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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2459–2463

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Are 5′-*O*-Carbamate-2′,3′-dideoxythiacytidine New Anti-HIV and Anti-HBV Nucleoside Drugs or Prodrugs?

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Received 3 April 2003; revised 14 May 2003; accepted 15 May 2003

Abstract—In contrast to 5′-*O*-carbonate 3TC derivatives (**23**, **24**), which are clearly 3TC prodrugs, the corresponding 3TC carbamates (**15**–**21** and **25**), found to be very stable compounds with respect to enzymatic hydrolysis (cellular lysates and culture cell media) and still active on both HIV-1 and HBV infected cells, may not be 3TC prodrugs. The antiviral properties as well as the mechanism of action of 3TC analogues have been studied and evaluated.

Most of the nucleoside prodrugs described so far were designed for various purposes: to decrease toxicity associated with nucleoside drugs, to release active species by the degradative metabolism of the prodrugs, and to allow higher quantities of drug to enter into the cell. For this last purpose lipophilic chemical moieties were linked to the 5′-*O* position of the nucleoside through enzymatically hydrolysable functions, such as ester[1] or carbonate bonds.[2,3] Although some 5′-*O*-carbamoyl-2′,3′-dideoxynucleoside derivatives were reported inactive as antiviral compounds because of the enzymatic stability of the carbamate bond,[4] recently various amino-acids coupled to AZT through 5′-*O*-carbamate bonds have been found to be active in HIV-1 infected PBMCs (peripheral blood mononuclear cells).[5] Furthermore, their antiviral activities were suggested to be unrelated to the release of AZT in the cell, suggesting that these 5′-*O*-carbamate analogues may act through an original pathway.[2,5]

To date only (−)-2′-deoxy-3′-thiacytidine or 3TC (Fig. 1) has been licensed as both an anti-HBV and an anti-HIV drug (Epivir® against HIV infection, and Zeffix® as anti-hepatitis drug), so we have synthesized new 3TC carbamate analogues (Fig. 2). In this paper we report the intriguing in vitro antiviral properties (HIV and HBV) associated with this new class of nucleosides, their enzymatic stabilities, and some preliminary studies on their mechanism of action in comparison with their corresponding carbonate analogues.

The syntheses of 3TC carbamate and carbonate analogues are given in Scheme 1. The 3TC protected

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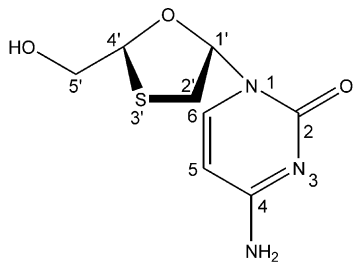
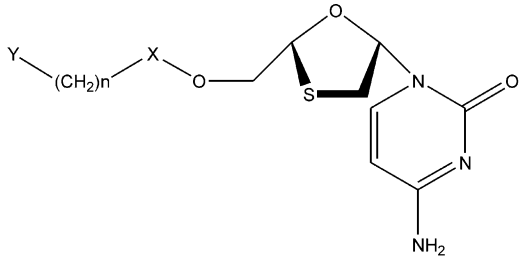
doi:10.1016/S0960-894X(03)00496-7



3TC, Epivir® (HIV) or Zeffix® (HBV)

Figure 1. Structure of 3TC, the only nucleoside drug licensed for HIV as well as HBV treatment.



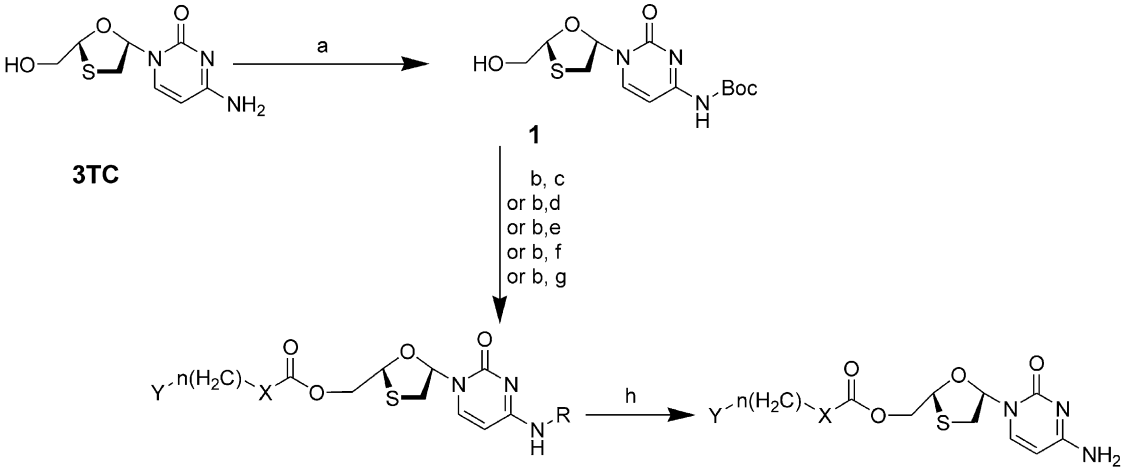
where, **X:** -NH-C(O)-, -O-C(O)-
Y: -NH_2, -OH, -CH_3, -P(O)-$(OEt)_2$, -P(O)-$(OH)_2$
n : 1 to 6

Figure 2. Structure of carbamate and carbonate analogues of 3TC.

nucleosides were treated with phosgene[6–8] in dichloromethane (DCM) at room temperature in the presence of diisopropylethylamine (DIEA) for several hours and then selected ω-functionalised primary amines were condensed. The yield of the corresponding 5′-*O*-3TC-carbamates ranged from 40 to 70%. The deprotection of *N*-Boc (*tert*-butyloxycarbonyl) group by trifluoroacetic acid (TFA) in DCM led to the final compounds in quantitative yields.

In parallel, we also accessed the 5′-*O*-carbamates through the use of ω-aminoalkyl-5′-*O*-3TC-carbonates. As shown in Figure 3, we demonstrated for the first time that 3TC-carbonate derivatives (**23** and **24**) could rearrange into the corresponding 5′-*O*-3TC carbamates through the intramolecular nucleophilic attack of the ω-amino group on the 5′-*O*-carbonate function. We found that ω-aminoalkyl-5′-*O*-3TC-carbonates rearranged partially into their corresponding carbamates in biological media (extra and intracellular media) as indicated in studies on stability and lipophilicity, but are chemically stable enough to be isolated in the experimental conditions of synthesis.

5′-*O*-3TC-carbamate drugs (compounds **15** to **22** and **25**) showed a less pronounced activity to inhibit the cytopathicity of HIV after MT-4[9] cells and PBMCs[10] acute infection, compared to that of the parent drug



5: X = -NH-, Y = -NH-Boc, n = 3, R = -Boc
6: X = -NH-, Y = -NH-Boc, n = 4, R = -Boc
7: X = -NH-, Y = -OH, n = 3, R = -Boc
8: X = -NH-, Y = -OH, n = 4, R = -Boc
9: X = -NH-, Y = -OH, n = 6, R = -Boc
10: X = -NH-, Y = -CH_3, n =3, R = -Boc
11: X = -NH-, Y = -CH_3, n =2, R = -Boc
12: X = -NH-, Y = -P(O)-$(OEt)_2$, n = 1, R = -Boc
13: X = -O-, Y = -NH-Boc, n = 3, R = -Boc
14: X = -O-, Y = -NH-Boc, n = 4, R = -Boc

i → **25**: X = -NH-, -P(O)-$(OH)_2$, n = 1, R = -H

15: X = -NH-, Y = -NH_2, n = 3
16: X = -NH-, Y = -NH_2, n = 4
17: X = -NH-, Y = -OH, n = 3
18: X = -NH-, Y = -OH, n = 4
19: X = -NH-, Y = -OH, n = 6
20: X = -NH-, Y = -CH_3, n =3
21: X = -NH-, Y = -CH_3, n = 2
22: X = -NH-, Y = -P(O)-$(OEt)_2$, n = 1
23: X = -O-, Y = -NH_2, n = 3
24: X = -O-, Y = -NH_2, n = 4

Scheme 1. Synthesis of the carbamate and carbonate analogues of 3TC. Reagents: (a) Boc_2O, anhydrous CH_2Cl_2, rt; (b) iPr_2NEt, Phosgene, anhydrous CH_2Cl_2, 0 °C and then rt; (c) iPr_2NEt, mono-*tert*-butoxycarbonyl diaminoalkyl, anhydrous CH_2Cl_2, rt; (d) iPr_2NEt, aminoalcohol, anhydrous CH_2Cl_2, rt; (e) iPr_2NEt, alkylamine, anhydrous CH_2Cl_2, rt; (f) diethylphophonoaminomethyl, anhydrous CH_2Cl_2, rt; (g) iPr_2NEt, *N*-*tert*-butoxycarbonyl aminoalcohol, anhydrous CH_2Cl_2, rt; (h) TFA, CH_2Cl_2; (i) Bromotrimethylsilane, anhydrous CH_2Cl_2, rt/MeO^-Na^+, MeOH, rt.

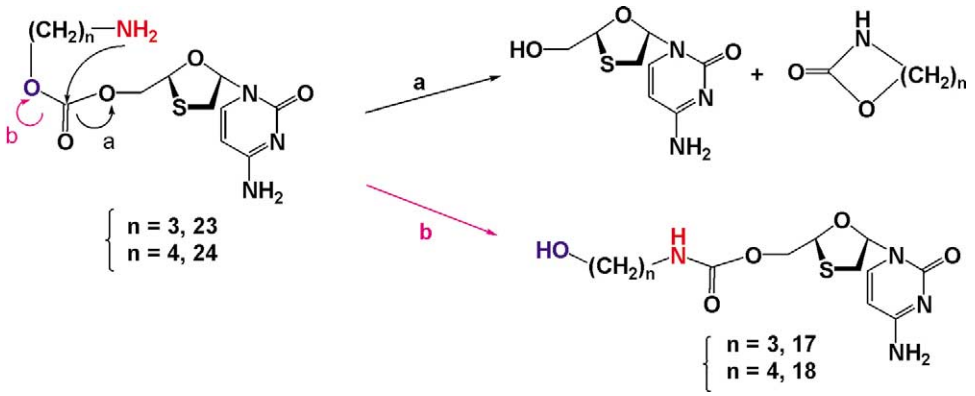
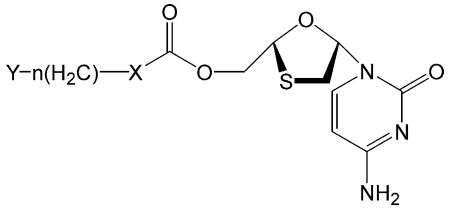


Figure 3. Nucleophilic intramolecular attack of ω-amino group of compounds **23** and **24** on its 5′-*O*-carbonate function leading to the generation of the 5′-*O*-carbamate (route b) analogues **17** and **18**.

3TC (Table 1). The tested 3TC-carbamate drugs showed anti-HIV activities within EC_{50} values ranging from 15 to 254 μM in MT-4 cell cultures and from 1 to 10 μM in PBMCs (Table 1). In contrast anti-HIV activities of 5′-*O*-3TC-carbonate drugs (**23** and **24**) were very similar to that of the parent drug 3TC (EC_{50} values of 0.30 and 0.84 μM in MT-4 cells, 0.01 and 0.1 μM in PBMCs) (Table 1). This last result, strongly suggests that 5′-*O*-3TC-carbonate nucleosides act as 3TC prodrugs, knowing that the carbonate bond is highly sensitive to enzymatic hydrolysis.

Anti-HBV activities and cytotoxicities of both 3TC carbamate and carbonate series were tested in the HepG2.2.15 infected cells using a known standard procedure.[11] Most of the tested compounds belonging to both series exhibited significant in vitro anti-HBV activities with EC_{50} values ranging from 1 to 5μM without significant toxicity up to 200 μM (Table 1). Interestingly similar anti-HBV activities were observed for any 5′-*O*-functions (carbamate or carbonate) linking the nucleoside core to the 5′-*O* side-chain and for any chemical structure of the side-chain. In contrast to their anti-HIV activities, 3TC-nucleoside-carbamates, which were found to be two orders of magnitude less potent than the corresponding carbonate analogues, demonstrated potent anti-HBV activities similar to that of their carbonate analogues or their parent drug 3TC.

Table 1. Antiviral potencies of 5′-*O*-carbamate and 5′-*O*-carbonate 3TC analogues



No.	Structure			HIV-1 III_B, MT-4			HIV-1 LAI, PBMCs			HBV, HEPG2.2.15
	X	Y	*n*	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	EC_{50}[d,e] (μM)
15	NH	NH_2	3	27±1.4	>282	>10	1±0.02	>10	>10	1±0.06
16	NH	NH_2	4	15±2.3	>218	>14	10±0.01	>10	>1	1±0.06
17	NH	OH	3	>254	>281	n.c.[g]	>10	>10	>1	1±0.06
18	NH	OH	4	>200	>217	n.c.[g]	>10	>10	n.c.[g]	5±0.1
19	NH	OH	6	75±30	>208	>3	10±0.02	>10	>1	5±0.1
20	NH	CH_3	3	26±5.8	>283	>11	5±0.03	>10	>2	1±0.06
21	NH	CH_3	2	32±6.8	>285	>9	10±0.01	>10	>1	5±0.1
22	NH	$PO(OEt)_2$	1	126±28	>233	>2	10±0.01	>10	>1	>5 (19%)
23	O	NH_2	3	0.3±0.1	>17	>57	0.01±0.01	>10	>1000	0.5±0.05
24	O	NH_2	4	0.8±0.2	>218	>260	0.1±0.02	>10	>100	1±0.06
25	NH	$PO(OH)_2$	1	n.a.[f]	>322	n.c.[g]	>10	>10	n.c.[g]	5±0.1
3TC				0.4±0.3	>44	>107	0.01±0.004	>10	>1000	0.4±0.02

[a]Concentration in μM required to inhibit by 50% the cytopathicity of HIV-1 on MT-4 cells or to inhibit by 50% HIV-1 replication on PBMCs, followed by standard deviation.
[b]Concentration in μM required to cause 50% death of uninfected MT-4 cells or PBMCs.
[c]Selectivity index = CC_{50}/EC_{50}.
[d]Concentration in μM required to inhibit 50% of HBV DNA secretion by HEPG2.2.15 stably transfected by HBV, followed by standard deviation.
[e]No apparent cytotoxicity was observed below 200 μM for each compound; no SI was given since the CC_{50} values could not be accurately determined in the conditions used for the antiviral assay.
[f]No activity.
[g]Not calculable.

Next we investigated the stabilities of these carbamate nucleosides in HIV and HBV cell culture media, and also in HepG2.2.15 and MT-4 cell lysates. Enzymatic hydrolysis half-lives ($t_{1/2}$) of the drugs conversion into the parent drug 3TC were determined by a HPLC method[2,3] (not shown). Our results indicated that the 5′-*O*-carbamate-3TC nucleosides, compounds **15** to **22** and **25**, were very stable under the different biological conditions tested. After 2 days of treatment no detectable conversion into the parent drug 3TC was detected. Furthermore, in order to determine the upper stability limit, selected compound **17** was incubated for 5 days in the above incubation conditions and release of 3TC was not observed. The enhanced stabilities of 5′-*O*-carbamate-3TC drugs are consistent with already reported literature data for 5′-*O*-AZT-carbamate drugs.[2,5] In contrast, 5′-*O*-carbonate-3TC drugs (compounds **23** and **24**) incubated in the same conditions were found to be highly sensitive to enzymatic hydrolysis; their half-lives were found to be between 13 min and 10 h for compound **23**, and 5 h and 24 h for compound **24** (not shown).

When (−)-2′-deoxy-3′-thiacytidin-5′-yl-*O*-(3-aminopropyl) carbonate **23** was incubated in biological media, formation of both the parent drug (3TC) and (−)-2′-deoxy-3′-thiacytidin-5′-yl-*O*-(hydroxypropyl) carbamate **17** was clearly identified by HPLC in the enzymatic extract, by comparison with the HPLC profiles of compounds 3TC and **17** (not shown). Formation of this later compound resulted from nucleophilic intramolecular attack of the ω-amino group on the 5′-*O*-carbonate function within the side-chain of compound **23** (Fig. 3). This result is consistent with the two possible hydrolytic cleavages (routes *a* or *b*) of carbonate prodrugs leading to the release of 3TC or to the generation of the carbamate function. Such rearrangements of ω-aminoalkyl carbonate into ω-hydroxyalkyl carbamate have only been reported in the case of AZT[2,3] and more recently in the case of HIV protease inhibitors.[12]

Results from our antiviral activities and stability studies on ω-functionalised alkyl-5′-*O*-3TC-carbamate drugs suggest that their antiviral activity mechanism could be different from that of 3TC (RT nucleoside inhibitor). However it must be underlined that the stability of these compounds in cell culture media or cell extracts can be different from that in intact cells, most nucleoside prodrugs being cleaved in these biological test conditions. Another hypothesis could be that these compounds are transported across the cellular membrane in their intact form, and that their antiviral potencies may not be associated with the release of free 3TC. Such hypothesis has been already suggested by Wagner et al.[13] in the case of phosphonoester amidates of nucleoside prodrugs. The accumulation of the unmodified nucleoside drug accumulated inside the cell might be at the origin of the observed antiviral effect. To this end we investigated the capacity of 3TC-carbamate drugs to inhibit exogenous RT, using standard HIV RT assays.[5,14] We showed that 3TC-carbamate drugs do not directly inhibit HIV-1 RT at concentrations as high as 5 mM (not shown). Taken together, the reported observations warrant further investigations in order to understand the mechanism of action of 5′-*O*-carbamate nucleosides.

In conclusion, 3TC-carbamate nucleosides are quite stable compounds in cell lysates or culture cell media, so that the release or formation of significant levels of free intracellular 3TC drug may be unlikely. Thus, hydrolysis of the carbamoyl moiety would not be a prerequisite for anti-HIV or anti-HBV activities. Since the carbamate analogues did not inhibit directly the HIV-1 RT activity this suggests that RT is not their biological target. Consequently, these compounds cannot be considered as classical 3TC prodrugs. Therefore, the antiviral activity of these compounds remains unclear. Nevertheless, 5′-*O*-3TC-carbamates have shown potent antiviral activities on HIV and particularly on HBV replication.

Acknowledgements

We gratefully acknowledge Pr. K. Dudley (INSERM U-382, Université Méditerranée, Luminy-Marseille, France) for his English corrections of the manuscript. This research was supported by grants from LAPHAL Laboratories and PACA regional Council (C.A.). INSERM is acknowledged for financial support. The following reagent was obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH: Reverse Transcriptase from the University of Alabama at Birmingham, Center for AIDS Research, Gene Expression Core Facility.

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