 by 10 cm) in *n*-propanol–water (3:7; vol/vol). The eluant was cooled to 3°C for 18 h, and the resulting precipitate was collected by filtration and dried under vacuum to give 0.348 g of compound 4: MS, ci (CH_4) 286 (M + 1), 154 [(M + 1)-pentose].

2-Amino-6-methylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 5). 2-Amino-6-methylaminopurine (0.5 g, 3 mmol) and uracil arabinoside (1.49 g, 6 mmol) were combined in 100 ml of 10 mM potassium phosphate (pH 7.4). Uridine phosphorylase (9,000 IU) and purine nucleoside phosphorylase (12,600 IU) were added, and the suspension was stirred at 35°C. Twenty-one days later, the reaction was filtered and the filtrate was pH adjusted to 10.5. The product was purified by chromatography on a column containing Dowex-1–formate resin (2.5 by 7 cm) in CH_3OH-H_2O (9:1). The residue was dissolved in *n*-propanol–water (3:7) and filtered through a 0.22-μm-pore-size filter, and the solvent was removed to give 0.27 g of compound 5: TLC R_f, 0.5 (cellulose, H_2O); MS, ci (CH_4) 297 (M + 1), 165 [(M + 1)-pentose].

2-Amino-6-dimethylamino-9-(β-D-arabinofuranosyl)-9*H*-purine (compound 6). 2-Amino-6-dimethylaminopurine (0.5 g, 2.8 mmol), uracil arabinoside (1.37 g, 5.6 mmol), uridine phosphorylase (9,000 IU), and purine nucleoside phosphorylase (12,600 IU) were combined in 100 ml of 10 mM potassium phosphate (pH 7.4). Reaction conditions and product isolation were identical to those described above for compound 1d, except that the reaction time was 15 days, the mobile phase for flash chromatography was $CHCl_3$-CH_3OH (8:2), and the product was dissolved in *n*-propanol–water (3:7) prior to lyophilization. This procedure resulted in 0.437 g of compound 6: TLC R_f, 0.42 (silica gel, $CHCl_3$-CH_3OH [85:15]); MS, ci (CH_4) 350 (M + 1), 218 [(M + 1)-pentose].

RESULTS AND DISCUSSION

Chemical methods for the synthesis of purine arabinosides involve multistep procedures (13, 14, 22, 26). An alternative procedure has been described that facilitates this synthesis by transferring the arabinosyl moiety from a pyrimidine arabinoside to the purine aglycone (15). The first step of this two-step synthesis uses the *Escherichia coli* enzyme uridine phosphorylase (EC 2.4.2.3) to catalyze the in situ generation of arabinose-1-phosphate. The desired purine arabinoside is generated in a second step from arabinose-1-phosphate and aglycone, as catalyzed by *E. coli* purine nucleoside phosphorylase (EC 2.4.2.1). Immobilization of these enzymes on DEAE-cellulose significantly increases their thermal stability, thereby allowing the reaction temperature to be increased and the reaction time to be decreased. In addition, the immobilization of the proteins facilitates their removal from the reaction vessel prior to isolation of the product and their reuse in subsequent reactions. The use of immobilized

TABLE 1. Physical properties of 6-*N*-substituted purine arabinosides

Compound	R_2	R_6	% Yield	Formula	Melting point (°C)	UV λ_{max} nm (ε, 10^3) at: pH 13	UV λ_{max} nm (ε, 10^3) at: pH 7	$[\alpha]_D^{20a}$
1a	H	$NHCH_3$	56	$C_{11}H_{15}N_5O_4 \cdot 0.2C_2H_6O$	192[b]	266.5 (16)[c]	266.5 (16)[c]	−2.1
1b	H	$N(CH_3)_2$	61	$C_{12}H_{17}N_5O_4 \cdot 0.3H_2O$	200	275.5 (19)[d]	275 (19)[d]	−2.4[e]
1c	H	$N(CH_3)_2$, 8-Br	17	$C_{12}H_{16}N_5BrO_4$	>215	273.5 (18.4)	280 (18.9)	
1d	H	$NHCH_2CH_3$	54	$C_{14}H_{21}N_5O_4 \cdot 0.8H_2O$	176	266.5 (18.9)	266.5 (17.5)	−10.0
1e	H	$N(CH_3)CH_2CH_3$	54	$C_{13}H_{19}N_5O_4$	159	275.5 (20.5)	275.5 (20.5)	−11.9
1f	H	$N(CH_2CH_3)_2$	64	$C_{14}H_{21}N_5O_4 \cdot 0.8H_2O$	156	276.5 (22.1)	276.5 (21)	−17.4
1g	H	$NHCH_2CH_2CH_3$	67	$C_{13}H_{19}N_5O_4 \cdot 1.2H_2O$	160	276 (21)	267 (20)	−10.4
1h	H	$NHCH(CH_3)_2$	70	$C_{13}H_{18}N_5O_4 \cdot 0.5H_2O$	90	268 (17.9)	268 (18)	−12.0
1i	H	$NH(CH_2)_5CH_3$	21	$C_{16}H_{25}N_5O_4$	155–157	267 (19)	268.5 (19.3)	−10.9[f]
1j	H	NHC_6H_{11}	42	$C_{16}H_{23}N_5O_4$	117–119	269 (20.4)	268.5 (19.8)	−11.6
1k	H	NHC_6H_5	15	$C_{16}H_{17}N_5O_4$	201–202	275[g]		
3	Cl	$NHCH_3$	4	$C_{11}H_{15}N_5ClO_4 \cdot H_2O$	224–225	272.5 (17.6)		+10.4[h]
4	F	NH_2	14	$C_{10}H_{12}N_5FO_4 \cdot H_2O$	>250[i]	262 (16.4)[j]	261 (17)[j]	−6.4
5	NH_2	$NHCH_3$	26	$C_{11}H_{16}N_6O_4 \cdot 2H_2O \cdot 0.1C_3H_6O$	235	279.5 (17.3) sh 260 (13.1)	279.5 (17.1) sh 260 (13.1)	+29.2
6	NH_2	$N(CH_3)_2$	47	$C_{12}H_{18}N_6O_4 \cdot H_2O \cdot 0.1C_3H_8O$	232	283.5 (15.4)	263 sh (10.6)	+28.6

[a] The concentration of the nucleoside was 0.5 g/100 ml in dimethylforamide (DMF).
[b] Literature reference (22): 201.5 to 210.5°C.
[c] Literature reference (22): pH 13, 266 nm (14.4); pH 7, 266 nm (13.8).
[d] Literature reference (22): pH 13, 276 nm (15.2); pH 7, 275 (15.7).
[e] c, 0.2, DMF.
[f] C, 0.46, DMF.
[g] Determined at pH 2.0
[h] c, 0.99, DMF.
[i] Literature reference (19): 265 to 267°C.
[j] Literature reference (19): pH 13, 262 nm (15.0); pH 7, 262 nm (15.0).

enzyme is exemplified with the synthesis of 6-dimethylaminopurine arabinoside (see Materials and Methods).

Chemical formulas, melting points, and UV absorbance data for the purine arabinosides prepared for this study are given in Table 1. Proton NMR data are given in Table 2.

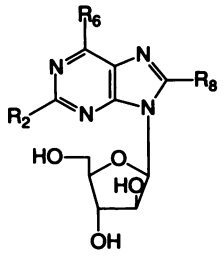
Cytotoxicity. The results of the growth inhibition assay for the 18 arabinosides in Table 3 show that only ara-A and compound 4 are appreciably toxic to cultured human D-98 and mouse L cells. The cellular toxicities of both of these analogs are well-known (2, 7, 20).

TABLE 2. ^1H NMR chemical shift assignments of 6-*N*-substituted purine arabinosides[a]

Compound	^1H NMR chemical shift assignments of 6-*N*-substituted purine arabinosides[a]: H_2 and H_8	$H_{1'}$	$H_{2'OH}$	$H_{3'OH}$	$H_{5'OH}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	Other
1a	8.19, 8.15	6.25	5.50	5.50	5.10	4.12	4.12	3.75	3.64	7.65 (b, NH), 2.96 (b, CH_3)
1b	8.19, 8.18	6.27	5.59	5.51	5.08	4.12	4.12	3.75	3.63	3.32 (bs, 2 CH_3)
1c	8.16	6.27	5.59	5.46	5.23	4.44	4.34	3.76	3.76	3.4 (b, 2 CH_3)
1d	8.17, 8.15	6.24	5.58	5.49	5.06	4.12	4.12	3.76	3.63	7.70 (b, NH), 3.5 (b, CH_2), 1.16 (t, J = 7.07 Hz, CH_3)
1e	8.18, 8.18	6.26	5.59	5.51	5.06	4.03	4.03	3.78	3.61	4.10 (b, CH_2), 3.36 (m, CH_3), 1.15 (t, J = 6.99 Hz, CH_3)
1f	8.18, 8.18	6.27	5.58	5.48	5.05	4.12	4.12	3.75	3.63	3.92 (b, 2 CH_2), 1.19 (t, J = 6.95 Hz, CH_3)
1g	8.16, 8.15	6.24	5.57	5.48	5.06	4.12	4.12	3.76	3.63	7.71 (b, NH), 3.4 (b, CH_2), 1.59 (m, J = 7.3 Hz, CH_2), 0.88 (t, J = 7.4 Hz, CH_3)
1h	8.16, 8.16	6.24	5.58	5.53	5.08	4.09	4.09	3.75	3.62	7.49 (d, J = 8.25 Hz, CH), 4.44 (b, NH), 1.21, 1.18 (m, CH_3)
1i	8.17, 8.17	6.26	5.61	5.52	5.10	4.14	4.14	3.78	3.67	7.74 (b, 1H NH), 1.60 (m, 2H, NH-CH_2), 1.29 (b, 8H, 4CH_2), 0.86 (t, 3H, CH_3)
1j	8.15, 8.15	6.24	5.57	5.49	5.07	4.18	4.18	3.75	3.6	7.48 (d, 1H, NH), 4.18 (b, 1H, CH-N), 1.5–2.0 (b, 5H, cyclohexyl), 1.0–1.45 (b, 5H, cyclohexyl)
1k[b]	8.40, 8.36	6.34	5.66	5.55	5.11	4.16	4.16	3.8	3.69	9.89 (s, NH), 7.96 (d, 2H, ϕ), 7.33 (t, 1H, ϕ), 7.04 (t, 1H, ϕ)
3[b]	8.20	6.12	—[c]	—	—	4.11	4.17	3.77	3.63	8.21 (q, NBH), 2.92 (d, CH_3)
4	8.15	6.08	5.60	5.49	5.03	4.10	4.10	3.72	6.61	7.75 (b, NH_2)
5	7.74	6.05	5.59	5.44	5.05	4.01	4.01	3.71	3.59	7.13 (b, NH), 2.88 (b, CH_3)
6	7.77	6.08	5.57	5.45	5.04	4.04	4.04	4.01	3.7	5.80 (s, NH_2), 3.34 (s, 6H, CH_3)

[a] Assignments were made from 200-MHz spectra, except where indicated. Coupling constants were determined by first-order analysis of the spectra. Assignments for compounds 1a and 1b were based on a COSY experiment. All $H_{1'}$ signals were doublets with J = 4.3 to 5.2 Hz.
[b] 300-MHz spectrum.
[c] —, NMR sample size was 0.4 mg, and the hydroxyl groups were exchange broadened.

TABLE 3. Biological activities and kinetic constants of substituted 6-aminopurine arabinosides



Compound	Substitution		Inhibition of:		VZV thymidine kinase[a]			Cell growth (% of control at 100 μM)	
	R_2	$R_{6\ (8)}$	VZV replication (IC_{50} [μM])[b]	MRC-5 growth (EC_{50} [μM])[c]	Relative V_{max}	K_i (μM)	Efficiency (relative V_m/K_i)	D-98 cells	L cells
1a	H	$NHCH_3$	3 (2.5[d])	86 (>100[e])	660	200	3.4	92 (100[f])	87 (86[f])
1b	H	$N(CH_2)_2$	1 (0.6[d])	87 (>100[e])	790	190	4.2	90 (95[f])	83 (76[f])
1c	H	$N(CH_3)_2$ (Br)	>100		4[g]	ND[h]		85	95
1d	H	$NHCH_2CH_3$	5	>100	460	900	0.51	87	84
1e	H	$N(CH_3)CH_2CH_3$	10	100 (100[e])	290	510	0.57	96	83
1f	H	$N(CH_2CH_3)_2$	>100		>200	>4,000	—[i]	65	89
1g	H	$NHCH_2CH_2CH_3$	>100		190	1,200	0.16	86	81
1h	H	$NHCH(CH_3)_2$	100	>100	>38	>6,500	—	93	87
1i	H	$NH(CH_2)_5CH_3$	>100		<5[g]	640		89	46
1j	H	NH-cyclohexyl	>100		<5[g]	880		100	87
1k	H	NH-phenyl	>100		<5[g]	760		78	69
2	NH_2	NH_2	28 (>100[d])	25 (>100[e])	>78	>5,000	—	100	96
3	Cl	$NHCH_3$	>100		ND	ND		97	87
4	F	NH_2	1	5 (6[e])	550	710	0.77	50	<20[j]
5	NH_2	$NHCH_3$	50	>100	>360	>5,000	—	91	96
6	NH_2	$N(CH_3)_2$	39		100	3,300	0.030	80	78
Ara-A			20–30 (1[d])	54 (7[e])	490	1,500	0.34	60 (40[f,k])	<20[j] (<20[f,k])
Ara-H			30	47 (42[e])	67	730	0.093	91	86
Acyclovir			15	>100	38	890	0.042	100	50

[a] Relative V_{max} is expressed as percent velocity of thymidine calculated from observed velocities at 1 mM and the measured K_i value $\{V_m = [v(S + K'_m)/S]\}$. Since these compounds were alternate substrates for the enzyme and all inhibition by them was linearly competitive, K_i values were equal to K'_m values (27, 30). The standard error of the K_i values was ±20% or less.

[b] Oka strain of VZV.

[c] Standard deviation of 50% effective concentration was ±20% or less. Results are averages of three determinations.

[d] The IC_{50}s were determined in the presence of 10 μM EHNA.

[e] The 50% effective concentration in the presence of 10 μM EHNA.

[f] Percentage of control cell growth in the presence of 10 μM EHNA.

[g] Relative substrate velocity (rate with thymidine, 100) at 1 mM rather than the relative maximal velocity.

[h] ND, not determined.

[i] Efficiency values were indeterminate.

[j] EC_{50} for cell growth of 30 μM for 4 and 35 μM for ara-A with L cells.

[k] The 50% effective concentration for cell growth in the presence of 10 μM EHNA was 90 μM for D-98 cells and 25 μM for L cells.

The growth inhibition of uninfected MRC-5 cells by selected nucleosides is also given in Table 3. The slight toxicities of compounds 1a and 1b and the more significant toxicity of compound 2 was reversed upon the addition of the adenosine deaminase inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) (25). As expected, the toxicity of ara-A was potentiated by blocking its deamination. Other than compound 4, none of the arabinosides tested were cytotoxic to uninfected MRC-5 cells.

There was no apparent correlation between the cytotoxicities of the 18 arabinosides tested and their antiviral effects.

Antiviral activities and enzyme correlations. The anti-VZV assay assessed the ability of compounds to inhibit replication of the virus in MRC-5 cells (5, 29). Ara-A was moderately inhibitory to the replicating virus (Table 3). However, its potency was significantly increased in the presence of EHNA (Table 3). Substitution of a methyl or a dimethyl group on the 6-amino group of ara-A (compounds 1a and 1b, respectively) resulted in analogs that were more active than ara-A in the absence of EHNA and that were equally as active as ara-A in the presence of EHNA (Table 3). Both compounds 1a and 1b were efficiently phosphorylated by the VZV-encoded thymidine kinase. These methyl substitutions also diminished the substrate efficiency of both analogs with ADA (Table 4).

Further increases in the bulk and hydrophobicity of the alkylamino substituent in the purine 6 position proved to be detrimental both to the antiviral activity and to the rate of phosphorylation by VZV thymidine kinase. Substitution of ethyl for methyl, exemplified by compound 1e, increased the 50% inhibitory concentration (IC_{50}) to 10 μM and decreased the substrate efficiency eightfold with VZV thymidine kinase. Further extension of the 6-substituent to diethylamino (compound 1f) resulted in the loss of all antiviral activity.

TABLE 4. Kinetic constants for ADA with adenosine and related arabinosides

Compound	K_i (μM)	K_m (μM)	Relative V_{max}	Relative substrate efficiency
Adenosine		30	100	100
Ara-A	66	66	15	6.6
6-Methylaminopurine arabinoside (compound 1a)		13	0.035	0.065
6-Dimethylaminopurine arabinoside (compound 1b)	>500		<0.00002	

TABLE 5. Viral and cellular nucleoside kinase substrate activity with the arabinosides of 6-methylaminopurine, 6-dimethylaminopurine, and adenine

Nucleoside kinase and source	Relative velocity[a]		
	6-Methylaminopurine arabinoside	6-Dimethylaminopurine arabinoside	Ara-A
Thymidine kinase from:			
VZV	550	660	200
Herpes simplex virus type 1	0.1	0.06	0.2
Human H-9 cells	<0.01	<0.02	<0.06
2′-Deoxycytidine kinase from calf thymus	<0.05	<0.005	14
Adenosine kinase from rabbit liver	<0.01	<0.01	0.1

[a] Relative velocity is the percentage of the rate observed with the substrate at 1 mM with thymidine for thymidine kinase, 1 mM 2′-deoxyguanosine for 2′-deoxycytidine kinase, and 0.1 mM adenosine for adenosine kinase.

Extension of a mono-substitution at the 6-amino position from methyl (compound 1a) to ethyl (compound 1d) caused a fivefold increase in the IC_{50} (Table 3). The higher IC_{50} with compound 1d was also reflected in a sixfold decrease in substrate efficiency. Further extension to *n*-propyl (compound 1g) eliminated any observed antiviral effect. However, compound 1g was a substrate for VZV thymidine kinase. This exception to the correlation of antiviral activity to the phosphorylation efficiency of VZV thymidine kinase suggests that additional metabolic steps after phosphorylation may be required to inhibit VZV. The trend of increasing IC_{50}s with increasing bulk was further exemplified by compound 1i. This analog was devoid of antiviral activity and was not detectably phosphorylated by the virally encoded thymidine kinase (Table 3). Branching of the α carbon of the 6-*N*-substituent (compound 1h) also decreased the antiviral activity compared with that of compound 1d. Cyclization of the *n*-hexyl chain (compound 1j) resulted in a compound devoid of antiviral activity. This lack of activity correlated with the absence of detectable phosphorylation by the VZV-encoded thymidine kinase (Table 3).

Compounds substituted at the purine 2 position were prepared in an attempt to limit catalysis by ADA and to enhance the antiviral activity. It is well documented that the rate of enzymatic deamination of adenosine and ara-A can be lowered by the substitution of a halogen in the purine 2 position (3, 4, 7, 19, 20). Compound 4 was efficiently phosphorylated by the virally encoded thymidine kinase and exhibited anti-VZV potency greater than that of ara-A and equivalent to that of ara-A in the presence of EHNA (Table 3). Although these properties are desirable for a potential anti-VZV candidate, clinical evaluation of compound 4 as an antileukemic agent has shown it to be more toxic than ara-A (8, 18).

Substitution at the 2 position of ara-A with an amino group to give compound 2 had no effect on the anti-VZV activity compared with that of ara-A. However, contrary to the result observed with ara-A, compound 2 was devoid of anti-VZV activity when it was tested in the presence of EHNA (Table 3). These data suggest that the activity observed in the absence of EHNA is most likely due to the generation of guanine arabinoside. Guanine arabinoside has been shown to have anti-VZV activity equivalent to that observed with compound 2 in the absence of EHNA (1).

The addition of an amino group to the 2 position of a purine arabinoside that is resistant to deamination by ADA and that is active against VZV proved to be detrimental. Compounds 5 and 6 had IC_{50}s that were 17- and 39-fold higher, respectively, than those of the corresponding 2-unsubstituted compounds (Table 3). Additionally, the VZV thymidine kinase substrate efficiencies of compounds 5 and 6 were significantly lower than those of the corresponding 2-unsubstituted analogs, compounds 1a and 1b. Substitution of chlorine in the 2 position of compound 1a (compound 3) resulted in the loss of all anti-VZV activity.

Substitution of a bromine at the 8 position of compound 1b was unfavorable (compound 1c). This result correlated with the inefficient phosphorylation observed for compound 1c with VZV thymidine kinase (Table 3).

Other viruses. The antiviral properties of the most potent anti-VZV analogs (compounds 1a, 1b, and 1d) against other viruses in the herpesvirus group were also examined. At concentrations below 100 μM, none inhibited the replication of herpes simplex virus type 1 or type 2, cytomegalovirus, or Epstein-Barr virus.

Studies with other nucleoside kinases. The arabinosides of adenine, 6-methylaminopurine (compound 1a), and 6-dimethylaminopurine (compound 1b) were also studied as substrates of thymidine kinases isolated from HSV type 1-infected cells and H9-uninfected cells (Table 5). The HSV type 1-encoded thymidine kinase catalyzed the slow phosphorylation of these arabinosides, while none of these compounds were phosphorylated with the thymidine kinase from uninfected H9 cells. In contrast, the VZV-encoded thymidine kinase readily catalyzed the phosphorylation of all three compounds (Table 5).

A major difference between ara-A and compounds 1a and 1b was that neither 2′-deoxycytidine kinase nor adenosine kinase catalyzed the phosphorylation of the latter two compounds (Table 5). Contrary to this observation, calf thymus 2′-deoxycytidine kinase catalyzed the rapid phosphorylation of ara-A. Substrate activity with adenosine kinase from rabbit muscle was also observed with ara-A but not with either compound 1a or 1b (Table 5) (1). The lack of observed substrate activity with these constitutive enzymes suggests that compounds 1a and 1b may be phosphorylated only in VZV-infected cells.

ACV is widely used for the treatment of HSV and VZV infections. In vitro, the IC_{50} for ACV inhibition of VZV replication is 15 μM (Table 3). Compounds 1a and 1b are clearly more potent than ACV and are worthy of further evaluation.

ACKNOWLEDGMENTS

We express appreciation to S. Wyckoff, C. Burns, J. Rideout, and S. Daluge for chemical support; S. Stanat and E. H. Dark for performing antiviral and cytotoxicity assays; W. Miller, J. Tuttle, and S. Mckee for providing some of the enzymatic data; and M. Mangum for help in preparation of the manuscript.

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