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Communications to the Editor

Anti-Human Immunodeficiency Virus and Anti-Hepatitis-B Virus Activities and Toxicities of the Enantiomers of 2′-Deoxy-3′-oxa-4′-thiocytidine and Their 5-Fluoro Analogues *in Vitro*

Tarek S. Mansour,*,† Haolun Jin,† Wei Wang,†
Elizabeth U. Hooker,‡ Clare Ashman,‡
Nick Cammack,‡ Horacio Salomon,§
Antonietta R. Belmonte,§ and Mark A. Wainberg§

BioChem Therapeutic Inc., 275 Armand-Frappier Boulevard, Laval, Québec, Canada H7V 4A7, Glaxo Research and Development Ltd., Greenford Road, Greenford, Middlesex, United Kingdom UB6 OHE, Lady Davis Institute, Jewis General Hospital, and McGill AIDS Center, McGill University, Montréal, Québec, Canada H3T 1E2

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Reverse transcriptase (RT) is a multifunctional enzyme that is essential for the replication of the human immunodeficiency viruses (HIV-1 and HIV-2), the causative agents of AIDS. Relevance of RT as a therapeutic target stems from the fact that no cellular homologue has been identified to date.[1] Inhibitors of RT have been shown to exhibit anti-HIV activities *in vitro* and *in vivo* and are broadly divided into 2′,3′-dideoxynucleoside analogues and non-nucleoside RT inhibitors (NNRTIs). The latter class comprises chemically distinct but mechanistically similar inhibitors acting directly on RT of HIV-1 and in most cases not HIV-2.[2] In clinical trials NNRTIs have caused a rapid selection of resistant virus.[3] Among the HIV-RT inhibitors, 3′-azido-3′-deoxythymidine (zidovudine, AZT), 2′,3′-dideoxyinosine (didanosine, ddI), 2′,3′-dideoxycytidine (zalcitabine, ddC), and, recently, 2′,3′-didehydro-3′-deoxythymidine (stavudine, d_4T) are used clinically for the treatment of AIDS patients.[4,5] However, significant side effects are associated with the above drugs during anti-AIDS chemotherapy coupled with the emergence of drug-resistant variants of HIV-1 isolated from patients treated with AZT, ddI, and ddC which limit their therapeutic use.[3,5] Recently, several members of the heterosubstituted class of 2′,3′-dideoxynucleoside analogues were discovered to be active against HIV and hepatitis-B viruses *in vitro*.[6-12] In particular (−)-2′-deoxy-3′-thiacytidine (lamivudine, 3TC) is currently in advanced stages of clinical trials for AIDS and hepatitis-B infections,[8] and its 5-fluoro analogue (−)-FTC is a promising candidate for antiviral therapy.[12] The chemical structures of 3TC and (−)-FTC differ from the clinically approved agents by having a sulfur atom in place of the 3′-carbon. Furthermore, these antiviral agents possess the β-L-sugar configuration and carry a cytosine and 5-fluorocytosine base moieties, respectively. Intensive efforts are currently directed toward the synthesis of L-nucleosides as potential antiviral agents. To date, asymmetric syntheses and antiviral testing of 1,3-dioxolane analogues (base = thymine, cytosine, adenine, and gaunine),[13-15] β-L-ddC, and β-L-5FddC have been recorded.[16-18] Notwithstanding the impressive contributions emerging from these studies, there is a need for new drugs with a low toxicity profile for use in monotherapy or in combination with other agents. Herein we describe the antiviral activities of both enantiomeric forms of novel optically active 2′-deoxy-3′-oxa-4′-thiocytidine (dOTC) and their 5-fluoro analogues (dOTFC). This class of 2,4-disubstituted 1,3-oxathiolane nucleosides is a hybrid of the 4′-thio and

Table 1. Anti-HIV-1 Activities, Cytotoxicities, and Selectivity Indices of Nucleoside Analogues **1**−**4** in Cord Blood Mononuclear Cells

	IC_{50}[a,b]			
compd	range	mean	CC_{50}[c,d]	SI[e]
1	0.02−0.6	0.287 ± 0.23	105	365
2	0.04−0.6	0.355 ± 0.25	>500	>1408
3	0.1−0.4	0.250 ± 0.18	>500	>2000
4	0.01−0.4	0.200 ± 0.20	>500	>2500
AZT		0.03 ± 0.02	45	1500

[a] The supernatant was assayed for RT activity (cpm) 7 days postinfection. [b] Data represent an average of five independent experiments and are expressed in μM. [c] Cell growth was determined by cell counting and viability by trypan blue exclusion 7 days post drug treatment. [d] CC_{50} in μM is expressed as the drug concentration which inhibits 50% of cell growth. [e] Ratio of CC_{50} to IC_{50}.

* Author to whom correspondence should be addressed.
† BioChem Therapeutic Inc.
‡ Department of Virology, Glaxo Research and Development Ltd.
§ Lady Davis Institute, Jewish General Hospital, McGill University.

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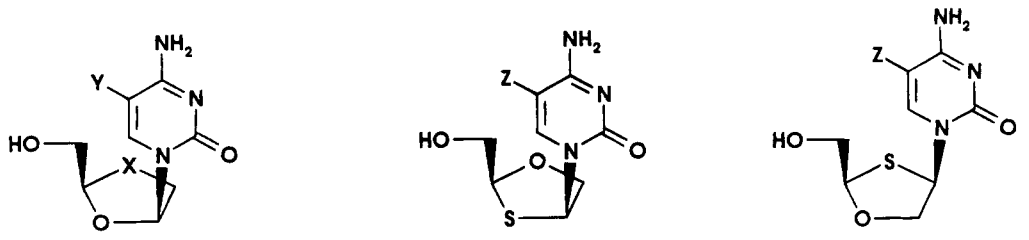


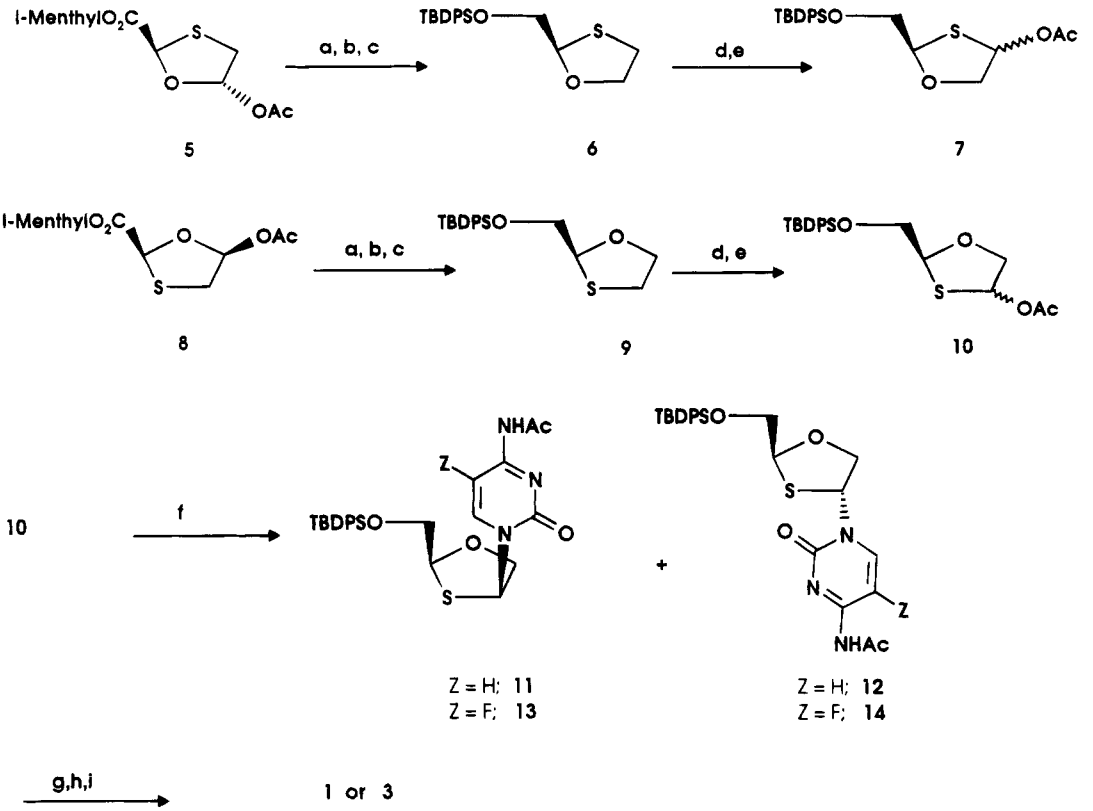
X = S; Y = H; 3TC™
X = S; Y = F; (-) - FTC
X = O; Y = H; (-) - BCH-204, (-) - dioxolane C
X = CH_2; Y = H; β-L-ddC

Z = H; 1; (+) - dOTC
Z = F; 3; (+) - dOTFC

Z = H; 2; (-) - dOTC
Z = F; 4; (-) - dOTFC

Figure 1. Absolute configuration of nucleosides **1**–**4** relative to heterosubstituted 2′,3′-dideoxynucleoside analogues and β-L-ddC.

Scheme 1[a]



[a] Reagents: (a) Et_3SiH, TMSOTf; (b) $NaBH_4$, EtOH; (c) TMDPSCl, Im, THF; (d) *m*-CPBA, CH_2Cl_2; (e) $(n\text{-}Bu)_4NOAc$, Ac_2O, 120 °C; (f) persilylated *N*-acetylcytosine or *N*-acetyl-5-fluorocytosine, TMSOTf, $ClCH_2CH_2Cl$, reflux; (g) preparative TLC; (h) TBAF, HOAc, THF; (i) K_2CO_3, MeOH.

isonucleoside families of compounds, isomeric to the 2,5-disubstituted 1,3-oxathiolanes by transposition of the heteroatom in the sugar moiety and can be considered as the 3-thia analogues of 1,3-dioxolanes, all of which exhibit antiviral activities. Specific choice of the cytosine analogues is based on precedent established in the heterosubstituted series[8,12–14] and 2′,3′-dideoxyfuranosides where D- and L-enantiomers differ in selectivity toward the viral target.[16–18]

The key features of the synthetic strategy include transposition of the acetoxy group from C-5 in oxathiolane **5** with defined absolute stereochemistry[19] to the C-4 center, reduction of the menthyl esters, and Vorbrüggen coupling with silylated cytosine and 5-fluorocytosine derivatives.[20] Transposition of the acetoxy group was achieved by a two-step reductive sequence in which the acetoxy group of **5** was first removed by reduction (Et_3SiH, TMSOTf) followed by reduction ($NaBH_4$, EtOH) of the menthyl ester and protection of the primary alcohol (TBDPCl, Im, THF). Subsequent oxidation of **6** with *m*-chloroperbenzoic acid in CH_2Cl_2 afforded a diastereomeric mixture of sulfoxides which underwent Pummerer rearrangement ($n\text{-}Bu_4OAc$, Ac_2O) to afford the transposed oxathiolanes **7** in 42% overall yield (five steps). In a similar fashion, **10** was obtained from **8** (Scheme 1).[21] The nucleosides **1** and **3** were synthesized by glycosylation of persilylated *N*-acetylcytosine or its 5-fluoro analogue with **10** under Vorbrüggen's conditions to furnish the *cis* and *trans* analogues in 1:1.5 ratio, which were separated and deprotected to afford the desired nucleosides free from their *trans* isomers. The isomeric purity of the final compounds was determined by chiral HPLC techniques. Similar transformations on **7** afforded **2** and **4**, respectively.

The anti-HIV-1 activity of **1**–**4** was initially assessed

Table 2. Anti-HIV-1 Activities, Cytotoxicities, and SI of Nucleoside Analogues 1–4 in Acutely Infected Cell Lines

	IC_{50}[a,b]					CC_{50}[c,d]					SI[e]				
compd	MT-4	Jurkat	H9	U937	CEM	MT-4	Jurkat	H9	U937	CEM	MT-4	Jurkat	H9	U937	CEM
1	0.9	1.8	0.08	0.3	0.4	>500	11	>500	22	103	>555	6	>6666	73	257
2	2.8	3.0	0.3	0.4	0.2	>500	450	>500	>500	>500	>178	150	>1667	>1250	>2500
3	3.0	3.5	0.1	0.6	0.2	>500	>500	>500	>500	>500	>166	>143	>5000	>833	>2500
4	3.2	6.0	1.0	0.4	0.3	>500	>500	>500	>500	>500	>156	>83	>500	>1250	>1667
AZT	0.005	0.01	0.07	0.04	0.01	110	>500	200	110	500	22000	>50000	2857	2750	50000

[a] Cell lines were infected with HIV-1 III_B ($TCID_{50}$ = 200) and then cultured with various concentrations of the drug. [b] IC_{50} in μM was determined by measuring p24 antigen levels in the supernatant of cultures at day 6 of infection. Data represents an average of five independent experiments and are expressed in μM. [c] Cell growth was determined by cell counting and viability by trypan blue exclusion 7 days post drug treatment. [d] CC_{50} in μM is expressed as the drug concentration which inhibits 50% of cell growth. [e] Ratio of CC_{50} to IC_{50}.

in cord blood mononuclear cells (CBMCs) infected with HIV-1 strain III_B ($TCID_{50}$ = 2000) by using production of RT as a measure of virus growth. The 50% inhibitory concentration (IC_{50}) ranges and mean values, 50% cytotoxic concentration, and selectivity indices (SI) are shown in Table 1. In that assay, all nucleosides, unexpectedly, had similar rank order of antiviral potency, with **4** being only slightly more potent than the others. In addition, distinction among the four nucleoside analogues is evident on the basis of their cytotoxicity profiles in CBMCs for which **2**, **3**, and **4** were not cytotoxic up to 500 μM and were at least 11-fold less cytotoxic than AZT.

The ability of the nucleosides to inhibit HIV-induced p24 antigen synthesis in a number of cell lines was compared with the inhibition ability of AZT. All of the nucleosides exhibited antiviral activities in the cell lines studied, most notably in Jurkat (human lymphoma), U937 (human monocyte), and CEM (human lymphoblastic leukemia) where AZT was less active (Table 2). Furthermore, nucleosides **1**–**4** exhibited well-defined dose–response curves and inhibited the HIV-induced syncytium formation in the T-lymphoblastoid cell line C8166 in the range of 0.35–1.2 μM with no cytotoxicity up to 200 μM. Among the four nucleosides, the difference in potency is relatively small with **1** being 3–4-fold more potent than **2**–**4**, equipotent to AZT in H9 (human T cells), and 180-fold weaker in MT-4 (human T helper) and Jurkat cells. The cytotoxicity of **1**–**4** for the five cell lines in cell proliferation assays indicated that **2**, **3**, and **4** were not cytotoxic up to 500 μM. In comparison, **1** is notably cytotoxic in Jurkat, U937, CEM, and CBMCs and is more cytotoxic than AZT in the former three cell lines (Table 2).

Since hepatitis-B virus encodes for an enzyme which possesses reverse transcriptase activity, we determined the anti-HBV activities of **1**–**4** in the transfected human hepatoma cell line 2.2.15. Although **1** displayed modest anti-HBV activity (IC_{50} = 17.5 μM, cytotoxicity >50 μM), it remains 1000-fold less potent than lamivudine. In comparison **2**, **3**, and **4** are not as potent up to 50 μM.

The results presented here demonstrate that **2**, **3**, and **4** are potent and selective inhibitors of HIV-1 replication *in vitro* and that **1** is a potent inhibitor of HIV-1 replication with modest activity against HBV *in vitro*. Despite the appreciable potency of **1**, it displays relatively low selectivity in four cell lines. The order of potency and selectivity of the other three nucleosides are similar.

For most anti-HIV nucleoside analogues, only one enantiomeric form is active as, for example, the cases of AZT,[22,23] ddI,[24] d_4T,[24] d_4C,[24] FLT,[23] dioxolane-T,[13] and 2′,3′-dideoxy-3′-*C*-(hydroxymethyl)cytidine.[25] On the other hand, the enantiomers of 2′-deoxy-3′-thiacytidine (BCH-189) represent the unique case of equipotency in anti-HIV activity but not with regard to cytotoxicity which was seen primarily with the D-enantiomer.[26–30] Recently, anti-HIV and anti-HBV activities were reported for β-L-ddC and its 5-fluoro analogue.[16–18] The potency of the enantiomers **1** and **2** and the greater selectivity of **2** represents the first example of such behavior in the 4′-thio class of antiviral nucleosides. Surprisingly, the similarity in potency and lack of cytotoxicities of the enantiomers **3** and **4** is unprecedented, to the best of our knowledge, in the nucleoside field. The effect of the 5-fluoro substituent versus hydrogen in the 2*R*,4*R* analogues, **2** and **4**, on potency and cytotoxicity profiles is negligible. However, this substituent decreased the cytotoxicity profile *in vitro* of the 2*S*,4*S* isomer **3** in comparison to **1**.

In conclusion, we report the first examples of enantiomerically pure 4′-thio nucleoside analogues with potent and selective activity against HIV-1 in a panel of cell lines and, as well, in lymphocytes of primary origin. Further details on resistance profiles and cellular metabolism will be reported in forthcoming publications.[31,32]

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Supplementary Material Available: Experimental procedures, including yields, melting points, combustion data, protocols for antiviral testing, and dose–response curves (35 pages). Ordering information is given on any current masthead page.

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(32) The *trans* isomers of **1**–**4** were tested in MT-4 cells and found to be inactive up to 400 μM and not cytotoxic. These compounds were also inactive against HBV replication *in vitro* up to 50 μM.

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