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Short Communication

Synthesis and antiviral activity of new carbonylphosphonate 2′,3′-dideoxy-3′-thiacytidine conjugates

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Abstract

The synthesis of new potential PFA-BCH-189 conjugate analogues is described and their molecular structure clearly identified through NMR and mass spectra techniques. The anti-HIV-1 activity was determined according to the inhibition of syncytium formation in MT-4 cells, while the anti-HBV activity was determined in infected duck hepatocytes. Both antiviral activities of the PFA-BCH-189 conjugates were much lower than those of the parent BCH-189 (2′,3′-dideoxy-3′-thiacytidine) (1). Whereas a prodrug effect, following cleavage and release of the free BCH-189 and PFA, cannot be ruled out, poor cellular permeation of the drug seems to be the most likely reason for the reduced activities against HIV and DHBV. The presence of the PFA moiety appears to be detrimental for both the anti-HIV and anti-DHBV activity of PFA-BCH-189 cases.

Keywords: 2′,3′-Dideoxy-3′-thiacytidine; 2′,3′-Dideoxy-3′-thiacytidine phophonoformic conjugates; HIV-1; DHBV

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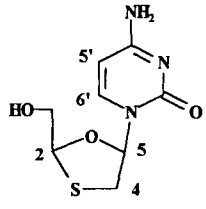
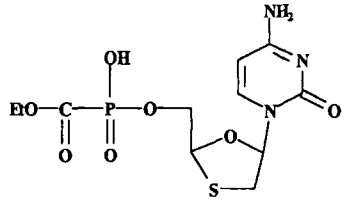
Despite intensive and continuing efforts in many laboratories, the most effective drugs presently available against human immunodeficiency virus type 1 (HIV-1) remain those directed against reverse transcriptase (RT). The most prominent class of RT inhibitors are 2′,3′-dideoxynucleosides, of which 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI) and 2′,3′-dideoxycytidine (ddC) are currently approved for the therapy of AIDS (Schinazi et al., 1992). However, anti-AIDS chemotherapy with AZT is associated with significant side effects (Richman et al., 1987), and clinical trials with ddC have demonstrated a painful peripheral neuropathy in patients treated with this drug (Merigan et al., 1989). 2′,3′-Dideoxy-3′-thiacytidine (BCH-189) (Belleau et al., 1989; Soudeyns et al., 1991; Coates et al., 1992), a nucleoside analogue in which the ribose is replaced by a 1,3-oxathiolane ring, has been claimed less toxic than AZT and not cross-resistant to AZT-resistant variants (Schinazi et al., 1991; Jeong et al., 1993). While the human hepatitis B virus (HBV) (Yoffe and Noonan, 1992) is considered as a DNA virus, it replicates via a reverse transcription step (Summers and Mason, 1982) and is therefore susceptible to reverse transcriptase inhibitors. Of significance is that BCH-189 and related compounds are also effective against human hepatitis B virus (HBV) production in the hepatoma cell line HepG2 transfected with HBV DNA (Doong et al., 1991; Chang et al., 1992). Anti-HIV activity was estimated by inhibition of syncytium formation, while the duck hepatitis B virus (DHBV)-infected primary duck hepatocyte cultures were choosen as the most relevant system for the in vitro screening of anti-HBV compounds (Lee et al., 1989; Lavine and Ganem, 1993; Niu et al., 1993; Offensperger et al., 1993). Indeed DHBV, which belongs to the Hepadnavirus family, is very similar to HBV and in standardized conditions (Pugh and Summers, 1989), duck hepatocyte primary cultures allow complete virus replication. Taking into account the results obtained by Rosowsky et al. (1990) concerning AZT-phosphonoformic conjugates, it occured to us that new models consisting of covalently linked BCH-189 and PFA through the phosphoryl group, could be of interest. Starting from the 2′,3′-dideoxy-3′-thiacytidine (BCH-189) we designed new analogues in which the oxygen atom at the 5′-position in the pseudoribose ring was linked to the phosphorous atom of PFA. On the basis of these considerations, we report in this paper the synthesis and the comparative antiviral evaluation (HIV and HBV) of the parent drug BCH-189 (1) and the corresponding BCH-189-PFA conjugates 2 and 3 (Fig. 1).

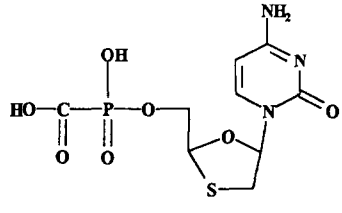
Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AMX-200 (^{1}H-NMR, ^{31}P-NMR, ^{13}C-NMR) spectrometer. Chemical-shifts were expressed in δ values (part per million) relative to tetramethylsilane as an external standard for ^{1}H and relative to H_3PO_4 as an external standard for ^{31}P. FAB$^+$ mass spectra were obtained on a JEOL DX-100 mass spectrometer (Laboratoire de Mesures Physiques, USTL, Montpellier, France) using a cesium ion source and a glycerol/HCl matrix. Elemental microanalyses (Service Central d'Analyse CNRS Vernaison-Lyon France) gave combustion values for C, H, N within 0.4% of the theoretical values. Preparative flash column chromatographies were performed using silica-gel Merck G60 230–240 mesh. It should be pointed out that all the analogues were synthesized starting from the racemic mixture of β-2′,3′-dideoxy-3′-thiacytidine, called BCH-189, which was synthetized according to known procedures (Doong et al., 1991; Belleau et al., 1989; Storer et al., 1993). The phosphorylated intermediate, dichloroethylphosphonoformate, was prepared using a



1
BCH-189



2
2-[[[(Ethoxycarbonyl)phosphonyl]-oxy]-methyl]-5-(cytosin-1'-yl)-1,3-oxathiolane



3
2-[[[(hydroxycarbonyl)hydroxyphosphonyl]-oxy]-methyl]-5-(cytosin-1'-yl)-1,3-oxathiolane

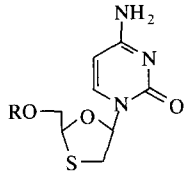
Fig. 1. Structures of the compounds 1, 2 and 3.

described procedure (Hata and Sekine, 1974; Vaghefi et al., 1986). Final conjugate analogues 2 and 3 have been prepared as follows:

Cis-isomers of 2-[[[(Ethoxycarbonyl)phosphonyl]-oxy]-methyl]-5-(cytosin-1'-yl)-1,3-oxathiolane (2).

Dichloroethylphosphonoformate (110 mg, 0.57 mmol, 1.3 eq.) was dissolved in 1 ml of anhydrous DMF. The solution was cooled to 0°C and BCH-189 (100 mg, 0.43 mmol, 1 eq.) was added. The mixture was stirred 1 h at 0°C and then 1 h at room temperature under nitrogen. After a work up, the crude compound was purified by flash chromatography (eluent nBuOH/H_2O (8:1)) to give 90 mg of a pure white solid, in 58% yield. mp: 227°C. ^{1}H-NMR (D_2O) $\delta = 1.16$ (dt, 3H, CH_3, $J^3 = 7.1$ and $J^5 = 0.8$ Hz); 3.12 (dd, 1H, H-4a, $J^2 = 12.2$ and $J^3 = 4.5$ Hz); 3.45 (dd, 2H, H-4b, $J^2 = -12.2$ and $J^3 = 5.4$ Hz); 4.20 (m, 4H, CH_2O and C2-CH_2O); 5.35 (t, 1H, H-2, $J^3 = 3.16$ Hz); 5.95 (d, 1H, CH-5', $J^3 = 7.6$ Hz); 6.23 (pseudo t, 1H, H-5, $J^3 = 4.9$ and $J^3 = 5.0$ Hz); 7.93 (d, 1H, H-6', $J^3 = 7.6$ Hz). ^{31}P-NMR (D_2O) $\delta = -4.80$. ^{13}C-NMR (D_2O) $\delta = 16.03$ (CH_3);

Table 1
Anti-HIV-1 activity of BCH-189 analogues.



N°	R	IC_{50} μmol [a]	TI [c]
2	EtO–C(O)P(O)(OH)–	inactive/toxic	–
3	HO–C(O)P(O)(OH)–	100–50	10
1	H–	0.1 ± 0.05	1000

[a] IC_{50}: Concentration required to inhibit syncytium formation by 50%.
[b] CC_{50}: Concentration required to cause 50% death of uninfected MT4 cells (cytotoxicity)
[c] TI: Therapeutic index, $TI = CC_{50}/IC_{50}$.

39.37 (C-4); 64.57 (CH_2O); 69.03 (C2-CHO_2); 86.58 (C-2); 89.29 (C-5); 98.62 (C-5′); 144.57 (C-6′); 153 (C-2′); 166.42 (C-4′). MS (FAB +): $[M + H]^+ = 366$;. Anal. ($C_{11}H_{16}N_3O_7PS$) C, H, N.

Cis-isomers of 2-[[[(hydroxycarbonyl)hydroxyphosphonyl]-oxy]-methyl]-5-(cytosin-1′-yl)-1,3oxathiolane (3).

Compound 2 (19 mg, 0.051 mmol) was dissolved in 2 ml of water and 285 μl (0.23 mmol, 4.5 eq.) of 0.4 N sodium hydroxide were added (pH 10). The mixture was neutralized with DOWEX (50WX8-100, H^+ form), previously washed with distilled water, filtered and lyophylized to give 10 mg of white solid, 51% yield. mp: 167–169°C. ^{1}H-NMR (D_2O) $\delta = 3.22$ (dd, 1H, H-4a, $J^2 = 12.6$Hz and $J^3 = 3.4$Hz); 3.52 (dd, 1H, H-H4b, $J^2 = 12.6$Hz and $J^3 = 5.4$Hz); 4.16 (m, 2H, C2-CH_2); 5.38 (br t, 1H, H-2); 6.08 (d, 1H, H-5′, $J^3 = 7.8$Hz); 6.25 (pseudo t, 1H, H-5, $J^3 = 4.7$Hz and $J^3 = 4.1$Hz); 8.18 (d, 1H, H-6′, $J^3 = 7.8$Hz). ^{31}P-NMR (D_2O) $\delta = 6.57$; ^{13}C-NMR (D_2O) δ: 37.14 (C-4); 63.92 (C2-CH_2O); 85.47 (C-2); 87.53 (C-5); 95.24 (C-5′); 143.77 (C-6′); 198.42 (C-2′); 178.34 (C-4′); 196.68 (O = C-P = O). Anal. ($C_9H_{12}N_3O_7PS$) C, H, N.

The new compounds were tested for their ability to inhibit HIV-1 infection in cell culture. The assays were performed according to well known procedures previously described (Harada et al., 1985; Rey et al., 1987; Rey et al., 1991). For toxicity testing, cell viability was determined by trypan blue exclusion, as previously described (Coates et al., 1992).

The anti HBV assays were conducted according to procedures previously described (Guillouzou and Guguen-Guillouzou, 1986) for duck hepatocytes culture, and according to Fourel et al. (Fourel et al., 1989) to estimate DHBV production.

As shown on Table 1, both conjugate analogues 2 and 3 appear to be less potent than the parent drug BCH-189 by at least two orders of magnitude. At first, this decrease of potency could be surprising, since one could expect rather similar inhibitory effect of the three drugs on viral replication. Indeed, as already reported (Rosowsky et al., 1990), in the case of AZT-phosphonoformic conjugates, conjugate analogues such as 2 or 3, could inhibit virus replication in MT4 cells via several pathways:

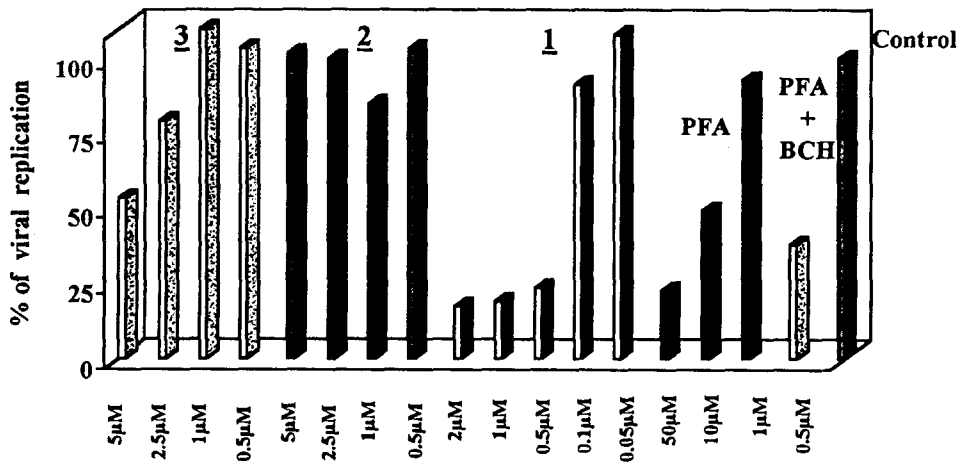


Fig. 2. Inhibition of DHBV replication by BCH-189, PFA and 2 and 3 phosphonate esters. Antiviral activity of BCH-189, PFA and phosphonate esters 2 and 3 against DHBV replication in duck hepatocyte primary cultures. Viral replication was estimated by the amount of DHBV DNA in the supernatant of cell culture in the presence of test compounds and are expressed as percent of that in the control cell cultures.

- direct binding to substrate and PPi sites
- cleavage into PFA and the parent drug BCH-189 during drug metabolism
- decarboxylation and oxidation leading to BCH-189 monophosphate.

However, as the conjugate drugs 2 and 3 have a much lower anti-HIV potency than BCH-189, these routes appear to be rather unlikely. The lower potency may reflect slower uptake into MT4 cells, as well as slow metabolism. Since MT4 cells culture medium contain serum factors which could catalyse the cleavage of the phosphoryl ester function leading to the release of BCH-189, our findings argue against this possibility.

As shown in Fig. 2, BCH-189 strongly inhibited intracellular DHBV DNA synthesis with a 50% inhibitory concentration (IC_{50}) between 0.5 and 0.1 μM. Both conjugate analogues 2 and 3 appeared to be less potent against DHBV than the parent drug BCH-189. Indeed no inhibitory effect of compound 2 was observed even at high concentration (5 μM), while conjugate analogue 3 was a weak inhibitor with an IC_{50} of approximately 5 μM. When PFA and BCH-189 were used together at the same concentration (0.5 μM) inhibition of viral replication was similar to that obtained with BCH-189 alone. As PFA had no clear inhibitory effect at this concentration, synergic effect of both compounds can be excluded.

Our rational approach to the development of drugs for the treatment of HIV-1 and HBV infection in patients is to identify those compounds that specifically inhibit HIV and HBV, because these viruses use both a reverse transcription step for the replication of their viral genome. The activities associated with BCH-189-PFA conjugates 2 and 3 against HIV and HBV were found lower than that of the active antiviral agent BCH-189. Several explanations could account for these results. First, the conjugates may have an intrinsic antiretroviral activity lower than the parent compound. Second, the conjugates may be acting as prodrug thereby releasing the active antiviral agent(s).

If this were the case, the prodrug conjugates should be active, the rate of hydrolysis being the limiting step.

Although these two explanations cannot be entirely ruled out, we believe that drug delivery represents the most likely reason for the lower potency of PFA-BCH-189 conjugates. Indeed previous observations (Camplo et al., 1993a; Camplo et al., 1993b; Kraus, 1993) have clearly shown that some N^4-substituted 2′,3′-dideoxy-3′-thiacytidine conjugates are not active, and, moreover, that partition coefficient of these analogues (Camplo et al., 1994) might have a significant role in their diffusion into the cells. Compounds which have the highest partition coefficient diffuse into the cells to a greater extent than other analogues. Due to the PFA moieties, the polar conjugate analogues 2 and 3 have significantly lower partition coefficients compared to those of the parent compound BCH-189. Hence, they should be less permeable to the cells. Similar observations have been already reported in the case of AZT (Aggarwal et al., 1990). AZT prodrugs incorporating polar species like glutamic acid were found to be less permeable to the cells in comparison to the corresponding analogues carrying less polar moieties such as isoleucine. Because of their polar character, compounds 2 and 3 may enter the cells very poorly. This lack in the delivery properties could account for the weak antiviral potency of these compounds.

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