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ACCELERATED COMMUNICATION

Potent and Selective Activity of 3′-Azido-2,6-diaminopurine-2′,3′-dideoxyriboside, 3′-Fluoro-2,6-diaminopurine-2′,3′-dideoxyriboside, and 3′-Fluoro-2′,3′-dideoxyguanosine Against Human Immunodeficiency Virus

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SUMMARY

Several sugar-modified 2,6-diaminopurine and guanine 2′,3′-dideoxyribosides were synthesized and evaluated *in vitro* for their ability to inhibit the cytopathic effect and replication of human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS). 3′-Azido-2,6-diaminopurine-2′,3′-dideoxyriboside (AzddDAPR), 3′-fluoro-2,6-diaminopurine-2′,3′-dideoxyriboside (FddDAPR), and 3′-fluoro-2′,3′-dideoxyguanosine emerged as potent and selective anti-HIV agents in MT4 cells (50% effective antiviral dose: 0.3–4.5 μM). Their selectivity indexes, based on the ratio of the 50% cytotoxic dose to the 50% antiviral effective dose, were 157, 80, and 96, respectively, as compared to 106 for 2,6-diaminopurine-2′,3′-dideoxyriboside (ddDAPR) and 132 for 2′,3′-dideoxyadenosine (ddAdo), two other potent anti-HIV agents. The 9-β-D-arabinoside and 9-β-D-2′-deoxyxyloside derivatives of 2,6-diaminopurine were devoid of any antiretrovirus activity. Both AzddDAPR and FddDAPR, like the parent compounds ddDAPR and ddAdo, proved susceptible to deamination by beef intestine adenosine deaminase (K_m, 11, 148, 29, and 73 μM, respectively). 2′-Deoxycoformycin, a potent inhibitor of adenosine deaminase, decreased the antiretrovirus and cytostatic activity of ddDAPR and FddDAPR to a greater extent than that of AzddDAPR. This suggests that ddDAPR and FddDAPR are primarily active as their guanine analogues, whereas AzddDAPR may be potentially active as a 2,6-diaminopurine derivative as well.

Several purine and pyrimidine nucleoside analogues with the 2′,3′-dideoxyribose sugar moiety have proven successful in the inhibition of replication of HIV *in vitro* (1–10). The most potent and selective inhibitors reported so far are ddCyd (1–3), ddThd (4), the 2′,3′-unsaturated 2′,3′-dideoxynucleoside analogues derived therefrom (D4C and D4T, also termed ddeCyd and ddeThd, respectively) (2–8), 5-fluoro-ddCyd (9), 3′-fluoro-2′,3′-dideoxythymidine (10), 3′-azido-2′,3′-dideoxyuridine (11), AzddThd (AZT, BW A509 U) (10, 12, 13), ddAdo (1), 3′-azido-2′,3′-dideoxyadenosine, (14, 15), 2′-fluoro-2′,3′-dideoxy-araA (16), ddGuo (1), and 2′,3′-dideoxyinosine (ddIno) (1). Recently, AzddGuo (17, 18) and ddDAPR (19) were also reported as potent anti-HIV agents. AzddGuo completely inhibits HIV-induced cytopathogenicity and antigen expression in MT4 cells at 5 μM, and is more selective than ddGuo in its anti-HIV activity (17). ddDAPR is, like ddAdo, a potent and selective inhibitor of HIV *in vitro*, and inhibits HIV replication in MT4 cells at a 50% effective dose (ED_{50}) of 2.5–3.6 μM (19). We now report four 2,6-diaminopurine nucleosides with modifications

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ABBREVIATIONS: HIV, human immunodeficiency virus; ddCyd, 2′,3′-dideoxycytidine; ddThd, 2′,3′-dideoxythymidine; AzddThd, or AZT (BW A509 U), 1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymidine; ddAdo, 2′,3′-dideoxyadenosine; ddGuo, 2′,3′-dideoxyguanosine; AzddGuo, 3′-azido-2′,3′-dideoxyguanosine; ddDAPR, 2,6-diamino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (2,6-diaminopurine-2′,3′-dideoxyriboside); AzddDAPR, 2,6-diamino-9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)purine; FddDAPR, 2,6-diamino-3′fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)purine; araDAPR, 9-β-D-arabinofuranosyl-2,6-diaminopurine; XylodDAPR, 2′-deoxy-9-β-D-xylofuranosyl-2,6-diaminopurine; FddGuo, 3′-fluoro-2′,3′-dideoxyguanosine; ddeAdo, 2′,3′-didehydro-2′,3′-dideoxyadenosine; MSV, Moloney murine sarcoma virus; SI, selectivity index; DCF, 2′-deoxycoformycin; ADA, beef intestine adenosine deaminase; Ado, adenosine; AIDS, acquired immunodeficiency syndrome.

in the sugar moiety, i.e., AzddDAPR, FddDAPR, araDAPR, and XylodDAPR, and compare the antiretroviral, cytostatic, and antimetabolic properties of these novel ddDAPR derivatives with their parent compound, ddDAPR, and their deaminated congeners AzddGuo and FddGuo.

Materials and Methods

Compounds. ddDAPR was synthesized according to a procedure recently used for conversion of adenosine into its 2′,3′-unsaturated derivative, ddeAdo, and subsequent hydrogenation of ddeAdo to give ddAdo (20–22). AzddDAPR was prepared in very low yield by the chemical transfer glycosylation procedure of Imazawa and Eckstein (23) using AZT and 2,6-diacetamidopurine (24). Work-up was followed by deprotection with methanolic aqueous ammonia and purification on columns of Dowex 1X2 (OH^-) followed by silica gel. AzddDAPR had m.p. 239–241° (dec.); MS *m/z* 291.1195 (M^+ [$C_{10}H_{13}N_9O_2$] = 291.1192); ^1H NMR (Me_2SO-d_6, Me_4Si) 6.15 ("t," $J_{1'-2',2''}$ = 6.5 Hz, 1, H1′) (25). AzddDAPR was also synthesized from 2,6-diamino-9-(2-deoxy-3-*O*-mesyl-β-D-*threo*-pentofuranosyl)purine with lithium azide in dimethylformamide. FddDAPR was synthesized from 2,6-diamino-9-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-2-*N*-tritylpurine with diethylaminosulfur trifluoride in dichloromethane, followed by deprotection with 80% acetic acid. Procedures for chemical synthesis of AzddDAPR and FddDAPR will be described in detail elsewhere. The synthesis of araDAPR has been described previously (26). The chemical synthesis of XylodDAPR will be described elsewhere. The structural formulas of AzddDAPR, FddDAPR, XylodDAPR, and araDAPR are depicted in Fig. 1. ddGuo was obtained from Pharmacia PL-Biochemicals (Uppsala, Sweden). AzddGuo was synthesized by Imazawa and Eckstein (23) or obtained upon deamination of 3′-azido-ddDAPR by ADA (Boehringer Mannheim, Mannheim, West Germany). The procedure for the chemical synthesis of FddGuo will be described in detail elsewhere. FddGuo was also obtained by deaminating FddDAPR with ADA. 2′,3′-dideoxyadenosine, and 2′,3′-dideoxycytidine were kindly provided by Dr. D. G. Johns (National Institutes of Health, Bethesda, MD).

Cells. The cultivation of Raji, Molt/4F, CEM, H9, HUT-78, and MT4 cells has been described elsewhere (27, 28). MT4 cells were a gift from Dr. N. Yamamoto, Yamaguchi University, Yamaguchi, Japan.

Viruses. HIV was obtained from the culture supernatant of an H9 cell line persistently infected with HTLV-III_B (29), which was kindly provided by Dr. R. C. Gallo (National Cancer Institute, Bethesda, MD). MSV was prepared from tumors induced by *in vivo* infection of 2- to 3-day-old NMRI mice (30).

Antiviral assays. The method for transformation of mouse embryo (C3H) cells by MSV has been described previously (19). Briefly, confluent monolayers of C3H cells in 1-ml wells of Tissue Culture Cluster plates were infected with 150 focus-forming units of MSV during 90 min, whereafter medium was replaced by 1 ml of fresh culture medium containing different concentrations of the test compounds. After 6–7 days of incubation at 37°C, transformation of the cell cultures was monitored microscopically.

Determination of the cytopathic effect of HIV in human T-lymphocyte MT4 cells has been described previously (19, 28). Briefly, MT4 cells, subcultured 1 day before the start of the experiment, were adjusted at 5×10^5 cells/ml and infected with HIV (HTLV-III_B) at 400 $CCID_{50}$/ml. Then, 100 μl of the infected cell suspension were brought into the wells of a microtiter tray containing 100 μl of varying dilutions of the test compounds. After 5 days' incubation at 37°, the number of viable cells was recorded microscopically in a hematocytometer by trypan blue exclusion.

The percentage of the protective effect of the test compounds on the survival of the MT4 cells exposed to the virus was determined by the following formula: 100 × [(number of total viable cells exposed to HIV and cultured in the presence of the test compound) − (number of total viable cells exposed to HIV and cultured in the absence of the test compound]/[(number of total viable cells not exposed to HIV and cultured in the absence of the test compound) − (number of total viable cells exposed to HIV and cultured in the absence of the test compound)]

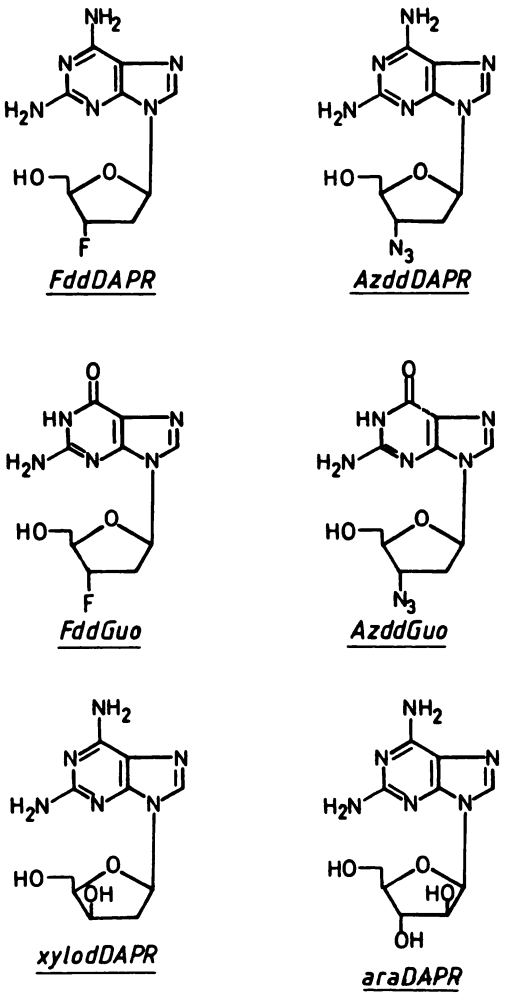

Fig. 1. Structural formulas of 3′-fluoro-2,6-diaminopurine-2′,3′-dideoxyriboside (*FddDAPR*), 3′-azido-2,6-diaminopurine-2′,3′-dideoxyriboside (*AzddDAPR*), 3′-fluoro-2′,3′-dideoxyguanosine (*FddGuo*), 3′-azido-2′,3′-dideoxyguanosine (*AzddGuo*), 2′-deoxy-9-β-D-xylofuranosyl-2,6-diaminopurine (*XylodDAPR*), and 9-β-D-arabinofuranosyl-2,6-diaminopurine (*araDAPR*).

The inhibitory effects of the test compounds on HIV antigen expression in infected H9 and HUT-78 cells were determined at days 8 and 14, respectively, after infection, by indirect immunofluorescence and laser flow cytofluorography using a polyclonal antibody as probe, as previously described (28).

Cytostatic effects of the test compounds. The cytostatic effects of the test compounds were determined by measuring inhibition of cell proliferation. The experimental procedures have been described previously (3, 31, 32).

Adenosine deaminase assay. The reaction mixture contained 800 μl of potassium phosphate buffer, 50 mM, pH 7.4, a 100-μl solution of the test compound (final concentrations varying between 10 and 150 μM), and 100 μl (0.1 unit for the AzddDAPR assays and 1.0 unit for the FddDAPR, araDAPR, and XylodDAPR assays) of beef intestine adenosine deaminase (Boehringer Mannheim).

Determination of the lipophilicity of AzddDAPR, ddDAPR, AzddThd, and ddThd. To estimate the lipid solubility of the test compounds, two different procedures were followed: (i) the R_f values of ddDAPR, AzddDAPR, ddThd, and AzddThd were determined by thin layer chromatography on Silicagel M 5735 with a mixture of chloroform/methanol (80:20) for ddDAPR and AzddDAPR, and chloroform/

methanol (95:5) for ddThd and AzddThd; or (ii) the partition of ddDAPR, AzddDAPR, ddThd, and AzddThd between 1-octanol (Merck, Darmstadt, West Germany) and 10 mM potassium phosphate buffer, pH 7.4 (Merck), was measured. Briefly, a 50 μM concentration of the test compound in the potassium phosphate buffer was thoroughly mixed with an equal volume of L-octanol for 30 min. Then, the mixture was further equilibrated at room temperature for 60 min, UV absorption was measured for the aqueous and alcoholic liquid phases, and the percentage of the test compound present in each liquid phase was calculated based on their absorption maxima.

Results

Antiretrovirus effect of sugar-modified purine 2′,3′-dideoxynucleoside analogues. A series of 2,6-diaminopurine and guanine 2′,3′-dideoxynucleoside analogues, in which the 3′-carbon of the 2′,3′-dideoxyribose moiety bears a 3′-fluoro or 3′-azido group, or in which the 2′,3′-dideoxyribose moiety is replaced by arabinose or 2′-deoxyxylose, was synthesized and evaluated for their effect on the transformation of C3H mouse embryo fibroblasts by MSV (Table 1) and the cytopathogenicity of HIV for human T4 lymphocyte MT4 cells (Table 1, Fig. 2). AzddDAPR and FddDAPR selectively inhibited HIV-induced cytopathogenicity in MT4 cells, AzddDAPR (ED_{50}, 0.3 μM) being 15- to 20-fold more effective than FddDAPR and ddAdo. AzddDAPR showed an anti-HIV activity that was 10-fold higher than that of its deaminated analogue, AzddGuo, whereas the anti-HIV activity of FddDAPR was comparable to that of FddGuo. AzddDAPR proved considerably more effective in inhibiting HIV infectivity than ddAdo and the parent compound ddDAPR, but it was also more toxic to the MT4 cells. Its SI [ratio of 50% cytotoxic dose (CD_{50}) to 50% antivirally effective dose (ED_{50})] was comparable to that of ddAdo and ddDAPR (157 versus 106, respectively) (Table 1). However, both the anti-HIV and cytostatic activities of AzddDAPR were almost similar to those obtained for the pyrimidine 2′,3′-dideoxynucleoside ddCyd, another well known potent inhibitor of HIV replication (1–3). Within the guanosine series, both FddGuo and AzddGuo proved effective in inhibiting HIV-induced cytopathogenicity (ED_{50}, 2.4 and 2.8 μM, respectively). There was no significant difference in the antiviral and cytotoxic properties of the chemically synthesized and the enzymatically prepared AzddGuo and FddGuo derivatives. The 9-β-D-arabinoside and 2′-deoxy-9-β-D-xyloside of ddDAPR were totally devoid of anti-HIV activity. When evaluated for their inhibitory effects on MSV-induced transformation of murine C3H cells, AzddDAPR was clearly more effective than FddDAPR, AzddGuo and FddGuo. AzddDAPR inhibited cell transformation by MSV at a concentration as low as 1.0 μM, that is, about 6-fold lower than the dose required for AzddGuo, and 35-, 19-, or 28-fold lower than the doses required for ddAdo, ddDAPR and FddDAPR, respectively. Also, AzddDAPR proved to be superior to ddCyd in inhibiting transformation of C3H cells by MSV. XylodDAPR and araDAPR did not exhibit any anti-MSV activity at concentrations up to 400 μM.

We also evaluated the 3′-substituted ddDAPR derivatives in two other human T4 lymphocyte cell lines (HUT-78 and H9) for their inhibitory effects on HIV-induced antigen expression. In these experiments, both AzddDAPR and FddDAPR achieved an inhibition of viral antigen expression within the concentration range of 0.09–1.0 μM (data not shown), and, thus, at concentrations which were even lower than those required to inhibit HIV-induced cytopathogenicity in MT4 cells.

Cytostatic and antimetabolic effects of sugar-modified purine 2′,3′-dideoxynucleoside analogues. When examined for their inhibitory effects on the growth of human B-lymphoblast Raji, T-lymphoblast Molt/4F, or T-lymphocyte CEM, H9, and MT4 cell lines (Table 2), none of the test compounds proved particularly cytostatic. In no case was the 50% inhibitory dose (ID_{50}) for cell proliferation below 60 μM. The higher ID_{50} values of the test compounds against MT4 compared to their CD_{50} values as presented in Table 1 are due to the shorter incubation time (3 days) of the cells with the test compounds. The CD_{50} values presented in Table 1 were recorded after 5 days of incubation. AzddDAPR was slightly more inhibitory to human cell proliferation than AzddGuo (ID_{50}, 68–382 μM, and 152–630 μM, respectively). In contrast, FddGuo was slightly more cytostatic than FddDAPR (ID_{50}, 190–228 μM, and 233–>1000 μM, respectively). XylodDAPR and araDAPR did not exhibit any cytostatic activity, even at a concentration of 1000 μM.

Cellular DNA synthesis, as monitored by the incorporation of [*methyl*-^{3}H]dThd into DNA of MT4 cells, was not markedly affected by any of the test compounds at a concentration of 200 μM.

Effect of 2′-deoxycoformycin on the antiretroviral and cytostatic effects of ddDAPR, AzddDAPR, and FddDAPR. Pretreatment of MT4 or C3H cells with 10 μM DCF for 3 hr resulted in a decrease of the antiretroviral effects of ddDAPR, AzddDAPR, and FddDAPR, being less pronounced for AzddDAPR (Table 3). Also, pretreatment of Molt/4F, CEM, and MT4 cells with DCF brought about a considerable decrease in the cytostatic activity of ddDAPR, a somewhat lesser decrease in the cytostatic effect of FddDAPR, and virtually no decrease in the cytostatic effect of AzddDAPR (Table 4).

Substrate activities of the ddDAPR analogues for beef intestine adenosine deaminase. The four ddDAPR analogues were examined for their susceptibility to deamination by ADA. The K_m values of ADA for the natural substrates Ado

TABLE 1

Antiretroviral and cytotoxic activity of sugar-modified purine 2′,3′-dideoxynucleoside analogues

Compound	HIV-induced cytopathogenicity in MT4 cells[a]			MSV-induced cell transformation in C3H cells[b]		
	ED_{50}[c]	CD_{50}[c]	SI	ED_{50}[c]	CD_{50}[c]	SI
	μM			μM		
ddDAPR	3.5 (±2.8)	404 (±45)	106	19 (±8.5)	>200	>11
AzddDAPR	0.3 (±0.04)	44 (±25)	147	1.0 (±0.6)	>200	>200
FddDAPR	4.5 (±1.6)	360 (±51)	80	28 (±10.6)	>400	>15
XylodDAPR	>125	>625		>400	>400	
araDAPR	>125	>625		>400	>400	
ddGuo	7.6[d]	486[d]	64[d]	72 (±65)	>400	>5.5
AzddGuo	2.8	165	59	6.2 (±0.75)	>400	>64
FddGuo	2.4 (±0.1)	237 (±6)	96	17 (±15.3)	>400	>23
ddAdo	6.3 (±2.4)	890[e]	141	35 (±27)	>100	>5.7
ddCyd	0.3 (±0.1)	41.4 (±0.6)	138	25.3 (±9)	>200	>7.9

[a] The effect of the test compounds on HIV-induced cytopathogenicity in MT4 cells and the cytostatic effects of the test compounds against MT4 cells were recorded at day 5 of the experiment by counting the number of viable cells by the trypan blue exclusion method.

[b] The effect of the test compound on MSV-induced transformation of C3H cells was examined microscopically at day 6 after infection.

[c] Data (±standard deviation) represent the average of four to six separate experiments.

[d] See Ref. 17.

[e] See Ref. 19.

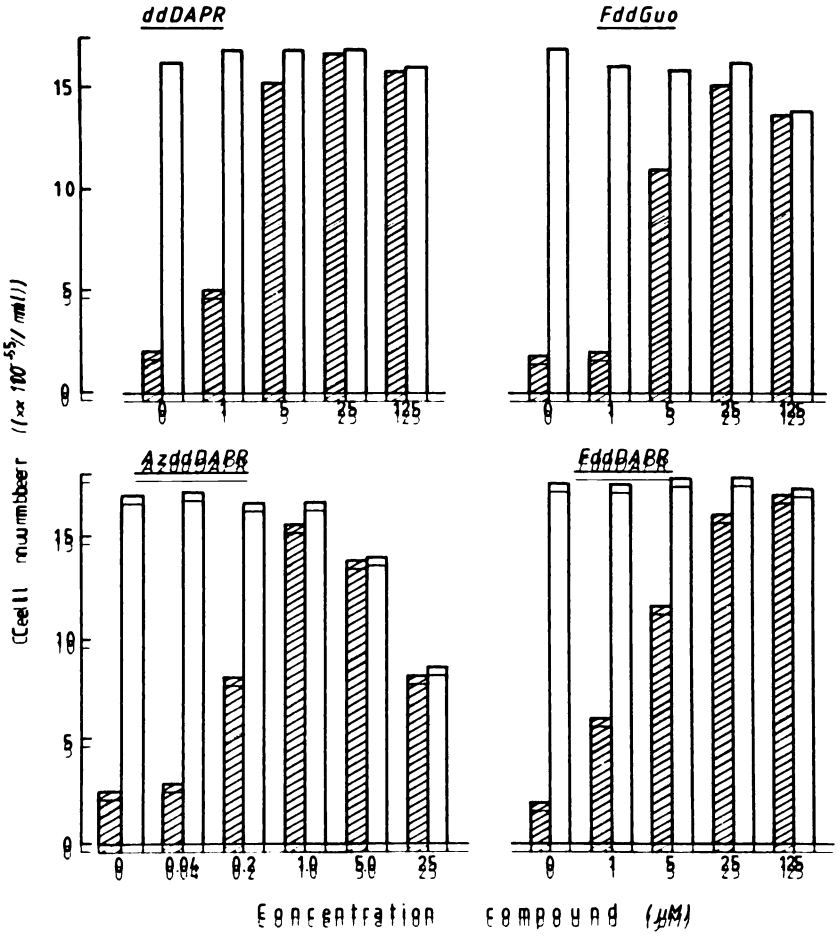

Fig. 2. Inhibition of the cytopathogenicity of HIV for MT4 cells by ddDAPR, FddGuo, AzddDAPR, and FddDAPR. Viability of the cells was measured by trypan blue exclusion after an incubation period of 5 days. □, mock-infected cells, incubated in the presence of different concentrations of the test compound; ▨, HIV-infected cells, incubated in the presence of different concentrations of the test compounds. Data represent the average of three to five separate experiments. The standard deviations of the data are presented in Table 1.

TABLE 2
Cytostatic effect of sugar-modified purine 2′,3′-dideoxynucleoside analogues against human B and T cell lines

Compound	ID_{50}[a,b]				
	Raji	Molt/4F	CEM	H9	MT4
	μM				
ddDAPR	324 (±105)	244 (±23)	125 (±19)	466 (±463)	644 (±319)
AzddDAPR	157 (±10)	72 (±23)	68 (±5)	69 (±16)	382 (±39)
FddDAPR	753 (±300)	425 (±64)	233 (±47)	377 (±81)	>1000
XylodDAPR	>1000	>1000	>1000	>1000	>1000
XylddDAPR	>1000	>1000	>1000	>1000	>1000
araDAPR	>1000	>1000	>1000	>1000	>1000
ddGuo	903 (±138)	380 (±170)	142 (±12)	531 (±98)	942 (±93)
AzddGuo	294 (±93)	165 (±29)	152 (±68)	304 (±48)	630 (±71)
FddGuo	107 (±27)	228 (±8)	119 (±3)	194 (±10)	197 (±10)

[a] Cell growth was recorded after 3 days of cultivation of the cells in the presence of test compound by counting the number of cells with a Coulter counter.
[b] Data (±standard deviation) represent the average of two to three separate experiments.

TABLE 3
Effect of DCF on the antiretroviral activity of ddDAPR, AzddDAPR, and FddDAPR

Compound	With or without 10 μM DCF[a]	ED_{50}[b]	
		HIV-induced cytopathogenicity in MT4 cells	MSV-induced transformation in C3H cells
		μM	
ddDAPR	−	3.8 (±2.8)	23 (±5.7)
	+	15 (±11)	84 (±13.1)
AzddDAPR	−	0.28 (±0.04)	1.3 (±0.24)
	+	0.70 (±0.40)	1.6 (±0.58)
FddDAPR	−	4.5 (±1.55)	31 (±2.8)
	+	39 (±24)	87 (±6.7)

[a] Cells were pretreated with 10 μM DCF for 3 hr before a continuous exposure of the cells with DCF (10 μM) and different concentrations of test compounds. HIV-induced cytopathogenicity in MT4 cells was recorded at day 5 after infection by counting the viable cell number by the trypan blue exclusion method. MSV-induced transformation of C3H cells was examined microscopically at day 6 after infection.
[b] Data (±standard deviation) represent the average of three to five separate experiments.

and 2′-deoxyadenosine were 29 μM and 15 μM, respectively (Table 5) (see also Ref. 33). ddDAPR had the same K_m as Ado but a V_{max} that was 35-fold lower. AzddDAPR had a 2.5-fold lower K_m (11 μM) and a 5-fold higher V_{max} (40 μmol/mg of protein/min) than ddDAPR. Thus, the ratio V_{max}/K_m obtained for AzddDAPR was about 10-fold higher than that for ddDAPR, similar to that for ddAdo, and only 3-fold lower than that for Ado (Table 5). FddDAPR had a 4- to 5-fold higher K_m and V_{max} than ddDAPR. XylodDAPR and araDAPR had similar K_m (74–83 μM) and V_{max} (3.7–5.9 μmol/mg of protein/min) values, and their V_{max}/K_m ratios were considerably lower than those obtained with ddDAPR and ddAdo.

Lipid solubility of ddDAPR, AzddDAPR, ddThd, and AzddThd. To estimate the lipid solubility of ddDAPR, AzddDAPR, ddThd, and AzddThd, R_f values for the respective compounds were determined by thin layer chromatography in a mixture of chloroform/methanol. Under these experimental conditions, ddDAPR and AzddDAPR had R_f values of 0.49 and 0.64, respectively, whereas the R_f values found for ddThd and AzddThd were 0.29 and 0.39, respectively (data not shown). In a second procedure, the partition of the test compounds be-

TABLE 4

Effect of DCF on the cytostatic activity of ddDAPR, AzddDAPR, and FddDAPR

Compound	Presence of 10 μM DCF[a]	ID_{50}[b,c]		
		CEM	Molt/4F	MT4
			μM	
ddDAPR	−	128 (±9.0)	220 (±69)	>1000
	+	>1000	>1000	>1000
AzddDAPR	−	68 (±3.2)	59 (±8.6)	315 (±13)
	+	96 (±6.0)	87 (±10.5)	357 (±19)
FddDAPR	−	241 (±105)	353 (±32)	469 (±69)
	+	739 (±229)	865 (±234)	520 (±21)

[a] An initial concentration of 10 μM DCF was added to the cells 1 hr before the addition of the test compounds.

[b] Cytostatic effects of the compounds were recorded at day 3 of the experiment by counting the cell number with a Coulter counter.

[c] Data (±standard deviation) represent the average of three separate experiments.

TABLE 5

Kinetic properties of ADA for 2,6-diaminopurine 2′,3′-dideoxynucleoside analogues

Compound	K_m[a]	V_{max}[a]	V_{max}/K_m
	μM	μmol/mg protein/min	
Ado	29[b]	287[b]	9.9
ddAdo	73[b]	262[b]	3.6
ddDAPR	29[b]	8.2[b]	0.28
AzddDAPR	11	40	3.6
FddDAPR	148	31	0.21
araDAPR	83	5.9	0.07
XylodDAPR	74	3.7	0.05

[a] K_m and V_{max} values were calculated from a Lineweaver-Burk diagram, constructed with the data of at least five to six different concentrations of the test compounds. Linear regression analysis on the individual values yielded a regression coefficient > 0.95 in each experiment.

[b] Data taken from Ref. 33.

tween *L*-octanol and potassium phosphate buffer was measured. We found that AzddDAPR and ddDAPR were present at 63% and 25.5%, respectively, in the *L*-octanol phase; AzddThd and ddThd were present at 50.7% and 20.1%, respectively, in the *L*-octanol phase. These data indicate that the increase of lipid solubility conferred on ddDAPR by the 3′-azido group is comparable to, if not higher than, that observed when a 3′-azido group is introduced in ddThd.

Discussion

ddDAPR is, like ddAdo, a potent and selective inhibitor of HIV *in vitro*, and may be considered as a potential chemotherapeutic agent against AIDS (19). We have now established that the 3′-azido derivative of ddDAPR, named AzddDAPR, is about 10- to 20-fold more effective than ddDAPR and ddAdo in inhibiting the cytopathogenicity of HIV in MT4 cells. However, since the cytotoxicity of AzddDAPR increased almost proportionally with the antiviral potency, it has an SI of the same order of magnitude as those of ddDAPR and ddAdo. AzddDAPR is also far more effective than the other purine 2′,3′-dideoxyribosides against transformation of murine cells by the murine retrovirus MSV. Because of its pronounced antiretroviral activities at nontoxic concentrations in cell culture, AzddDAPR must be considered as a novel, promising antiretroviral agent that should be further pursued for its efficacy in the treatment of retrovirus infections including AIDS. FddDAPR and FddGuo, although less effective than AzddDAPR, possess SIs comparable to those of ddDAPR and ddAdo (19), and, thus, may also be considered as two novel candidate anti-HIV drugs.

Taking into account the similarity in the biological properties of ddDAPR, AzddDAPR, and FddDAPR and those of ddGuo, AzddGuo, and FddGuo (Tables 1 and 2), and the fact that ddDAPR, AzddDAPR, and FddDAPR are susceptible to deamination by beef intestine ADA, one may postulate that these 2,6-diaminopurine derivatives act, at least in part, as prodrugs of ddGuo, AzddGuo, or FddGuo, respectively. Indeed, it is well documented that 2,6-diaminopurine derivatives are subject to deamination by ADA and thus behave as prodrugs of the corresponding guanosine analogues [i.e. 2,6-diaminopurine ribonucleoside (33–36), araDAPR (34), the acyclic 2,6-diaminopurine derivatives 2,6-diamino-9-[(2-hydroxyethoxy)methyl] purine and 2,6-diamino-9-[(1,3-dihydroxy-2-propoxy)methyl] purine (37, 38), 2,6-diaminopurine deoxyriboside (39)]. Moreover, we have found that the principal metabolites of [^{3}H] ddDAPR in Molt/4F cells are predominantly phosphorylated ddGuo metabolites.[1]

When we compared the four ddDAPR analogues (listed in Table 5) with the parental ddDAPR for their substrate affinities for ADA, we found that AzddDAPR had a significantly higher relative substrate efficacy (10- to 15-fold) for ADA than ddDAPR and FddDAPR. However, when evaluated for their cytostatic and antiretroviral activity in combination with a potent inhibitor of ADA (DCF), the biological effects of ddDAPR and FddDAPR were counteracted to a greater extent than those of AzddDAPR. These observations suggest that ddDAPR and FddDAPR may act primarily as prodrugs of ddGuo and FddGuo, respectively, whereas AzddDAPR may be active as its 2,6-diaminopurine analogue as well. The extent by which AzddDAPR (or its phosphorylated products) is converted intracellularly to the corresponding guanine metabolites remains a subject for further study.

The SI of FddGuo, as based on the ratio of the 50% cytotoxic dose to the 50% antiviral effective dose, was within the range of that recorded for ddGuo, AzddGuo, FddDAPR and ddDAPR. However, as stated above, FddDAPR may primarily act as a prodrug of FddGuo, and in this respect, it is not clear whether FddDAPR is advantageous over FddGuo from a chemotherapeutic viewpoint.

The mechanisms by which AzddDAPR, FddDAPR, AzddGuo and FddGuo inhibit HIV replication remain subjects for further study. For some 2′,3′-dideoxynucleosides (i.e., AzddThd, ddCyd, ddThd, ddAdo, and ddGuo) it has been ascertained that their corresponding 5′-triphosphate derivatives selectively interfere with the HIV reverse transcriptase and may inhibit further DNA chain elongation by serving as DNA chain terminators (40–43). From this perspective, it would seem imperative to synthesize the 5′-triphosphate derivatives of the ddDAPR and ddGuo analogues and to examine their substrate inhibitor properties with HIV reverse transcriptase and mammalian DNA polymerases.

It is also not clear why AzddDAPR has a substantially greater antiretrovirus and cytostatic effect than ddAdo, ddDAPR, and FddDAPR. One possible explanation may be the more rapid entry into cells of AzddDAPR *versus* ddDAPR by virtue of a greater lipid solubility conferred on ddDAPR by the introduc-

[1] J. Balzarini and D. G. Johns, unpublished results.

tion of a 3′-azido group. Indeed, it has recently been shown that 3′-azido-ddThd permeates the cell membrane chiefly by nonfacilitated diffusion and not via the nucleoside transport carrier present in the cell membrane (44). It was suggested that the unusual ability of AzddThd to diffuse across the cell membranes independently of the nucleoside transport system may be attributed to the considerable lipophilicity introduced in this molecule by the replacement of the 3′-hydroxyl group of thymidine with an azido substitute [partition coefficients (L-octanol/0.1 M sodium phosphate, pH 7.0)] of AzddThd and dThd were 1.26 and 0.064, respectively [(44). As a parameter for the impact of the 3′-azido substituent on the lipophilicity of the parental nucleoside ddDAPR, we estimated the R_f values of AzddDAPR versus ddDAPR by thin layer chromatography in a chromatography mixture of chloroform/methanol and found that AzddDAPR was considerably more lipophilic than ddDAPR (R_f 0.64 and 0.49, respectively). The increased lipophilicity conferred by the azido group in the ddDAPR molecule was comparable to the increased lipophilicity conferred by the azido group in the ddThd molecule (R_f = 0.29 and 0.39 for ddThd and AzddThd, respectively). Moreover, we also found that in a mixture of L-octanol-potassium phosphate buffer, 63% of AzddDAPR was present in the L-octanol phase versus 25.5% of ddDAPR. These data reflect a partition of the compound between the lipophilic and hydrophilic phase that is comparable to AzddThd and ddThd (50.7% and 20.1% in the L-octanol phase, respectively). Thus, since AzddDAPR is considerably more lipophilic than ddDAPR, AzddDAPR might, as has been suggested for AzddThd, enter into the cells more rapidly than its unsubstituted derivative ddDAPR. Uptake studies with radiolabeled AzddDAPR are required to confirm this hypothesis.

In conclusion, from our study, three novel purine 2′,3′-dideoxynucleoside analogues have emerged as potent and selective antiretrovirus agents *in vitro*, i.e., AzddDAPR, FddDAPR, and FddGuo. Their potency and selectivity as anti-HIV agents are comparable to that of ddDAPR, which, in turn, is as potent and selective an inhibitor of HIV as ddAdo (19), a compound that is now entering clinical trials for the treatment of AIDS.

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