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Lipophilic halogenated congeners of 2′,3′-dideoxypurine nucleosides active against human immunodeficiency virus *in vitro*

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ABSTRACT **Four 2-amino-6-halo- and four 6-halo-2′,3′-dideoxypurine ribofuranosides (ddPs) were synthesized and tested for *in vitro* activity to suppress the infectivity, cytopathic effect, Gag protein expression, and DNA synthesis of human immunodeficiency virus (HIV). The comparative order of *in vitro* anti-HIV activity of the eight 6-halo-ddPs was as follows: 2-amino-6-fluoro, 2-amino-6-chloro, 6-fluoro > 2-amino-6-bromo > 2-amino-6-iodo, 6-chloro > 6-bromo > 6-iodo. 2-Amino-6-fluoro-, 2-amino-6-chloro-, and 6-fluoro-ddPs showed a potent activity against HIV comparable to that of 2′,3′-dideoxyinosine (ddI) or 2′,3′-dideoxyguanosine (ddG) and completely blocked the infectivity of HIV without affecting the growth of target cells. The lipophilicity order was as follows: 2-amino-6-iodo > 2-amino-6-bromo > 2-amino-6-chloro > 2-amino-6-fluoro >> ddG > ddI. All eight 6-halo-ddPs were substrates for adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4). The relative rates of hydrolysis by ADA were as follows: ddA, 2-amino-6-fluoro >> 2-amino-6-chloro, 2-amino-6-bromo > 2-amino-6-iodo. Taken together, these compounds may represent an additional class of lipophilic prodrugs for ddI and ddG and may also provide a strategy for endowing therapeutic purine nucleosides with desirable lipophilicity.**

Several drugs active against human immunodeficiency virus (HIV) have now been transferred from laboratory to clinical settings to produce therapeutic benefits in patients with HIV infection (1, 2). One such drug, 3′-azido-2′,3′-dideoxyribosylthymine (AZT or zidovudine) has been formally proven to reduce the morbidity and mortality of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) (3, 4). Other members of the dideoxynucleoside family, including 2′,3′-dideoxycytidine (ddC) (5), 2′,3′-didehydro-2′,3′-dideoxyribosylthymine (M. Browne, G. Curt, and Brown University Phase I working group, personal communication), and 2′,3′-dideoxyinosine (ddI or didanosine) (6), have recently been shown to be active against HIV in some patients with AIDS and ARC in short-term phase I clinical trials. However, the lipophilicity of 2′,3′-dideoxypurine ribofuranosides (ddPs) including ddI is generally low and perhaps in part this limits their penetration into the central nervous system (CNS). One of the devastating features of AIDS and its related disorders is HIV-induced neurological abnormalities. It is of note that HIV-associated neurological disorders in both adults and children with AIDS or ARC have been substantially improved during therapy with AZT (7, 8). Thus, development of lipophilic antiretroviral drugs might have a direct clinical relevance in the therapy of HIV infection.

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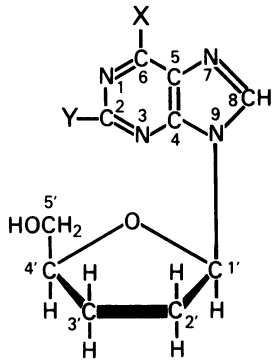

	X	Y		X	Y
1	F	NH_2	10	SH	H
2	Cl	NH_2	11	NH_2	NH_2
3	Br	NH_2	12	NH_2	Cl
4	I	NH_2	13	Cl	Cl
5	F	H	14	OH	OH
6	Cl	H	15	H	H
7	Br	H	16	NH_2	H
8	I	H	17	OH	H
9	SH	NH_2	18	OH	NH_2

FIG. 1. Structures of nucleosides tested. Numbers refer to positions in the base; primed numbers refer to positions in the sugar moiety.

MATERIALS AND METHODS

Viruses and Cells. HIV-1 was pelleted by ultracentrifugation from the culture supernatants of HIV-$1/III_B$-producing H9 cells (9). The 50% tissue culture infective dose ($TCID_{50}$) per cell of the cell-free HIV-1 preparation was determined by an endpoint titration method in which $CD4^+$ T cells (ATH8) were used (see below) as described by Leland and French (10).

The supernatant of monocyte/macrophage (M/M) culture after exposure to HIV-1_{Ba-L} was collected and used as a source of infectious monocytotropic virus as described (11).

Three $CD4^+$ T-cell lines—ATH8 (12), MT2 (13), and H9 (9)—were used as target cells for infection by HIV-1 in this study.

Abbreviations: ADA, adenosine deaminase; ARC, AIDS-related complex; AZT, 3′-azido-2′,3′-dideoxyribosylthymine; CNS, central nervous system; ddA, 2′,3′-dideoxyadenosine; ddG, 2′,3′-dideoxyguanosine; ddI, 2′,3′-dideoxyinosine; ddP, 2′,3′-dideoxypurine ribofuranoside; HIV-1, human immunodeficiency virus type 1; M/M, monocyte(s)/macrophage(s).

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Reagents. 2-Amino-6-chloropurine and 2-amino-6-iodopurine were purchased from Sigma. 2-Amino-6-fluoropurine, 2-amino-6-bromopurine, and 2',3'-dideoxyuridine were synthesized as described (14–16). Four 2-amino-6-halo-ddPs, four 6-halo-ddPs, and several dideoxynucleoside derivatives were synthesized by using a method developed by K.M., H.Y., and E.K. (unpublished data) with pelleted live *Escherichia coli* JA-300 cells (17) as a source of pyrimidine and purine nucleoside phosphorylases.

Structures of compounds tested are depicted in Fig. 1. Compounds **6, 11,** and **12** in Table 1 have been described elsewhere (18–21), while compounds **1–5, 7–10, 13–15** were newly synthesized and will be described in detail elsewhere. All synthesized ddPs were >99% pure (except 2,6-diamino-ddP, whose purity was ≈95%) as assessed by nuclear magnetic resonance spectra and high-performance liquid chromatography (HPLC). Satisfactory elementary analyses have been obtained for all the compounds described in this paper. 2',3'-Dideoxyadenosine (ddA), ddI, and 2',3'-dideoxyguanosine (ddG) were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, while AZT was purchased from Sigma. Adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) from calf intestine was purchased from Boehringer Mannheim.

HIV Cytopathic Effect Inhibition Assay. The HIV cytopathic effect inhibition assay was performed as described (2, 12).

Determination of HIV-1 Gag Protein Expression. Determination of HIV-1 Gag protein expression was performed as described (12). Briefly, H9 cells (2×10^5) were pretreated for 2 hr with various drugs, exposed to a dose of 4.3×10^3 $TCID_{50}$ of HIV-1 per cell, and cultured at 37°C in 5% CO_2/95% humidified air. On days 5 and 7, the percentage of the H9 cells expressing p24 Gag protein was determined by an indirect immunofluorescence assay with a murine monoclonal antibody reactive against HIV-1 p24 Gag protein (M26).

Southern Blot Hybridization. Southern blot hybridization was performed as described by Southern with minor modifications (1). Relative levels of the detected viral DNA were compared by densitometry (X-Rite, 301; X-Rite Inc., Grand Rapids, MI). The percentage reduction of HIV proviral DNA content was determined by the following formula: 100 × [1 − (OD for a sample DNA − the background OD)/(OD for no drug control DNA − the background OD)], where OD represents optical density reading and the background represents the lowest density site within each lane.

Table 1. *In vitro* antiretroviral activity of 2',3'-dideoxynucleosides tested

Compound	Target	Concentration, μM	Protective effect, %	Cytotoxicity, %
2-Amino-6-halo-ddPs				
(1) 2-amino-6-fluoro-ddP	ATH8	2, 5, 20, 50, 100, 200	48, 62, 100, 100, 77, 70	0, 0, 0, 0, 26, 32
	MT2	2, 20, 50, 100, 200	0, 100, 89, 57, 54	0, 0, 0, 33, 42
(2) 2-amino-6-chloro-ddP	ATH8	2, 5, 20, 50, 100, 200	38, 48, 100, 100, 84, 78	0, 0, 0, 0, 19, 25
	MT2	2, 20, 50, 100, 200	0, 100, 100, 62, 38	1, 0, 2, 30, 63
(3) 2-amino-6-bromo-ddP	ATH8	2, 5, 20, 50, 100, 200	35, 35, 100, 100, 78, 64	0, 0, 0, 0, 20, 33
	MT2	2, 20, 50, 100, 200	0, 100, 66, 62, 27	0, 0, 32, 35, 71
(4) 2-amino-6-iodo-ddP	ATH8	2, 5, 20, 50, 100, 200	21, 21, 100, 93, 73, 47	0, 0, 0, 9, 26, 47
	MT2	2, 20, 50, 100, 200	0, 97, 70, 34, 7	2, 5, 25, 63, 96
6-Halo-ddPs				
(5) 6-fluoro-ddP	ATH8	2, 20, 50, 100, 200	64, 100, 87, 61, 43	0, 5, 10, 32, 52
(6) 6-chloro-ddP	ATH8	2, 20, 50, 100, 200	28, 100, 69, 56, 49	8, 1, 20, 41, 55
(7) 6-bromo-ddP	ATH8	2, 20, 50, 100, 200	25, 80, 63, 48, 36	7, 17, 17, 52, 61
(8) 6-iodo-ddP	ATH8	2, 20, 50, 100, 200	17, 41, 39, 30, 20	4, 39, 37, 55, 81
6-Mercapto-ddPs				
(9) 2-amino-6-mercapto-ddP	ATH8	2, 20, 200	6, 1, 2	29, 83, 78
(10) 6-mercapto-ddP	ATH8	2, 20, 200	0, 2, 8	59, 46, 49
Other ddP analogues				
(11) 2,6-diamino-ddP	ATH8	2, 20, 200	17, 90, 100	0, 0, 0
	MT2	2, 20, 200	0, 3, 77	2, 13, 27
(12) 2-chloro-6-amino-ddP	ATH8	2, 20, 200	1, 1, 0	0, 77, 95
	MT2	2, 20, 50, 200	2, 0, 0, 0	0, 99, 100, 100
(13) 2,6-dichloro-ddP	ATH8	2, 20, 200	0, 0, 0	0, 45, 100
(14) 2',3'-dideoxyxanthosine	ATH8	2, 20, 200	1, 0, 0	3, 2, 11
(15) ddP	ATH8	2, 20, 200	0, 0, 3	0, 0, 5
(16) ddA	ATH8	5, 20, 100, 200, 500	10, 100, 100, 100, 66	0, 0, 0, 0, 0, 28
(17) ddI	ATH8	1, 10, 100, 200, 1000	7, 97, 100, 100, 46	9, 6, 0, 0, 40
	MT2	2, 5, 10, 20, 50	25, 81, 95, 92, 100	0, 0, 0, 0, 0
(18) ddG	ATH8	5, 10, 100, 200, 500	34, 90, 100, 91, 66	0, 0, 0, 1, 41
	MT2	5, 10, 50, 100, 500	0, 5, 40, 76, 38	4, 8, 12, 12, 59
(19) AZT	ATH8	0.5, 1, 5, 10, 50	56, 100, 93, 93, 48	0, 0, 4, 4, 51
	MT2	1, 2, 5, 10, 20	28, 100, 95, 100, 81	0, 0, 5, 0, 12

ATH8 or MT2 cells (2×10^5) were exposed to 4.3×10^3 $TCID_{50}$ of HIV-1/III_B per cell (1000 viral particles per cell) for 1 hr and cultured in the presence of various concentrations of each compound. On days 5–7, the total viable cells were counted. Data obtained by using ATH8 or MT2 cells are indicated. Orders of numbers in the column for concentrations correspond to the orders of numbers in other columns. The percentage of protective effect of each compound on the survival and growth of target cells exposed to the virus was determined by the following formula: 100 × [(no. of viable cells exposed to HIV-1 and cultured in the presence of the compound − no. of viable cells exposed to HIV-1 and cultured in the absence of the compound)/(no. of viable cells cultured alone − no. of viable cells exposed to HIV-1 and cultured in the absence of the compound)]. By this formula, when the number of viable cells exposed to the virus and the compound is the same as or more than the number of viable cells cultured alone, 100% is given. Calculated percentages equal to or less than zero are expressed as 0%. The percentage of toxicity of each compound on target cells was determined by the following formula: 100 × [1 − (no. of total viable cells cultured in the presence of the compound)/(no. of total viable cells cultured alone)]. Calculated percentages equal to or less than zero are expressed as 0%. AZT is listed as a reference compound.

Determination of HIV-1 Gag Protein Expression in M/M. HIV-1 Gag protein expression in M/M was determined as described (22). Briefly, target M/M (10^6) were preincubated with drugs for 20 min, exposed to 100 μl of HIV-1_{Ba-L} preparation (1 μl of the supernatant represented the minimum infectious dose), and cultured in 1 ml of complete medium in the presence or absence of the drug. On day 6 in culture, the cells were extensively washed and further cultured in 1 ml of fresh medium. On day 12 and beyond, the amount of p24 Gag protein in the supernatant was assessed by radioimmunoassay (DuPont).

Partition Coefficient Determination. *n*-Octanol/water partition coefficients (P) were determined by a microshake-flask procedure. A 20-μl aliquot of a 0.5-mg/ml dimethyl sulfoxide solution of a given compound was dissolved in 1 ml of octanol-saturated (pH 7.0) 0.01 M potassium phosphate buffer and thoroughly mixed with 1.0 ml of buffer-saturated *n*-octanol in a 2-ml Mixxor apparatus (Lidex Technologies, Bedford, MA) at 24–26°C. The phases were separated and centrifuged individually, and the relative concentration of sample in a 50-μl aliquot of each phase was determined by HPLC analysis. The partition coefficient was calculated by dividing the absolute area of the appropriate integrator peak from the octanol phase by that of the buffer phase. The values were expressed as log P.

Studies of Enzymatic Hydrolysis by ADA. Relative rates of hydrolysis of ddPs by ADA and characterization of their products were determined as described (23).

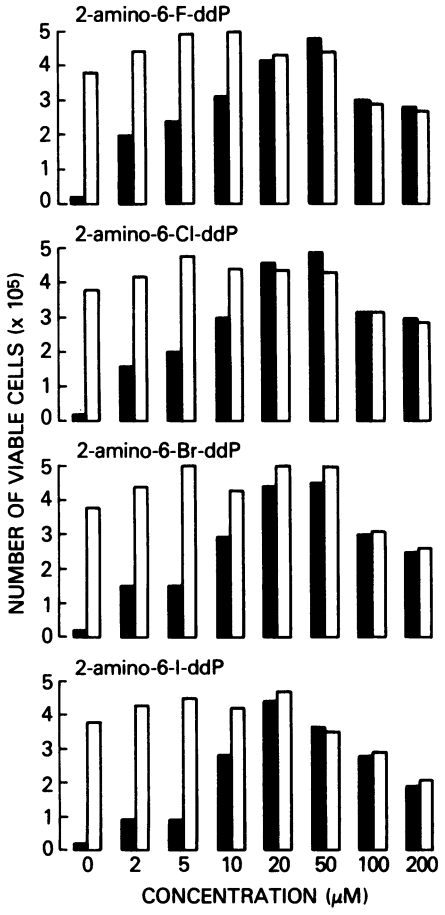

FIG. 2. Inhibition of the cytopathic effect of HIV-1 by 2-amino-6-halo-ddPs in ATH8 cell system. ATH8 cells (2×10^5) were exposed to a dose of 4.3×10^3 $TCID_{50}$ of HIV-1 per cell, in the presence or absence of various concentrations of 2-amino-6-halo-ddPs (solid bar). Control cells (open bar) were not exposed to the virus. On day 6, the total viable cells were counted.

RESULTS

***In Vitro* Antiretroviral Activity of 2-Amino-6-halo- and 6-Halo-ddPs Against HIV.** Four 2-amino-6-halo-ddPs (compounds **1–4** in Table 1) and three 6-halo-ddPs (compounds **5–7**) exerted a potent anti-HIV-1 activity *in vitro*. In the HIV cytopathic effect inhibition assay, almost all ATH8 cells were destroyed by the virus by day 7 after exposure to HIV-1 in the absence of drugs (Fig. 2). However, when the cells were cultured in the presence of each compound at 2 and 5 μM, a partial protective effect on ATH8 cells was obtained. At 20 and 50 μM, the cells were virtually completely protected against HIV-1 and grew comparably to virus-uninfected cells (Fig. 2; Table 1). These compounds exhibited comparable antiviral activity when MT2 cells were used as the target cells (Table 1). At concentrations $\geq$100 μM, however, the drugs appeared to be somewhat more suppressive to cell growth as compared to the reference compounds ddI and ddG (compounds **17** and **18** in Table 1). Among ddPs tested, compounds substituted with an iodine were the most toxic for cell growth. Other structurally related ddP congeners (compounds **9, 10,** and **12–15**) were not active against HIV under the conditions described above. It was of note that, contrary to a previous report (20), 2-chloro-6-amino-ddP (compound **12** in Table 1) was toxic and failed to exert significant protective effect against HIV-1 in both ATH8 and MT2 cells.

In this initial screening assay, 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs were the most potent and least toxic among the ddPs tested and were further investigated for their antiviral activity against HIV-1.

2-Amino-6-fluoro- and 2-Amino-6-chloro-ddPs Inhibit HIV-1 Gag Protein Expression *in Vitro*. When $CD4^+$ H9 cells were exposed to HIV-1, by day 5 in culture, $\approx$40% of the H9 cells expressed p24 Gag protein as assessed by an indirect immunofluorescence technique, and on day 7, $\approx$60% of the cells became positive for Gag protein (Fig. 3). However, 10 μM 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs suppressed Gag expression by 75–100% and at $\geq$20 μM both compounds virtually completely blocked the expression of HIV-1

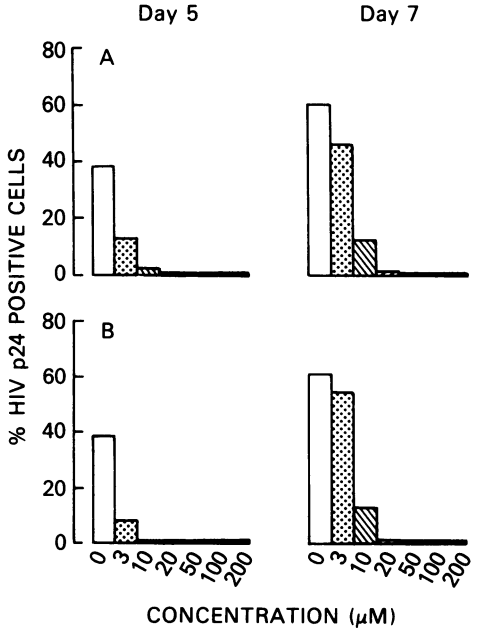

FIG. 3. Inhibition of expression of HIV-1 p24 Gag protein in H9 cells. H9 cells were pretreated with or without 2-amino-6-fluoro-ddP (*A*) or 2-amino-6-chloro-ddP (*B*) for 2 hr, exposed to HIV-1, resuspended, and cultured in the presence or absence of various concentrations of the drug. On days 5 and 7 in culture, the percentage of H9 cells expressing HIV-1 p24 Gag protein was assessed.

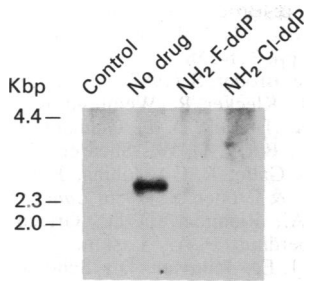

FIG. 4. Inhibition of HIV-1 DNA synthesis in peripheral blood mononuclear cells exposed to the virus. Peripheral blood mononuclear cells stimulated with phytohemagglutinin (1×10^7) were pretreated with or without 80 μM 2-amino-6-fluoro- (NH_2-F-ddP) or 2-amino-6-chloro-ddP (NH_2-Cl-ddP) for 2 hr, exposed to HIV-1 (1000 viral particles per cell), and cultured in the presence or absence of the same concentration of the drugs. On day 2 after exposure to the virus, high molecular weight DNA was extracted from the cells, and 40 μg of such DNA was digested with *Kpn* I and subjected to Southern blot hybridization. Ethidium bromide staining of DNA in an agarose gel visualized under UV light ensured that the same amount of DNA digest was loaded in each lane. Note that the viral DNA was detected as a 2.7-kilobase-pair (Kbp) env-containing internal fragment.

throughout the 7 days in culture. At concentrations used in this experiment, the viability of H9 cells was always 90–100%.

Inhibition of HIV-1 DNA Synthesis by 2-Amino-6-halo-ddP *in Vitro*. To further characterize the antiviral activity of 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs, the amount of proviral DNA synthesized in phytohemagglutinin-stimulated peripheral blood mononuclear cells after exposure to HIV-1 was assessed by the Southern blot hybridization technique. In the absence of the drugs, proviral DNA was readily detectable on day 2 (Fig. 4). However, in the presence of 80 μM 2-amino-6-fluoro- or 2-amino-6-chloro-ddP, the synthesis of proviral DNA was virtually completely suppressed. Densitometric analysis of the exposed film revealed that 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs reduced HIV-1 DNA synthesis by 98% and 94%, respectively, as compared to the amount of proviral DNA detected in the cells cultured in the absence of drugs.

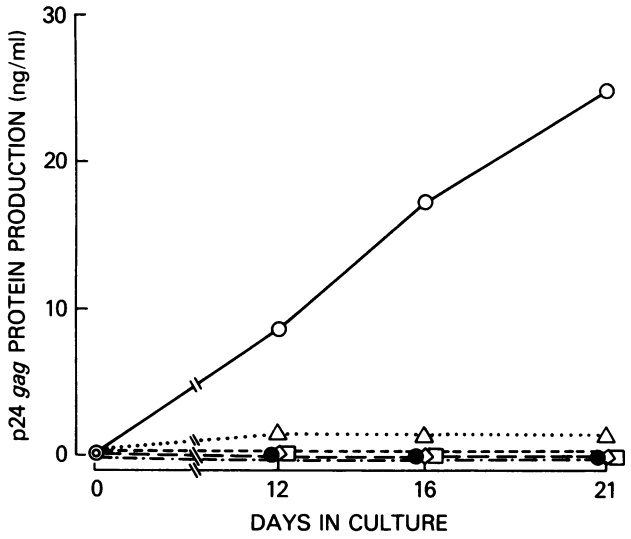

FIG. 5. Inhibition of expression of p24 Gag protein in M/M by 2-amino-6-fluoro-ddP and 2-amino-6-chloro-ddP. M/M were exposed to HIV-$1_{Ba\text{-}L}$ and cultured alone (○) or with 2 μM 2-amino-6-fluoro-ddP (△), 2 μM 2-amino-6-chloro-ddP (◇), or 50 μM ddI (□). Control M/M (●) were similarly treated but not exposed to the virus.

Table 2. Octanol/water partition coefficient

Compound	Log P
2-Amino-6-halo-ddPs	
(1) 2-amino-6-fluoro-ddP	−0.050 ± 0.007
(2) 2-amino-6-chloro-ddP	0.211 ± 0.011
(3) 2-amino-6-bromo-ddP	0.338 ± 0.007
(4) 2-amino-6-iodo-ddP	0.523 ± 0.008
6-Halo-ddPs	
(5) 6-fluoro-ddP	−0.002 ± 0.005
(6) 6-chloro-ddP	0.237 ± 0.007
(7) 6-bromo-ddP	0.354 ± 0.006
(8) 6-iodo-ddP	0.526 ± 0.012
(16) ddA	−0.287 ± 0.005
(17) ddI	−1.242 ± 0.028
(18) ddG	−1.091 ± 0.006
(19) AZT	0.052 ± 0.009

AZT is listed as a reference compound.

***In Vitro* Inhibition of Monocytotropic HIV-1 Replication in M/M by 2-Amino-6-halo-ddP.** We asked if 2-amino-6-fluoro- and 2-amino-6-chloro-ddPs could also block the replication of a monocytotropic HIV-1 strain, HIV-1_{Bal}, in M/M *in vitro*. In the absence of drugs, by day 12 in culture, M/M after exposure to HIV-1_{Bal} began to produce a detectable amount of HIV-1; and by day 21, they produced as much as 25 ng of p24 Gag protein per ml as assessed by radioimmunoassay (Fig. 5). However, when M/M were cultured in the presence of 2 μM 2-amino-6-fluoro- or 2-amino-6-chloro-ddP, replication of the virus was virtually completely inhibited.

Lipophilicity of 2-Amino-6-halo- and 6-Halo-ddPs. We further asked whether 2-amino-6-halo- and 6-halo-ddPs had a high level of lipophilicity (Table 2). It was found that all eight 2-amino-6-halo-ddPs had substantially higher octanol partition coefficients than the reference compounds, ddA, ddI, and ddG. The partition coefficients of 2-amino-6-fluoro-ddP and 6-fluoro-ddP were close to that of AZT; however, other 2-amino-6-halo- and 6-halo-ddPs had higher coefficients than AZT.

The 2-Amino-6-halo- and 6-Halo-ddPs Are Substrates for ADA. Since some 2- and 6-substituted purine ribonucleosides are readily hydrolyzed by ADA (24), we asked whether the corresponding dideoxynucleosides were the substrate for this enzyme. This turned out to be the case shown in Table 3, which depicts the rate constants of selected 2-amino-6-halo- and 6-halo-ddPs as substrates of ADA. When the kinetic experiments were performed in culture media containing 15% fetal calf serum, the 2-amino-6-halo-ddPs were still hydrolyzed to ddG, but at a rate that was ≈60 times slower than the rate in the presence of excess enzyme. Thus, both ddA and 2-amino-6-fluoro-ddP had a half-life in RPMI 1640 medium of ≈2 hr.

The antiviral activity of all these compounds at a concentration of 50 μM was completely abrogated in the presence of 2′-deoxycoformycin (5 μM), a potent inhibitor of ADA, and

Table 3. Rate constants of 2-amino-6-halo- and 6-halo-ddPs as substrates of ADA

Substrate	K_m,	Relative V_{max}
Adenosine	6.9×10^{-5}	100
2-amino-6-fluoro-ddP (1)	1.3×10^{-3}	127
2-amino-6-chloro-ddP (2)	6.2×10^{-3}	50
6-fluoro-ddP (5)	1.0×10^{-3}	76
ddA (16)	1.6×10^{-4}	65

ADA activity was assessed as described. For comparison, the relative V_{max} of adenosine, the reference compound, was defined to be 100.

essentially all the target ATH8 cells were destroyed by the virus (data not shown).

DISCUSSION

HIV not only causes pathologic effects in immunocompetent cells but also often causes a variety of neurological disorders, including acute and chronic meningitis, inflammatory and sensory neuropathy and myelopathy, and encephalopathy. HIV in the CNS may replicate more actively than in other tissues, and the CNS may serve as a principal reservoir of the virus in the whole body (11). Thus, the capacity of antiviral agents against HIV to penetrate into the CNS may constitute an important feature of therapeutics against HIV. We describe here that the substitution with a halogen atom at the 6 position of the base can confer substantial lipophilicity on 2',3'-dideoxypurine nucleosides without reducing their *in vitro* antiretroviral activity. It should be noted, however, that the principal determinants of entry of any drugs into the CNS include lipophilicity, protein binding, and carrier systems (25); and the lipophilicity of a given congener *per se* may not necessarily determine its penetration potential into the CNS. It is also possible that a drug with a high lipophilicity may not have an improved therapeutic index and may even exert increased toxicity. Only *in vivo* studies can resolve these issues.

The 6-halo versions of ddPs are of interest in view of their activity/structure relationships. 2-Amino-6-halo- and 6-halo-ddPs appear to exert antiviral activity only upon conversion to ddG and ddI, respectively. In this regard, replacement of a phenolic oxygen (e.g., the oxygen in position 6 of purine) has been shown to increase the lipophilicity under certain circumstances (26) and some 2- and 6-substituted purine ribonucleosides have been shown by Chassy and Suhadolnik (24) to be substrates for ADA. However, it should be noted that there is still no reliable algorithm for predicting which congeners will exert more antiretroviral activity or less cellular toxicity. For example, replacement of a phenolic oxygen of ddI by a hydrogen, generating ddP, negates the potent antiretroviral activity of ddI (27). The same replacement of ddG, generating 2-amino-ddP, also abolishes the antiretroviral activity of ddG (27). Indeed, in the present study, we also found that neither of two 6-mercapto-ddPs (compounds **9** and **10** in Table 1) exerted antiretroviral activity *in vitro*. This may be due to the fact that these 6-mercapto-ddPs are not good substrates for ADA (unpublished data) and may not convert to ddG or ddI.

We now have data that each of 6-halo-ddPs discussed in this study is as sensitive as ddI and ddA to solvolysis in acid reactions and decomposes to a purine base and a dideoxyribose, thus losing its antiretroviral activity (unpublished data). However, it has been shown that ddI is orally bioavailable when administered with antacids, and plasma concentrations higher than those that exert potent antiviral activity *in vitro* can be achieved (6).

Taken together, these newly synthesized 6-halo-substituted ddPs may represent a class of lipophilic antiretroviral drugs against HIV-1. Our current observations may also provide a strategy to develop lipophilic purine nucleoside derivatives for different clinical applications.

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