Biochemical Pharmacology, Vol. 37, No. 19, pp. 3543–3548, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

COMPARATIVE ACTIVITY OF 2',3'-SATURATED AND UNSATURATED PYRIMIDINE AND PURINE NUCLEOSIDES AGAINST HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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(Accepted 13 July 1988)

Although a number of compounds have been identified as inhibitors of human immunodeficiency virus type 1 (HIV-1) *in vitro*, nucleosides seem to be the most potent and promising class of compounds for the treatment of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) [1]. These include 3'-azido-3'-deoxythymidine (AZT; Zidovudine; Retrovir) [2-5], 2',3'-dideoxycytidine (D2C) and 2',3'-dideoxyadenosine (D2A) [6,7], 3'-azido-2',3'-dideoxyuridine (CS-87) [8,9], 2',3'-dideoxy-2',3'-didehydrocytidine (D4C) [10-13], 3'-deoxy-2',3'-didehydrothymidine (D4T) [11,13-15], and ribavirin [16,17]. These nucleosides are currently in various stages of development, and AZT has been licensed in the United States for the treatment of certain HIV-1 infections. Although AZT has been reported to reduce the mortality and morbidity of AIDS [4], its bone marrow suppression, resulting anemia and neutropenia [5] may preclude its use for long term therapy for AIDS/ARC. Thus, it is necessary to search for new antiviral agents with high potency and fewer side effects.

Recently, the anti-HIV-1 activities of 2',3'-dideoxy-2',3'-didehydronucleosides such as D4C [10-13], D4T [11,13-15], 2',3'-dideoxy-2',3'-didehydroadenosine (D4A) and the corresponding guanine analogue, d4G, were reported [18,19]. As a part of our continuing efforts to study nucleosides as anti-HIV-1 agents, the structure-activity relationships of various 2',3'-dideoxy-2',3'-didehydro-pyrimidine and purine nucleosides, including some C-nucleosides, are reported. A human peripheral blood mononuclear (PBM) cell screening system was used to determine the antiviral activity. Since 3'-azido-5-ethyl-2',3'-dideoxyuridine (CS-85)§ and CS-87 [8,9] are compounds that have been reported to have antiviral activity and low bone marrow toxicity in culture, it was of interest to synthesize the 3'-unsubstituted 2',3'-saturated and 2',3'-unsaturated analogues of CS-85 and CS-87. The effects of these compounds on the growth of uninfected PBM cells are also reported.

MATERIALS AND METHODS

Synthetic Methodology

2',3'-Dideoxy analogues of uridine (**1**, D2U), thymidine (**2**, D2T), 5-ethyluridine (**3**, D2EtU), and cytidine (**4**, D2C) were prepared by deiodination of 3'-iodo-nucleosides or by deoxygenation of the ribonucleosides to the corresponding 2',3'-unsaturated nucleosides following reduction of the 2',3'-double bond [CK Chu,

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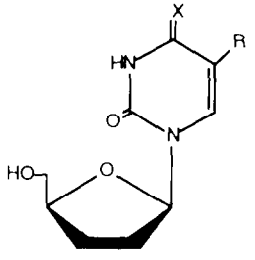
§ Schinazi RF, Chu CK, Feorino P and Sommadossi JP, 3'-Azido-2',3'-dideoxy-5-ethyluridine (CS-85) - a new potent selective anti-HTLV3/LAV compound. 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, Sept. 28 - Oct 1, 1986.

unpublished results] (Fig. 1). 2',3'-Dideoxy-2',3'-didehydro-analogues of uridine (**5**, D4U), thymidine (**6**, D4T), 5-ethyluridine (**7**, D4EtU), and cytidine (**8**, D4C) were prepared according to the method of Horwitz and his coworkers [20] or from the corresponding ribonucleosides. 2',3'-Dideoxy-nucleoside analogues of inosine (**9**, D2I), adenosine (**10**, D2A), N^6-methyladenosine (**11**, D2MeA), and guanine (**12**, D2G) as well as 2',3'-dideoxy-2',3'-didehydronucleoside analogues of inosine (**13**, D4I), adenosine (**14**, D4A), N^6-methyladenosine (**15**, D4MeA), and guanosine (**16**, D4G) were also prepared from the corresponding ribonucleosides (Fig. 1). The preparation of 2',3'-unsaturated purine nucleosides **13**, **14**, and **16** has been reported previously by dehalogenation of 2'- or 3'-halo nucleosides with chromous ions [21,22]. C-Nucleoside analogues of D2C and D4C, **17** and **18**, respectively, were synthesized from pseudouridine [23]. All the new compounds were characterized by ^{1}H-NMR spectroscopy and elemental analysis.



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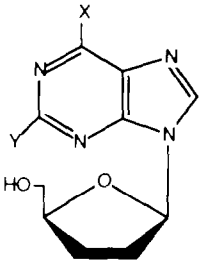

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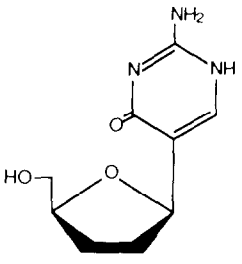

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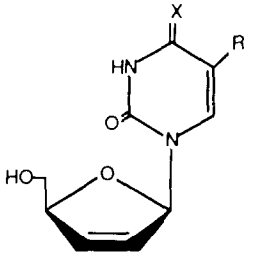

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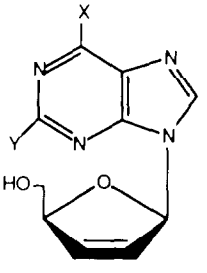

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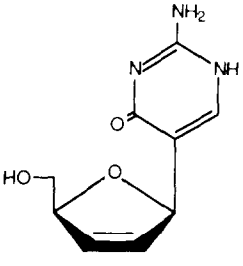

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Fig. 1. Structures of 2',3'-deoxypyrimidine and purine nucleosides

Antiviral Assay In Human PBM Cells

Three-day-old phytohemagglutinin-stimulated PBM cells (10^6 cells/ml) from hepatitis B and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 50% tissue culture infectious dose per ml and cultured in the presence and absence of various concentrations of compounds. The drugs were added about 45 min after infection. Five days later the supernatant was clarified and the virus pelleted. The reverse transcriptase activity associated with the disrupted virus was determined. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal *et al.* [24] and Spira *et al.* [25], except that fungizone was not included in the medium. The virus infected control had about 2 x 10^5 dpm per ml of reverse transcriptase activity. The blank and uninfected cell control values were about 300 and 1000 dpm, respectively. Drugs with potent activity were retested using different PBM cells.

The effects of drugs on the growth of uninfected human PBM cells were also established. Mitogen-stimulated PBM cells (3.8 x 10^5 cells/ml) were cultured in the presence and absence of drugs under the same conditions as those used for the antiviral assays described above. The cells were counted using a hemacytometer on day 5 using the trypan blue exclusion method. The median effective (EC_{50}) and inhibitory (IC_{50}) concentrations were determined by the median effect method [26]. The correlation coefficients for the dose response curves were greater than 0.90.

RESULTS

The results of antiviral assays of the new pyrimidine and purine nucleosides in PBM cells compared to known antiviral compounds are shown in Table 1. The data indicated that any modifications at the C5-position on the pyrimidine ring of 2',3'-dideoxynucleosides such as D2U (**1**) or D2EtU (**3**) resulted in a marked reduction of antiviral activity when compared to D2T (**2**) and D2C (**4**). As previously reported in PBM cells [10,14], the dideoxydidehydro analogues of thymidine (**6**) and cytidine (**8**) exhibited the most potent anti-HIV-1 activity among the compounds tested (Table 1). In contrast, in an ATH8 system, 2',3'-dideoxycytidine (**4**, D2C) is one of the most potent anti-retroviral agents [13].

Table 1. Median effective (EC_{50}) and inhibitory (IC_{50}) concentrations of various pyrimidine and purine nucleosides in PBM cells[a]

Compound	R	X	Y	EC_{50} (μM)	IC_{50} (μM)
1. D2U	H	O	-	96.8 (48)[b]	> 100 (> 250)[b]
2. D2T	CH_3	O	-	0.17 (0.2)	> 100 (> 125)
3. D2EtU	C_2H_5	O	-	4.9	> 100
4. D2C	H	NH	-	0.011 (0.046)	> 100 (9.1, 37)
5. D4U	H	NH	-	73.8 (> 125)	> 100 (27)
6. D4T	CH_3	O	-	0.009 (0.01)	70.0 (1.2)
7. D4EtU	C_2H_5	O	-	75.7	> 100
8. D4C	H	NH	-	0.005 (0.13)	65.0 (7.9)
9. D2I	-	OH	H	4.31	> 100
10. D2A	-	NH_2	H	0.91 (6.4)	> 100 (890)
11. D2MeA	-	$NHCH_3$	H	0.20	> 100
12. D2G	-	OH	NH_2	0.88 (7.6)	> 100 (486)
13. D4I	-	OH	H	>100	> 100
14. D4A	-	NH_2	H	0.76 (> 125)	> 100 (9.5, 19)
15. D4MeA	-	$NHCH_3$	H	4.06	> 100
16. D4G	-	OH	NH_2	>100 (> 5)	> 100 (11)
17. C-nucleosides				>100	> 100
18. C-nucleosides				>100	> 100
AZT				0.006 (0.006)	> 100 (3.5)
AZDDU (CS-87)				0.20 (0.36)[c]	> 100 (244)[c]

[a] Values represent the mean of at least two separate experiments with different donor PBM cells.

[b] Values in parentheses were obtained in MT4 assays [15,19].

[c] Ref. 27.

For the dideoxydidehydro-nucleosides, minor modifications at the 5-position of the pyrimidine moiety (compounds **5** and **7**) again resulted in a marked reduction of antiviral activity. The purine analogues D2A (**10**) and D4A (**14**) exhibited significant anti-HIV-1 activity, which was in agreement with the results of Balzarini and colleagues in the ATH8 and MT4 systems [13,19]. The guanosine analogue, D2G (**12**), was at least 100-fold more potent than the corresponding unsaturated compound D4G (**16**). The ranking of these two compounds was also in agreement with the report in MT4 cells [19]. However, D4G was significantly less toxic in PBM than in MT4 cells (IC_{50} > 100 μM *vs* 11 μM). D2MeA was consistently one of the most potent purine analogues tested in the PBM system. In contrast, the unsaturated analogue D4MeA (**15**) was 20-fold less potent than the saturated analogue and about 7-fold less potent than D4A (**14**).

Other 2',3'-unsaturated purine derivatives such as D4I (**13**) and D4G (**16**) did not show any significant antiviral activity in PBM cells. Similarly, the C-nucleoside analogues of D2C and D4C (**17** and **18**) were found to be inactive and non-toxic. These compounds are related to the antileukemic compound 5-azacytidine and pseudoisocytidine [28].

DISCUSSION

The relative anti-HIV-1 potencies of several new and known 2',3'-saturated and unsaturated pyrimidine and purine nucleosides were determined in human PBM cells. The data presented above indicated that none of the newly synthesized compounds were more potent than AZT, D2C, D4C, or D4T [10,14]. There was a good correlation in the ranking of antiviral activity between the PBM system (Table 1) and the MT4 system described by a Belgian group [15,19]. However, compounds were invariably more active and less toxic in PBM cells than in MT4 or ATH8 cells. The greater potency in PBM cells may be attributed to the lower multiplicity of infection used and to other characteristics of the cells; MT4 and ATH8 cells contain the HTLV-I genome and are biochemically different from primary cells such as PBM cells. The lower toxicity could be related to the slower growth rate of mitogen-stimulated PBM cells than of continuous cell lines.

Among the purine analogues evaluated, the new compound D2MeA (**11**) was more potent than D2A; its cell culture therapeutic index was greater than 500. Although the complete mechanism of action of this drug is still unknown, like D2A, the phosphorylated form of the drug may act as a chain terminator in the synthesis of proviral DNA [19]. Recent data indicate that D2MeA is not a substrate for adenosine deaminase and, hence, its potent antiviral activity could be related to its resistance to deamination to D2I.[#] The detailed biochemical pharmacology as well as the structure-activity relationship of D2MeA and related compounds will be reported elsewhere. It is significant that minor modifications of known anti-retroviral agents (e.g. N^6-methylation of D2A) can result in a compound with a higher therapeutic index than the parent drug and emphasizes the need to perform additional minor chemical transformations of nucleosides for additional quantitative structure-activity relationship evaluation.

ACKNOWLEDGEMENTS

This work was supported by United States Public Health Service Grants AI 26055 and AI 25899 from the National Institute of Allergy and Infectious Diseases, the Veterans Administration, and a grant (BSRG SO7 RR05364) awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

[#] Schinazi RF, Eriksson BF and Chu CK, 2',3'-Dideoxy-N^6-methyladenosine (D2MeA) selectively inhibits HIV-1 replication and is resistant to adenosine deaminase. To be presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, October 24-26, 1988.

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