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DIHYDROPYRIMIDINE ANALOGS OF ACYCLONUCLEOSIDES. I. SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2′,3′-DIHYDROXYPROPYL DERIVATIVES OF 5-NITRO-2,5- AND 1,6-DIHYDROPYRIMIDINES

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The considerable interest displayed in recent years in the acyclic analogs of natural nucleosides is explained by the fact that compounds have been discovered in this group showing powerful antiviral action on a whole series of DNA- and RNA-containing viruses [8, 11, 12]. In 1978 De Clercq and A. Holy [9, 10] reported the antiviral activity of 9-(2,3-dihydroxypropyl)adenine (DHPA), a racemic mixture of which possesses practically the same activity as the (S)-enantiomer. Some N- and C-(2,3-dihydroxypropyl) substituted azines have been synthesized and their pharmacological properties investigated. In particular it was shown that the pyrimidine analogs of DHPA did not possess significant antiviral or anticancer activity [6]. However there is no information in the literature on acyclonucleosides containing a residue of reduced pyrimidine as the heterocyclic fragment.

Previously [3] we studied the alkylation of acetonyl anionic σ-complexes of the 5-nitropyrimidine series which may be considered as a model reaction for the synthesis of "twice modified" nucleosides. In the present study anionic σ-complexes were chosen as starting compounds for obtaining 5-nitrodihydropyrimidine analogs of DHPA (see scheme).

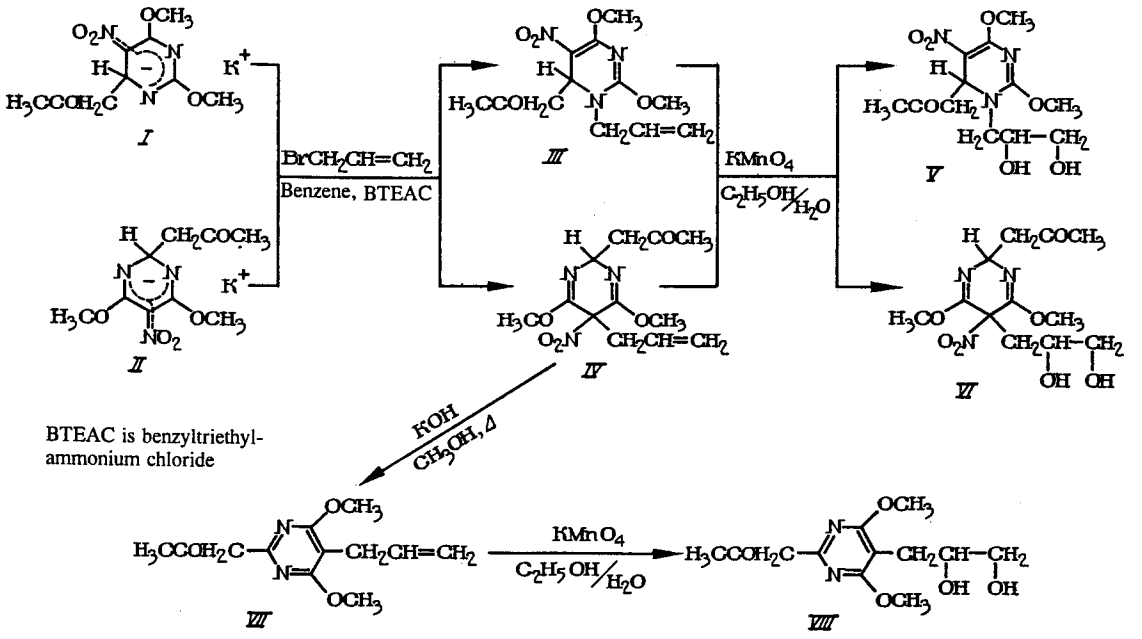

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TABLE 1. Effect of Compounds (V), (VI), and AZT on Viable MT-4 Cells Infected with HIV-1/IIIB

Concentration, μg/ml	Number of viable cells, %		
	VI	V	AZT
10	37,3	85,1	78,6
50	48,6	86,4	74,2
100	52,1	83,2	69,7
Control (intact cells)		87,5	
Viral control		18,7	

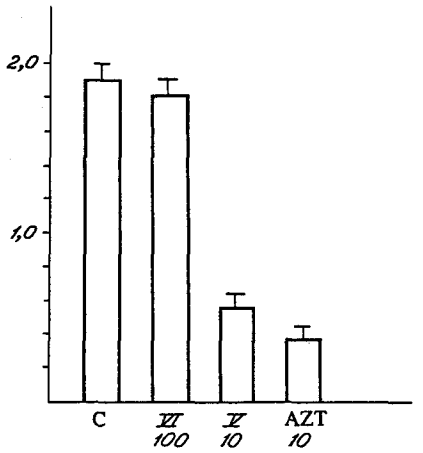

Fig. 1. Effect of Compounds (V), (VI), and AZT on p24 antigen content in the culture fluid of MT-4 cells. Concentration of p24 in ng/ml is on the ordinate. Compound concentration in μg/ml is on the abscissa here and in Fig. 2.

On reacting the acetonyl anionic σ-complexes (I) and (II) with allyl bromide in benzene in the presence of equimolar amounts of benzyltriethylammonium chloride (BTEAC), 1-allyl-1,6-dihydro- (III) and 5-allyl-5-nitro-2,5-dihydropyrimidines (IV) respectively were obtained. The presence in the PMR spectrum of compound (IV) of two triplets (5.49 and 5.71 ppm) and two doublets (2.53 and 2.73 ppm) proves the retention of the geminal unit at position 2 of the pyrimidine ring on going from (II) to (IV). The double set of signals in (IV) indicates the formation of a mixture of Z and E diastereomers. The position of the signals for the atoms C-2 and C-5 of compound (IV) in the ^{13}C NMR spectrum (see chemical experimental section) indicates their sp^3 hybrid character, which also confirms the 2,5-dihydropyrimidine structure of this compound. In the PMR spectrum of compound (III) the protons of the methylene group of the acetonyl fragment (2.86 ppm) are displayed as the AB portion of an ABX system due to nonequivalence. The chemical shifts of the allyl group characterize its link with a nitrogen atom of the pyrimidine ring. The chemical shift of the C-5 signal (105.91 ppm) in the ^{13}C NMR spectrum of compound (III) indicates the retention of its hybridization which also confirms the direction of the electrophilic attack on alkylation of the σ-complex (I).

The dihydropyrimidines (III) and (IV) obtained were used as starting materials for the synthesis of analogs of pyrimidine acyclonucleosides. On reacting these compounds with potassium permanganate in aqueous alcohol (by analogy with [7]) oxidation of the allyl fragment occurs which leads to the formation of N- and C-(2,3-dihydroxypropyl)dihydropyrimidines (V) and (VI) respectively. The spectral characteristics of the acyclonucleosides (V) and (VI) (see chemical experimental section) confirms their dihydropyrimidine structure.

Under alkaline conditions the 2,5-dihydropyrimidine (IV) is converted into pyrimidine (VII) by elimination of nitric acid. On oxidation with potassium permanganate compound (VII) forms the C-carbacyclonucleoside (VIII).

We have therefore for the first time obtained N- and C-pyrimidine analogs of DHPA from anionic σ-complexes of 5-nitropyrimidine. These contain a 1,6- or 2,5-pyrimidine fragment as heterocyclic fragment.

EXPERIMENTAL (CHEMICAL)

The ^1H and ^{13}C NMR spectra were recorded on a Varian VXP-300 spectrometer in $CDCl_3$, internal standard was hexamethyldisiloxane. The IR spectra were recorded on an M-80 spectrometer (KBr) and the UV spectra on an M-40 spectrometer (methanol). A check on the composition of reaction mixtures was effected by TLC on Silufol UV 254 plates (Czech Republic), the eluent was chloroform, and visualization was by scanning with UV light. The data of elemental analysis agreed with calculated values.

Compound (I) was synthesized according to [4], and compound (II) according to [5].

6-Acetonyl-1-allyl-2,4-dimethoxy-5-nitro-1,6-dihydropyrimidine (III). Allyl bromide (1.00 g, 8.3 mmole) was added with stirring to a suspension of the σ-complex (I) (1.96 g, 7.0 mmole) and benzyltriethylammonium chloride (1.59 g, 7.0 mmole) in absolute benzene (20 ml). After 24 h, the solid was filtered off, and the mother liquor evaporated to dryness at reduced pressure. The residue was chromatographed on a column of silica gel (eluent was chloroform). Yield was 0.69 g (35%) of mp 104-105°C (ethanol). UV spectrum, λ_{max}, nm (log ε): 203 (3.7), 257 (3.4), 380 (3.8). IR spectrum, ν_{max}, cm^{-1}: 1720 (C=O), 1300, 1520 (NO_2). ^{13}C NMR spectrum, δ, ppm: 30.50 (CH_3); 46.62 (CH_2CO); 49.60 (NCH_2); 53.92, 54.99 (OCH_3); 56.34 (C-6); 105.91 (C−NO_2); 118.84 (=CH_2); 130.90 (CH); 159.34, 163.48 (C-2, C-4); 205.98 (C=O). PMR spectrum, δ, ppm: 2.03 (3H, s, CH_3); 2.86 (2H, d q, CH_2); 3.75-4.17 (2H, m, NCH_2); 3.88, 3.92 (6H, 2 s, 2 OCH_3); 5.11-5.19 (3H, m, 6-CH + =CH_2); 5.76 (1H, m, CH). $C_{12}H_{17}N_3O_5$.

2-Acetonyl-5-allyl-4,6-dimethoxy-5-nitro-2,5-dihydropyrimidine (IV) was obtained similarly to (III) from the σ-complex (II). Yield was 62% of an oil. UV spectrum, λ_{max}, nm (log ε): 250 (3.0). IR spectrum, ν_{max}, cm^{-1}: 1710 (C=O); 1550, 1340 (NO_2). ^{13}C NMR spectrum, δ, ppm: 30.02, 31.08 (CH_3); 35.23, 35.67 (CH_2CO); 51.33, 52.51 (5-CH_2); 53.85 (OCH_3); 71.26, 71.44 (C-2); 80.82 (C-5); 121.77, 121.86 (=CH_2); 127.73, 131.49 (−CH=CH_2); 153.32, 153.52 (C-4, C-6); 205.77, 206.25 (C=O). PMR spectrum, δ, ppm: 2.17 (3H, s, CH_3); 2.53, 2.73 (2H, 2 d, CH_2); 3.08 (2H, m, CH_2); 3.65 (6H, s, OCH_3); 5.13 (3H, m, −CH=CH_2); 5.49, 5.71 (1H, 2 t, CH). $C_{12}H_{17}N_3O_5$.

6-Acetonyl-1-(2,3-dihydroxypropyl)-2,4-dimethoxy-5-nitro-1,6-dihydropyrimidine (V). A mixture of $KMnO_4$ (0.55 g, 3.5 mmole) and $MgSO_4 \cdot 7H_2O$ (0.86 g, 3.5 mmole) in water (60 ml) was added with stirring during 30 min to a cooled (−10°C) solution of compound (III) (1.00 g, 3.5 mmole) in ethyl alcohol (50 ml). After 4 h the solid was filtered off, the mother liquor evaporated to dryness, and the residue chromatographed on a column of silica gel (eluent was chloroform−methanol, 10:1). Yield was 0.43 g (38%) of a foam. UV spectrum, λ_{max}, nm (log ε): 255 (3.2), 386 (3.6). IR spectrum, ν_{max}, cm^{-1}: 3560 (OH); 1720 (C=O); 1580, 1375 (NO_2). PMR spectrum, δ, ppm: 1.70 (2H, br s, OH); 2.18, 2.19 (3H, 2 s, CH_3); 3.02 (2H, m, CH_2CO); 3.78-3.93 (4H, m, NCH_2 + CH_2OH); 3.95, 4.02 (6H, 2 s, 2 OCH_3); 4.98 (1H, m, 6-CH); 5.39 (1H, m, CHOH). $C_{12}H_{19}N_3O_7$.

2-Acetonyl-5-(2,3-dihydroxypropyl)-4,6-dimethoxy-5-nitro-2,5-dihydropyrimidine (VI) was obtained similarly to (V) from (IV). Yield was 42% of an oil. UV spectrum, λ_{max}, nm (log ε): 263 (3.1). IR spectrum, ν_{max}, cm^{-1}: 1720 (C=O); 3440 (OH); 1570, 1360 (NO_2). PMR spectrum, δ, ppm: 2.16, 2.17 (3H, 2 s, CH_3); 2.45 (CH_2OH); 2.53, 2.73 (2H, 2 d, CH_2CO); 3.26-3.42 (4H, m, 5-CH_2 + OH); 3.58, 3.61 (6H, 2 s, OCH_3); 5.44 (1H, m, CHOH); 5.49, 5.71 (1H, 2 t, CH). $C_{12}H_{19}N_3O_7$.

2-Acetonyl-5-allyl-4,6-dimethoxypyrimidine (VII). A solution of sodium methylate (0.22 g, 4.0 mmole) in methanol (3 ml) was added with stirring to a solution of the 2,5-dihydropyrimidine (IV) (1.0 g, 3.5 mmole) in absolute methanol (10 ml). After boiling the reaction mixture for 6 h the solvent was removed at reduced pressure. The residue was purified on a column of silica gel (eluent was chloroform). Yield was 0.68 g (82%) of mp 74-76°C (ethanol). UV spectrum, λ_{max}, nm (log ε): 246 (3.9), 289 (3.0). IR spectrum, ν_{max}, cm^{-1}: 1720 (C=O). ^{13}C NMR spectrum, δ, ppm: 26.10 (5-CH_2); 30.15 (CH_3); 54.67 (CH_2CO); 55.15 (OCH_3); 67.83 (C-2); 71.54 (CH=CH_2); 98.48 (=CH_2); 99.35 (C-5); 162.44 (C-4, C-6); 168.22 (C=O). PMR spectrum, δ, ppm: 2.24 (3H, s, CH_3); 3.20 (2H, d t, CH_2); 3.81 (2H, s, CH_2CO); 3.91 (6H, s, 2 OCH_3); 4.96 (2H, m, =CH_2); 5.82 (1H, m, CH). $C_{12}H_{16}N_2O_3$.

2-Acetonyl-5-(2,3-dihydroxypropyl)-4,6-dimethoxypyrimidine (VIII) was obtained similarly to (V) and (VI) from (VII). Yield was 40% of mp 117-118°C (ethanol). UV spectrum, λ_{max}, nm (log ε): 246 (3.9), 291 (3.3). IR spectrum, ν_{max}, cm^{-1}: 3200 (OH), 1710 (C=O). PMR spectrum, δ, ppm: 2.20 (3H, s, CH_3); 2.60 (2H, m, CH_2); 3.03 (3H, br s, OH); 3.40 (3H, m, CHOH + CH_2OH); 3.77 (2H, s, CH_2); 3.85 (6H, s, OCH_3). $C_{12}H_{18}N_2O_5$.

TABLE 2. Effect of Compounds (V), (VI), and AZT on the Reproduction of HIV-1/IIIB in Cultures of MT-4 Cells (EIA Data)

Concentration, μg/ml	Number of HIV-positive cells, %		
	VI	V	AZT
10	19,7 ± 2,9	3,6 ± 1,1	2,9 ± 0,7
50	15,3 ± 2,7	3,4 ± 1,1	2,2 ± 0,5
100	12,7 ± 1,9	2,8 ± 0,7	
Viral control (infected cells without the preparation)		24,9 ± 2,3	

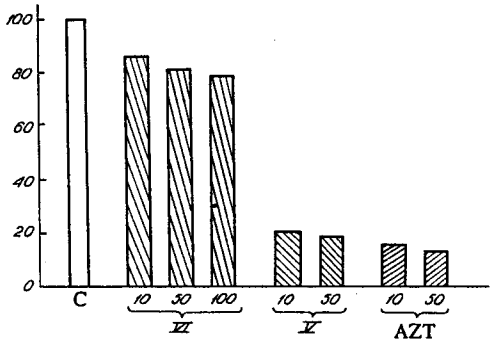

Fig. 2. Effect of compounds (V), (VI), and AZT on RT activity. Letter C denotes MT-4 cells infected with HIV-1/IIIB (control). Percentage of control is given on the ordinate.

EXPERIMENTAL (BIOLOGICAL)

Study of the antiviral activity of compounds (V) and (VI) was carried out using a model of MT-4 cells infected with HIV-1/IIIB at an infectivity of 0.05-0.10 log CTD_{50} per cell. Virus-containing culture fluid from transplanted cultures of H9/IIIB human T-lymphocytes was used for infection. These lymphocytes were of cell line H9 chronically infected with HIV-1. After adsorption of the virus for 1 h at 37°C the unbound virus was removed by centrifugation and subsequent washing of the cells with fresh medium. Cells ($1 \cdot 10^6$) in medium (2 ml) were placed in each well of a 24-well planchet. Control intact and infected cells were cultured for 7 d in the presence of preparations at concentrations of 10, 50, and 100 μg/ml for 7 days. Azidothymidine (AZT: Wellcome) was used as reference preparation.

The level of viral production in cultures was determined from the presence in the culture fluid of viral antigen p24, shown by enzyme immunoassay (EIA), and also from the reverse transcriptase (RT) activity in the culture medium [1]. The cytotoxic effect of the virus was assessed from the content of viable cells in the cultures by staining them with 0.25% Trypan Blue solution. The number of infected cells expressing viral antigens on the membrane was determined by an indirect immunofluorescence method using HIV-infected serum [2].

The number of viable cells in MT-4 cultures on day 7 after infection was 18.7 ± 1.8% (Table 1). A marked protection of cells from the cytodestructive action of HIV was observed on infecting cells simultaneously with the introduction of compound (V) into the culture medium. This was so at all the concentration used, viz. 10, 50, and 100 μg/ml, (83-85% viable cells). A similar effect was seen with AZT (65-78% viable cells). On the other hand compound (VI) possessed weak antiviral activity. The number of viable cells in cultures after infection was only 20-30% above the level recorded in control infected cultures.

Suppression of the cytotoxic action of the virus in cells coincided generally with the reduction in the content of p24 antigen for HIV-1 in the culture fluid. The results of a comparative study of the inhibitory action of compounds (V), (VI), and AZT on the production of p24 nitrogen of HIV-1 in a culture of MT-4 cells is shown in Fig. 1. It is evident that compound (V) at 10 μg/ml possessed a significant inhibiting ability (70-73%) which is close to the action of AZT at the same

concentration (80-85%). Compound (VI) did not show a significant effect on the content of p24 antigen for HIV in the culture fluid.

Similar results were obtained when studying the expression of HIV antigens on cell surfaces using EIA (Table 2). Simultaneous treatment of MT-4 cells with HIV-1 and with compound (V) led to a reduction in viral reproduction of 70-80%. Under the action of AZT the quantity of cells expressing HIV antigens was reduced by 85-95% compared to control infected cells. At the same time compound (VI) caused a reduction in the number of antigen-positive cells by 40-45% only at 100 μg/ml.

The results of studying the effect of compounds (V), (VI), and AZT on the level of RT activity are given in Fig. 2. The level of enzyme activity of each sample (mean of 3 replicates) is compared with the RT activity in control infected cultures taken as 100%. The RT activity in the culture fluid of MT-4 (viral control) on day 7 was 5.27 $\pm$ 0.38 imp/min/ml. It was established that compound (V) at 10 and 50 μg/ml, like AZT at the same concentrations, inhibited RT activity by 85-90%, while compound (VI) only inhibited by 20-30%.

The results of the experiments carried out show that compounds (V) and (VI) are characterized by different abilities to exert an effect on the replication of HIV-1 in sensitive cells. Compound (V), which possesses an antiviral activity similar to AZT, was not toxic at the concentrations necessary to protect cells from infection. Compound (VI) displayed insignificant antiviral activity and then only at 100 μg/ml, which is an order of magnitude greater than the concentrations of (V) and AZT. The results obtained indicate that AZT is not superior to compound (V) in practically all the features investigated.

It must be noted that 1-N-(2,3-dihydroxypropyl)-5-nitrouracil, described in [6], does not display significant antiviral activity. However, as the current investigation has shown, 5-nitrodihydropyrimidine analogs of acyclonucleosides have definite promise in the further search for highly effective antiviral preparations.

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