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Synthesis and antiviral activity of N-4'-dihydropyridinyl and dihydroquinolinylcarbonyl-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane derivatives against human immunodeficiency virus and duck hepatitis B virus

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Summary — Dihydropyridine and dihydroquinoline derivatives of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane ((±)-3TC) have been prepared. The *N*-4-nicotinate or the *N*-4-quinoline-carboxylate amides were obtained by reacting nicotinic or quinoline-carboxylic acids with (±)-3TC in the presence of DCC and HOBT. These derivatives were converted into their corresponding *N*-methylpyridinium and *N*-methyl quinolinium salts by treatment with MeI in acetone. Reduction of the latter with $Na_2S_2O_4$ gave dihydropyridine and dihydroquinoline compounds. The *N*-4-trifluorotoluidinonicotinate derivative was obtained from the coupling of niflumic acid and (±)-3TC using BOP and DIEA. The anti-HIV-1 activities of seven derivatives were determined in MT-4 infected cell cultures. Of these compounds, the IC_{50} values ranged from 0.1–100 μM, while the IC_{50} for (±)-3TC was 0.1 μM. The anti-HBV activities were determined in infected duck hepatocytes. Anti-HBV activities of the (±)-3TC derivatives were half that of the parent drug (±)-3TC. The lipophilicity (partition coefficients) of these compounds were determined. The dihydroquinoline prodrugs had greater lipophilicity than the dihydropyridine analogues.

2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane analogue / pyridinyl derivative / quinolinyl derivative / anti-HIV activity / prodrug / antiviral activity / duck hepatitis B virus

Introduction

The discovery of human immunodeficiency virus (HIV) has stimulated considerable effort in the search for new effective clinically antiviral drugs. The complications resulting from HIV infection of the central nervous system (CNS) are major problems of AIDS, especially in the advanced disease [1–4]. The HIV virus is transported to the CNS by infected macrophages/monocytes where it serves as a major reservoir for infection [5, 6]. Because HIV exhibits tissue tropism for the CNS and macrophages/monocytes in addition to T lymphocytes, the virus is able to cross the brain capillary wall, the blood–brain barrier (BBB) [7]. However, the mechanism of HIV-induced CNS dysfunction remains unclear. This invasion of the nervous system by HIV-1, coupled with the possibility that it could create a reservoir of persistent infection even if peripheral clearance was realized [8], has led to general agreement that an ideal chemotherapeutic agent for HIV should penetrate and be highly active in the CNS [9].

While most nucleosides, as polar molecules, do not readily cross the BBB, 3'-azido-3'-deoxythymidine (AZT, Zidovudine®) has been found in the cerebrospinal fluid of patients on AZT therapy [10, 11]. Moreover, the clinical data appear to be at odds with the results of a study which showed that, in carotid artery-injected rats, AZT is not measurably transported through the BBB [12]. Consequently, the distribution of AZT into brain interstitial fluid, as suggested by Terasaki and Pardridge [12], may be minimal [7].

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Various strategies for increasing drug delivery to the brain have been considered [13]. Among the different reported strategies, the prodrug approach appears to be the most suitable for HIV nucleosides [14]. Bodor and colleagues have achieved brain-specific delivery of dopamine, phenylethylamine *N*-pyridinium-2-carbaldoxime, testosterone, estradiol, tryptamine and other pharmacological agents, by means of a redox delivery system based on the chemistry of a dihydropyridine (DHP)–pyridinium salt interconversion [15–19]. In this system, a biologically-active compound linked to a lipophilic DHP carrier can readily penetrate the BBB. This concept has been investigated for several anti-HIV nucleosides drugs. For example AZT (Zidovudine®, d4T (Stavudine®), AZdU (3'-azido-2',3'-dideoxyuridine) and ddC (Zalcitabine®) have been transformed in vitro into their dihydropyridine derivatives and in vivo studies have also been conducted [7, 14, 20–25]. In vivo studies have shown that, in comparison to the parent drug, significantly greater amounts of AZT, AZdU and d4T could be delivered to the brain following prodrug administration [7, 14, 23–25].

There is a general belief that all anti-HIV drugs which are currently in clinical trials tend to lose their efficiency after six to twelve months of continual use. By combining drugs, there is the potential to extend the period of time for which the drugs are effective. However it is generally assumed that, since none of the combinations tested to date have completely suppressed viral replication, the virus will become resistant to all of them. Therefore it is likely that patients will alternate from one drug combination to another. This alternating will become more rapid and prominent as the viral load measures become more widely used in routine practice.

Recently it has been reported [26] that mutants resistant to AZT became phenotypically sensitive in vitro by mutation of the residue 184 of the viral reverse transcriptase to valine, which also induced resistance to (–)-β-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (3TC). Furthermore AZT-3TC coresistance was not observed during the extensive in vitro selection with both drugs. In vivo AZT-3TC combination therapy resulted in a more marked decrease in serum HIV-1 RNA concentration than treatment with AZT alone, even though valine-184 mutants rapidly emerged. Most samples assessed from the combination group remained AZT-sensitive at 24 weeks of therapy, consistent with in vitro mutation studies.

Accounting for the above considerations, it appears that AZT-3TC represents one of the most useful combination. AZT-3TC combinations may lead to a better response rate for both drugs. These two drugs could have complementary activities in different types of cells. Indeed, some drugs work better in chronically-infected cells, whereas others, like AZT, work better in acutely-infected cells. Since HIV is known to produce neuropsychiatric deficits in AIDS patients, the drugs must be able to penetrate into the brain. There is evidence that AZT can penetrate cerebrospinal fluid [11]. In contrast ddI, ddC [27] and 3TC do not seem to penetrate the BBB to any significant extent.

Torrence et al [20, 28] have reported the synthesis of dihydropyridine esters of AZT and ddC which bear the 1-4-dihydro-1-methyl-3-pyridinylcarbonyl moiety at both the cytidine exocyclic amino moiety and the sugar 2-hydroxymethyl function. According to these authors it appears that by applying the Bodor redox drug delivery system to dihydropyridine ddC derivatives, there is no significant increase of brain exposure to ddC after prodrug administration. However, with dihydropyridine AZT derivatives, a significant increase in brain exposure to AZT was possible. The instability observed for the redox prodrug forms of ddC may partly explain the failure of this particular prodrug approach. The 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane nucleoside analogue represents one of the most potent dideoxynucleosides [26]. However, based on the measurements of cerebrospinal fluid concentration in the CNS, this nucleoside seems to be less effective than AZT.

In this study we have investigated different strategies to achieve the synthesis of dihydropyridinyl derivatives of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane, including a niflumyl derivative. We have extended this methodology to the synthesis of the corresponding dihydroquinolinyl analogues. Determination of the partition coefficient of these newly synthesized compounds according to Hansch's method [29] confirmed the greater lipophilicity of dihydroquinoline-carbonyl prodrugs of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane compared to that of dihydropyridine-carbonyl analogues. Since it has been postulated that a lipophilic enhancement of compounds having a molecular weight less than 400 would improve brain permeability [30], these new dihydroquinoline-carbonyl derivatives of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane could be of pharmaceutical interest. Anti-HIV and anti-HBV activities of both series of analogues are determined and discussed.

Results and discussion

Chemistry

One potential approach to provide 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane with a dihydropyridine or dihydroquinoline redox system would be to

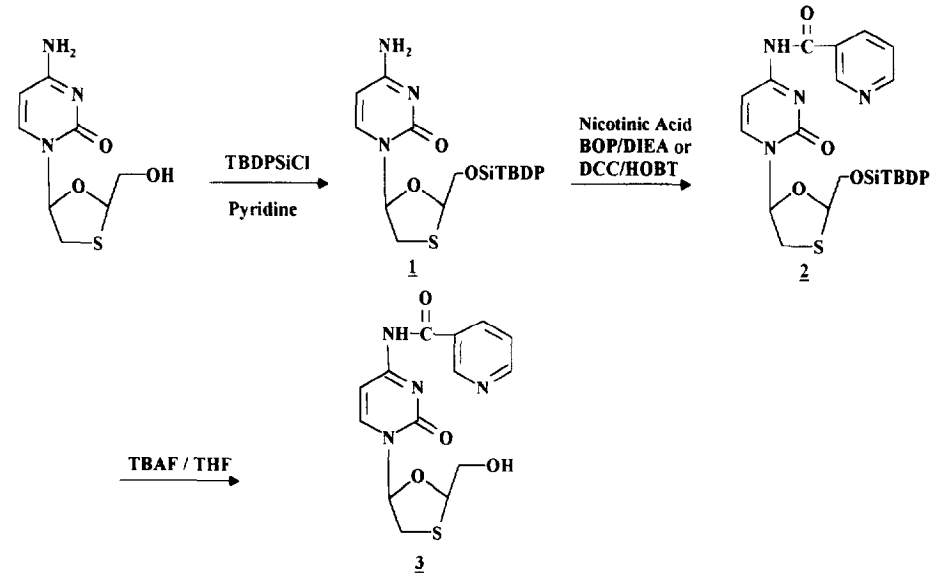
synthesize nicotinate or quinolinate ester at the C_2-hydroxymethyl position of the oxathiolane ring and/or synthesize the corresponding *N*-4'-acylated derivatives [31] Our group has shown that the anti-HIV activity of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane analogues resulting from the substitution at the *N*-4' position of the cytosine base by an active anti-HIV protease peptide, was greater than the corresponding C_2-hydroxymethyl esters [32]. Taking into account this observation, *N*-4'-dihydronicotinyl and *N*-4'-dihydroquinoline-carbonyl-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane **5** and **8** were selected as the final targets.

Target compounds **5** and **8** were synthesized following the general approach summarized in scheme 1. The synthesis of the key nucleoside 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (±)-3TC was achieved according to a procedure reported previously [33–35]. It should be emphasized that when the nucleoside 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane was coupled to nicotinic acid or 3-quinoline carboxylic acid using DCC (dicyclohexylcarbodiimide) in DMF in the presence of HOBT (1-hydroxy-benzotriazole), only the corresponding *N*-4' acylated derivatives **3** and **6** were isolated in reasonable yields. This result was confirmed by reacting 2-(*tert*-butyldiphenylsilyloxymethyl)-5-(cytosine-1'-yl)-1,3-oxathiolane and nicotinic acid, in the presence of DCC and HOBT (scheme 1). Deprotection of the silylated group with tetrabutylammonium fluoride gave the same *N*-4' acylated derivative **3**. When niflumic acid was coupled with 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane in the presence of BOP and DIEA in DMF, only the *N*-4' derivative **9** was obtained. In contrast, when nicotinic acid methyliodide was coupled to dideoxycytidine (ddC) in the presence of DCC, Torrence et al [28] observed exclusively the formation of *N*-4' and *O*-disubstituted ddC trigonellate intermediates.

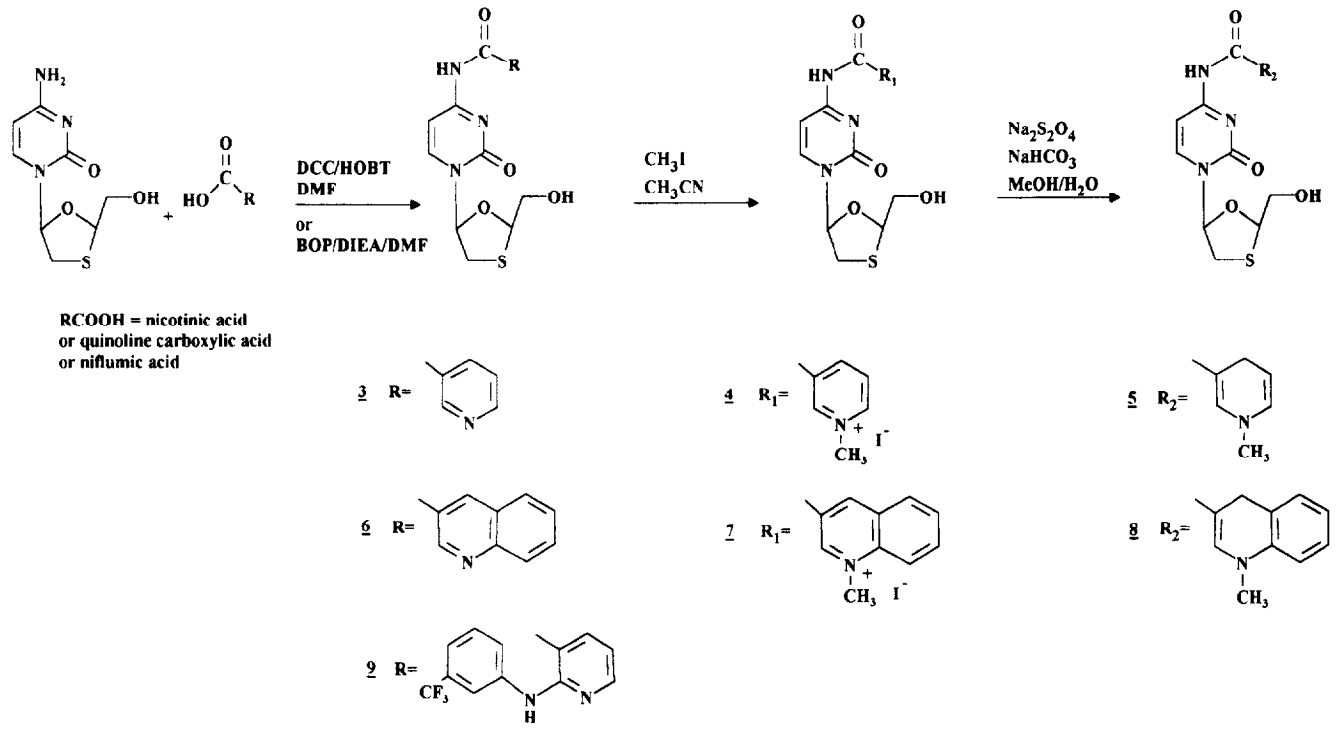
Compounds **3** and **6** were transformed into their methiodide forms **4** and **7** by treatment with iodomethane in acetonitrile. Due to the presence of the amine function in the trifluoro-toluidino-nicotinyl group, **9** was not submitted to the next reactions of the synthetic scheme 2. Reduction of the quaternized species, with sodium dithionite gave the dihydropyridinyl **5** and dihydroquinolinyl **8** derivatives. These latters were reasonably stable considering their dihydropyridine- and dihydroquinoline-like nature. They remain unchanged when stored at 0 °C in an aqueous DMSO solution for 48 h.

Partition coefficient measurements

Lipophilic non-ionic low molecular weight materials generally appear to have the best passive diffusion properties in the CNS [36]. Hansch and coworkers have pioneered the use of the octanol/water partition coefficient (log *P*) to correlate compound structure with CNS penetration [29]. Log *P* found for AZT (0.05) [37] indicates that AZT is neither lipophilic nor hydrophilic. In contrast, ddC (log *P* = –1.33) was



Scheme 1.



Scheme 2.

found to be more hydrophilic. AZT is one of the most lipophilic compound investigated clinically. It enters the CNS better than ddC. The most uncertain part of the problem is the retention of the anti-HIV activity when appropriate modifications have been realized.

Since the activity of dideoxynucleosides is critically dependent on a series of enzymatic events, any one of these might be adversely affected by a structural change. Therefore, the partition coefficients of the newly synthesised *N*-4'-amides were first determined by equilibrating their solution in *n*-octanol with phosphate buffer (0.2 M, pH = 7.4) at room temperature according to the procedure published by Fujita et al [38]. The data published in table I indicate that, as expected, *N*-4'-dihydro-nicotinyl **5** and *N*-4'-dihydro-quinoline-carbonyl **8** derivatives were the most lipophilic compounds. Their lipophilicities were approximately 200 times greater than that of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane. In contrast, the corresponding methyl-pyridinium iodide salts **4** and **7** are 3.5-times less lipophilic than 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane.

Anti-HIV activity in vitro

2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane, its nicotinyl and quinolinecarbonyl counterparts **3**, **4**, **5**, **6**, **7** and **8** were evaluated against HIV-1 induced cytopathogenicity in MT-4 cells. The antiviral activity was expressed as the concentration (μM) that inhibits viral replication by 50% (table I). The assays were performed according to known procedure previously described [39, 40].

Compounds **3**, **4** and **5**, which belong to the nicotinyl family, were approximately as active against HIV-1 as the corresponding parent drug 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane. This result could be related to published results revealing that the anti-HIV activities of nicotinyl analogues of AZT [20] and ddC [28] were roughly similar to those of their corresponding parent drugs. Therefore all three derivatives **3**, **4** and **5** could be transformed to the parent drug 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane in cell culture. It should be underlined that the trifluorotoluidinonicotinyl analogue **9** was significantly less

active (IC_{50} = 10 μM) than its nicotinyl congener **3** (IC_{50} = 0.1–1μM). The same phenomenon was observed for quinolinyl 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane congeners. The increased lipophilicity of these derivatives appears to be detrimental to their antiviral activity.

For some incomprehensible reasons, in cell culture, the quinolinyl series of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane seemed to be more resistant to hydrolysis into the parent drug than the pyridinyl analogues. We have already observed that others *N*-4'-acyl-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane, such as *N*-4'-retinoyl [41] or *N*-4'-acetyl [42] derivatives, were significantly less active against HIV-1 than the parent drug (±)-3TC. This observed decrease in anti-HIV activity was attributed to the greater stability of these 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane nucleoside conjugates to plasma enzymes. Moreover from the cytoxicity results reported in table I, apart from the trifluorotoluidinonicotinyl analogue **9**, *N*-4'-nicotinyl or *N*-4'-quinolinecarbonyl-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane derivatives exhibit very low toxicities on MT-4 cells. At the highest concentration tested (200 μM), only compound **7** displayed a detectable alteration of uninfected cells after a seven-day incubation period. In contrast, under the same experimental conditions, the other tested compounds **3**, **4**, **5**, **6** and **8** and the parent drug 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane showed no cytotoxicity at 200 μM.

Anti-HBV activity in vitro

Both series of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane derivatives were also tested for their ability to inhibit DHBV infection in cell culture. The anti-HBV assays were conducted according to procedure previously described for duck hepatocytes cultures [43, 44] to estimate DHBV DNA production. Since the cytosine derivatives exhibit in general, more potent anti-HBV activity than the other analogues [45–48], the anti-HBV evaluation of these new derivatives was of interest. The results reported in table I showed that the reference compound was the most active inhibitor of DHBV replication. In contrast, the whole congeners *N*-4'-nicotinyl and *N*-4'-quinolinecarbonyl-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane elicited anti-DHBV activity half that of the parent drug 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane. Moreover, *N*-4'-nicotinyl candidates and their *N*-4'-quinolinyl counterparts showed identical anti-DHBV activities under the in vitro testing conditions. This observation suggests that during drug treatment and infection of cultured hepatocytes, the *N*-4'-substituted drugs, *N*-4'-nicotinyl as well as *N*-4'-quinolinyl, were converted into the parent drug (±)-3TC. It should be underlined that the observed effect of compound **9** in DHBV DNA synthesis was long-lasting even if the formation of CCC-DNA (covalently closed DNA) from virion would presumably have occurred. Due to its long-lasting effect, the trifluoro-toluidinonicotinyl analogue **9** will be submitted to in vivo studies on Peking ducklings.

In conclusion, we have achieved the synthesis of new *N*-4'-dihydropyridinyl and dihydroquinolinylcarbonyl-2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane derivatives. These new derivatives appear to be as active as the parent drug (2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane) on both viral strain HIV-1 and DHBV in vitro. These results should stimulate further studies, mainly in order to explore the possibility that, through a dihydropyridine/pyridinium redox delivery system, a significant brain uptake enhancement of compounds **5** and **8** may be observed.

Experimental protocols

Chemistry

Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AMX-200 (^{1}H NMR, ^{13}C NMR) spectrometer. Chemical shifts were expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard for ^{1}H. FAB$^+$ mass spectra were performed by R Astier on a JEOL

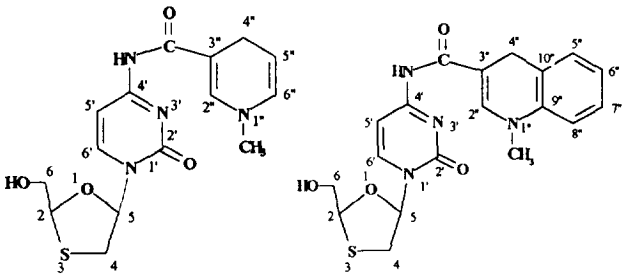
Table I. Physical and biological properties of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane derivatives.

Compound	*log P*[e]	*HIV-1 IC_{50}*[a] *(μM)*	*HIV-1 MCD*[b] *(μM)*	*DHBV DNA IC_{50}*[c] *(μM)*
1	–0.37	0.1–1	≥200	2 ± 0.5
2	≤–2	0.1–1	≥200	2 ± 0.5
3	+0.84	0.05–0.1	≥200	2 ± 0.5 (30%)[d]
4	+0.27	10 ± 5	≥200	2 ± 0.5
5	≤–2	≥100	≥100	2 ± 0.5
6	+0.87	5 ± 2	≥200	2 ± 0.5
7	–0.87	4 ± 3	≥ 50	2 ± 0.5
(±)-3TC	–1.45	0.1–1	≥200	1 ± 0.5

[a]IC_{50} values represent the drug concentration (μM) required to inhibit 50% of HIV-1 replication in MT-4 cells; [b]minimum cytoxic dose or dose required to cause a microscopically-detectable alteration of normal MT-4 cells morphology; [c]such concentration caused a 50% reduction of DHBV DNA in the supernatant of cell culture medium; [d]in this case, the observed reduction of DHBV DNA in the supernatant of cell culture was 30%; [e]log *P* values have been determined according to Hansch's procedure [29].

DX-100 mass spectrometer (Laboratoire de mesures physiques-RMN, USTL, Montpellier, France) using a cesium ion source and a glycerol/HCl matrix. Infrared spectra were obtained using a 1605 FT-IR (Perkin Elmer) spectrophotometer. Elemental microanalyses were determined by the Service central d'analyse, CNRS, Vernaison-Lyon, France, and gave combustion values for C, H, N within 0.4% of the theoretical values. Preparative flash column chromatography [49] was performed using silicagel Merck G60 230-240 mesh. Analytical thin-layer chromatography was performed on silicagel plates 60F 254 aluminium (Merck, Darmstadt) 0.2 mm thickness. The synthesis of the key intermediate 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (±)-3TC was performed according to a procedure reported previously [33, 50].

The numeration of the compounds is as follows:



*Synthesis of cis isomers of 2-(hydroxymethyl)-5-[N-4'-(3"-nicotinyl) cytosine-1'-yl]-1,3-oxathiolane **3** (scheme 1)*

*2-(tert-Butyldiphenylsilyloxymethyl)-5-(cytosine-1'-yl)-1,3-oxathiolane **1**.* To a solution of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (105 mg, 0.45 mmol) in pyridine (6 mL) was added *tert*-butyldiphenylsilyl chloride (TBDPSiCl) (140 µL, 0.5 mmol) under N_2 atmosphere. The mixture was stirred for 24 h at room temperature and then evaporated, washed with water (30 mL) and extracted with ethyl acetate (3 × 20 mL). Organic phases were dried over Na_2SO_4 and concentrated to give a white solid which was recrystallized in EtOAc (quantitative yield). ^{1}H NMR ($CDCl_3$) δ = 1.09 (s, 9H, *t*Bu), 3.2 (dd, 1H, H-4, *J* = 12.4 and 2.9 Hz), 3.5 (dd, 1H, H-4, *J* = 12.4 and 4.4 Hz), 4.08 (dd, 2H, 2H-6), 5.25 (t, 1H, H-2, *J* = 6 Hz), 5.51 (d, 1H, H-5', *J* = 7.6 Hz), 6.37 (dd, 1H, H-5, *J* = 5.4 and 2.9 Hz), 7.44 (m, 5H, arom), 7.69 (m, 5H, arom), 8.04 (d, 1H, H-6', J= 7.6 Hz).

*2-(tert-Butyldiphenylsilyloxymethyl)-5-[N-4'-(3"-nicotinyl) cytosine-1'-yl]-1,3-oxathiolane **2**.* Nicotinic acid (27 mg, 0.21 mmol), BOP (93 mg, 0.23 mmol), compound **1** (39 mg, 0.23 mmol), and DIEA (146 µL, 0.84 mmol) were stirred in a mixture of dichloromethane (7 mL) and dimethylformamide (2 mL) overnight under N_2 atmosphere at room temperature. The mixture was then washed with a 5% citric acid solution (10 mL), a 5% aqueous solution of sodium bicarbonate (10 mL), extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, evaporated and purified by preparative layer chromatography to give the desired compound (48 mg, 0.09 mmol, 39%). ^{1}H NMR ($CDCl_3$) δ = 1.1 (s, 9H, *t*Bu), 3.1–3.6 (m, 3H, H_2-6), 3.7–4.2 (m, 2H, H_2-4), 5.25 (t, 1H, H-5), 5.5 (d, 1H, H-5'), 6.35 (q, 1H, H-2), 7.4–7.8 (m, 10H, arom.), 7.6 (d, 1H, nicotinyl), 8.0 (d, 1H, H-6'), 8.3 (d, 1H, nicotinyl), 8.8 (q, 1H, nicotinyl), 9.1 (d, 1H, nicotinyl).

*2-(Hydroxymethyl)-5-[N-4'-(3"-nicotinyl)cytosine-1'-yl]-1,3-oxathiolane **3**. Method 1.* Compound **2** (25 mg, 0.05 mmol) was dissolved in anhydrous tetrahydrofuran (3 mL) and tetrabutylammonium fluoride (1 M in THF) was added (135 µL, 0.15 mmol). The mixture was stirred for 3 h under N_2 atmosphere at room temperature. After solvent evaporation, the crude compound was purified by preparative layer chromatography (eluent toluene/MeOH, 15%) to give the desired compound (13 mg, 0.04 mmol, 78%). ^{1}H NMR (DMSO-d_6) δ = 3.27 (dd, 1H, H-4, *J* = 12.2 and 2.8 Hz), 3.62 (dd, 1H, H-4, *J* = 12.2 and 5.4 Hz), 3.87 (d, 2H, H_2-6), 5.32 (t, 1H, OH), 5.49 (t, 1H, H-2, *J* = 5.6 Hz), 6.28 (dd, 1H, H-5, *J* = 5.0 and 2.8 Hz); 7.35 (d, 1H, H-5', *J* = 7.5 Hz), 7.56 (dd, 1H, H-5", *J* = 7.8 and 4.9 Hz), 8.35 (d, 1H, H-6", *J* = 7.9 Hz), 8.51 (d, 1H, H-6', *J* = 7.5 Hz), 8.78 (d, 1H, H-4", *J* = 3.7 Hz), 9.12 (s, 1H, H-2"). ^{13}C NMR (DMSO-d_6) δ = 38.2 (C-4), 61.8 (C-6), 87.1 (C-2), 88.2 (C-5), 95.7 (C-5'), 123.4 (C-5"), 128.5 (C-3"), 136.2 (C-4"), 141.2 (C-6'), 149.9 (C-6"), 152.9 (C-2"), 154.0 (C-2'), 163 (C-4'), 165.6 (CO). Analysis (C, H, N).

*2-(Hydroxymethyl)-5-[N-4'-(3"-nicotinyl)cytosine-1'-yl]-1,3-oxathiolane **3**. Method 2.* Compound **3** was also prepared directly according to scheme 2:

In anhydrous DMF, nicotinic acid (112 mg, 0.87 mmol), DCC (200 mg, 0.96 mmol) and HOBT (130 mg, 0.96 mol) were stirred for 1 h under N_2 atmosphere at room temperature. 2-Hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (200 mg, 0.87 mmol) was then added and the mixture stirred overnight. DMF was evaporated under reduced pressure, brine was added and the product extracted three times with EtOAc. Organic phases were washed with brine, dried over Na_2SO_4, filtered and evaporated. The crude product was purified by flash chromatography (eluent MeOH/CH_2Cl_2, 2–5%) to give a white solid (55 mg, 0.16 mmol, 19%).

*cis Isomers of 2-(hydroxymethyl)-5-[N-4'-(1"-methyl-3"-nicotinyl) cytosine-1'-yl]-1,3-oxathiolane iodide **4***

In anhydrous acetonitrile (4 mL) compound **3** (46 mg, 0.14 mmol) and methyliodide (77 µl, 1.25 mmol) were heated for 24 h at 40 °C under N_2 atmosphere. Solvent was evaporated and the obtained residue purified by flash chromatography (eluent *n*BuOH/H_2O/AcOH, 5:2.5:2.5). A yellow solid was obtained (55 mg, 0.11 mmol, 83%). ^{1}H NMR (DMSO-d_6) δ = 2.6 (s, 3H, CH_3-1"), 3.3 (dd, 1H, H-4, *J* = 12.2 and 4.9 Hz), 3.6 (dd, 1H, H-4, *J* = 12.2 and 10.8 Hz), 3.9 (dd, 2H, H_2-6), 5.20 (dd, 1H, H-2), 6.22 (dd, 1H, H-5), 6.92 (d, 1H, H-5', *J* = 7.4 Hz), 7.35 (m, 1H, H-6"), 7.84 (d, 1H, H-5", *J* = 7.4 Hz), 8.02 (d, 1H, H-6', *J* = 7.4 Hz), 9.00 (br s, 1H, H-4"), 9.46 (s, 1H, H-2"). ^{13}C NMR (DMSO-d_6) δ = 21.8 (CH_3), 36.2 (C-4), 62.5 (C-6), 85.8 (C-2), 86.8 (C-5), 93.9 (C-5'), 126.2 (C-5"), 127.4 (C-3"), 138.2 (C-4"), 140.9 (C-6'), 143.6 (C-6"), 145.9 (C-2"), 155.0 (C-2'), 166.1 (C-4'), 172.6 (CO). MS: (FAB$^+$) 349 $[M-I]^+$. Analysis (C, H, N).

*cis Isomers of 2-(hydroxymethyl)-5-[N-4'-(1"-methyl-1",4"-dihydro-3"-nicotinyl) cytosine-1'-yl]-1,3-oxathiolane **5***

Compound **4** (30 mg, 0.063 mmol) was dissolved in 3 mL of a degassed MeOH solution containing 10% water. Sodium bicarbonate (15 mg) and sodium dithionite (60 mg) were added and the mixture was stirred for 3 h under N_2 atmosphere at room temperature. The solvent was evaporated and the crude product purified by preparative layer chromatography (eluent toluene/MeOH, 5:5) to give a solid (7 mg, 0.018 mmol, 30%). ^{1}H NMR (DMSO-d_6) δ = 3.2 (m, 5H, CH_3-1" and H-4"), 3.3 (dd, 1H, H-4), 3.6 (dd, 1H, H-4), 3.9 (dd, 2H, H-6), 5.2 (dd, 1H, H-2), 6.2 (dd, 1H, H-5), 7.0 (d, 1H, H-5'), 7.2 (m, 1H, H-6'), 7.8 (d, 1H, H-5"), 8.0 (d, 1H, H-6"), 9.2 (s, 1H, H-2"). MS: (FAB$^+$) 350 $[M+H]^+$. Analysis (C, H, N).

cis Isomers of 2-(hydroxymethyl-5-[N-4'-(3"-(quinolinecarbonyl)cytosin-1'-yl]-1,3-oxathiolane ***6***

In anhydrous dimethylformamide (2 mL), 3-quinolinecarboxylic acid (74 mg, 0.43 mmol), 1,3-dicyclohexylcarbodiimide (96 mg, 0.47 mmol) and hydroxybenzotriazole hydrate (63.4 mg, 0.47 mmol) were added. The mixture was stirred for 1 h at room temperature under nitrogen. When a white precipitate of dicyclohexylurea appeared, 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (100 mg, 0.43 mmol) was added. The mixture was left stirring overnight. DMF was then evaporated under reduced pressure, the residue washed with brine (10 mL) and extracted twice with EtOAc (2 × 10 mL). The organic phases were combined, dried over $MgSO_4$, filtered and the solvent evaporated. The crude product was purified by flash chromatography (eluent EtOAc/MeOH, 98:2) to give a white solid (78 mg, 0.20 mmol, 46%). mp: 116–118 °C. IR: (KBr) ν =1656 cm^{-1} (C=O amide). ^1H NMR (DMSO-d_6) δ = 3.55 (dd, 2H, H_2-4), 3.98 (t, 2H, H_2-6), 5.55 (t, 1H, H-2), 6.35 (t, 1H, H-5), 7.45 (d, 1H, H-5'), 7.80 (m, 1H, H-7"), 8.00 (m, 1H, H-6"), 8.20 (m, 2H, H-8" and H-5"), 8.65 (d, 1H, H-6'), 9.15 (d, 1H, H-4"), 9.40 (d, 1H, H-2"), 11.75 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ = 37.14 (C-4); 61.95 (C-6); 87.28 (C-2); 88.29 (C-5); 95.85 (C-5'); 122.21 (C-3"); 126.28 (C-6"); 127.71 (C-5"); 129.63 (C-10"); 129.87 (C-7"); 132.08 (C-8"); 137.65 (C-4"); 138.89 (C-6'); 148.91 (C-9"); 149.29 (C-2"); 163.16 (C-2'); 164.51 (C-4'), 170.20 (CO). MS: (FAB$^+$) 385 $[M+H]^+$. Analysis (C, H, N).

cis Isomers of 2-(hydroxymethyl)-5-[N-4'-(1"-methyl-3"-quinolinecarbonyl)cytosin-1'-yl]-1,3-oxathiolane iodide ***7***

Compound **6** (78 mg, 0.20 mmol) and methyl iodide (112 µL, 1.8 mmol) were mixed in acetonitrile (4 mL) and the solution heated at 40 °C for 24 h under nitrogen. The solvent was evaporated, the crude compound dissolved in a minimum of acetonitrile and precipitated with ether to give an orange solid (60 mg, 55%). mp: 156–159 °C. ^1H NMR ($CDCl_3$) δ = 3.56 (dd, 1H, H-4, J = 12.5 and 5.5 Hz), 3.68 (dd, 1H, H-4, J = 12.5 and 5.3 Hz), 3.95 (dd, 1H, H-6, J = 13.0 and 3.5 Hz), 4.10 (dd, 1H, H-6, J = 13.0 and 3.1 Hz), 4.80 (s, 3H, CH_3-1"), 5.38 (dd, 1H, H-2, J = 3.5 and 3.1 Hz), 5.99 (d, 1H, H-5', J = 7.6 Hz), 6.33 (m, 1H, H-5), 8.13 (m, 1H, H-7"), 8.20 (d, 1H, H-5"), 8.39 (m, 1H, H-6'), 8.60 (m, 1H, H-8"), 8.72 (d, 1H, H-6", J = 7.6 Hz), 9.79 (d, 1H, H-4", J = 2.1 Hz), 9.95 (d, 1H, H-2", J = 2.1 Hz). MS: (FAB$^+$) 399 $[M-1]^+$, 799 $[2(M-1)]^+$. Analysis (C, H, N).

cis Isomers of 2-(hydroxymethyl)-5-[N-4'-(1"-methyl-1",4"-dihydro-3"-quinolinecarbonyl)-cytosin-1'-yl]-1,3-oxathiolane ***8***

To an aqueous degassed solution of MeOH (3 mL, 10% H_2O) were added compound **5** (30 mg, 0.06 mmol), sodium bicarbonate (15 mg) and sodium dithionite (60 mg). The mixture was stirred for 1 h under nitrogen. The solvent was evaporated and the crude compound purified by preparative layer chromatography (eluent EtOAc/MeOH, 1:1). A yellow glassy solid was obtained (10 mg, 46%). ^1H NMR (D_2O) δ = 3.24 (m, 5H, CH_3-1" and H_2-4"), 3.48 (m, 2H, H_2-4), 3.93 (m, 2H, H-6), 5.29 (pseudo t, 1H, H-2, J = 3.8 and 3.5 Hz), 6.11 (dd, 1H, H-5, J = 5.3 and 2.9 Hz), 6.96 (d, 1H, H-5', J = 7.5 Hz), 7.13 (m, 2H, H-6" and H-7"), 7.27 (m, 2H, H-5" and H-8"), 7.45 (d, 1H, H-2", J = 4.7 Hz), 8.23 (d, 1H, H-6', J = 7.5 Hz). MS: (FAB$^+$) 400 $[M-1]^+$. Analysis (C, H, N).

cis Isomers of 2-hydroxymethyl-5-[N-4'- (2-(α,α,α-trifluoro-m-toluidino) nicotinyl)cytosin-1'-yl]-1,3-oxathiolane ***9***

In anhydrous DMF (5 mL), 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane (50 mg, 0.2 mmol), benzotriazol-1-yl-oxy-hexafluorophosphate tris(dimethylamino)phosphonium (BOP, 93 mg, 0.23 mmol) and diisopropylethylamine (DIEA, 146 µL, 0.84 mmol) were added. The mixture was stirred overnight at room temperature, washed with a 5% citric acid solution (10 mL) and then washed with a 5% sodium bicarbonate aqueous solution (10 mL). The resulting mixture was extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (eluent EtOAc/toluene, 1:9) to give a white solid (49 mg, 48% yield). ^1H NMR ($CDCl_3$) δ = 3.1 (dd, 1H, H-4), 3.6 (dd, 1H, H-4), 4.7 (m, 2H, H_2-6), 5.6 (t, 1H, H-6'), 6.3 (t, 1H, H-2), 7.0 (m, 1H, arom), 7.1–7.7 (m, 4H, arom), 7.8 (d, 1H, H-5'), 7.9 (m, 1H, arom), 8.3 (m, 1H, arom), 10.1 (s, 1H, NH). MS: (FAB$^+$) 494 $[M+1]^+$. Analysis (C, H, N).

Pharmacology

Anti-HIV-1 activity in vitro

Representative compounds were tested for their ability to inhibit HIV-1 infection in cell culture. The fusogenic effect of HIV in the MT_4 cell line [39] was determined as described by Rey et al [40, 51]. A total of 3 × 10^5 MT_4 cells was infected with 100 µL of diluted virus for 1 h at 37 °C. After three washes, the infected cells were cultured in 24-well cell-culture plates in the presence of the inhibitor. The appearance of syncytia was measured with an inverted optical microscope five days after infection. The inhibitory concentration was expressed as the concentration of the tested compound which causec 50% inhibition of syncytia formation (IC_{50}) but was not toxic for the cells. For toxicity testing, three replication cultures of all uninfected MT_4 cells (2 × 10^5 cells) were incubated with various concentrations of 2-hydroxymethyl-5-[cytosin-1'-yl]-1,3-oxathiolane analogues. Cell viability was determined by trypan blue exclusion six days after drug addition.

Anti-HBV activity in vitro

Three-week old ducklings chronically infected by duck hepatitis B virus (DHBV) were used for the preparation of fresh hepatocytes. The ducks were killed under anesthesia and the isolation of the hepatocytes was carried out by in situ two-step collagenase perfusion of the liver [44]. At the end of the perfusion, the cell suspension was filtered through gauze and enriched by three differential centrifugations. Cells were suspended in Leibowitz medium supplemented with 5 µg per mL of bovine insulin, 7 × 10^{-5} M hydrocortisone hemisuccinate and 10% FCS. The cell density was 5 × 10^6 cells plated in 100 × 20 mm tissue culture dishes for duck hepatocyte cultures. Medium was changed every day and saved for further testing. Cultures were treated with antiviral compounds for ten days. DHBV DNA was detected by a DNA-spot hybridization as previously described [44]. To quantify viral DNA, 0.8 mL of culture medium and cloned DHBV DNA ranging from 100–0.1 pg were spotted in duplicate onto nitrocellulose filters. After denaturation and neutralization, filters were hybridized with a specific DHBV DNA probe and liquid-scintillation counting of each individual spot was performed.

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