Published in final edited form as:

Antiviral Res. 2017 September ; 145: 175–183. doi:10.1016/j.antiviral.2017.07.011.

Dual-targeted anti-TB/anti-HIV heterodimers

Liudmila Alexandrova¹, Sonia Zicari², Elena Matyugina¹, Anastasia Khandazhinskaya^{1,*}, Tatiana Smirnova³, Sofia Andreevskaya³, Larisa Chernousova³, Christophe Vanpouille^{2,*}, Sergei Kochetkov¹, and Leonid Margolis²

¹Engelhardt Institute of Molecular Biology, Vavilova Str., 32, Moscow, 119991, Russia

²Eunice Kennedy-Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

³Central Tuberculosis Research Institute, Yauzskaya Alley 2, Moscow 107564, Russia

Abstract

HIV and *M. tuberculosis* are two intersecting epidemics making the search for new dual action drugs against both pathogens extremely important. Here, we report on the synthesis and suppressive activities of five dual-targeted HIV/TB compounds. These compounds constitute heterodimers of AZT, as anti-HIV molecules, and 5-arylaminothymine derivatives or 2'-deoxyuridine monophosphates analogs, as anti-TB molecules. We found that these compounds inhibit the growth of *M. tuberculosis* and suppress the replication of HIV in human cell cultures and human lymphoid tissues *ex vivo*. We identified one particular heterodimer that inhibited most potently both HIV and the drug-resistant TB strain MS –115. This compound demonstrated low toxicity and had no cytostatic effect on cells in culture. This is a leading candidate for future development and further *in vivo* testing.

Keywords

HIV; TB; heterodimers; human tissues

1. Introduction

Infection by HIV and *M. tuberculosis* are among the main causes of death from infectious diseases worldwide. According to 2015 WHO estimates, 2.1 million people became newly infected with HIV and 1.1 million people died from AIDS-related illnesses. In 2015, among the causes of death from infectious diseases, tuberculosis (TB) ranked above HIV/AIDS with an estimated 10.4 million new cases and 1.8 million deaths [1].

HIV and TB epidemics fully intersect. On one hand, the risk of death in TB/HIV-coinfected individuals is twice that of HIV-infected individuals without TB, even when CD4 cell counts

*Correspondence to: Christophe Vanpouille: vanpouic@mail.nih.gov; Anastasia Khandazhinskaya: khandazhinskaya@bk.ru.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and antiretroviral therapy are taken into account [2]. On the other hand, HIV infection is the strongest known risk factor for progressing to TB disease for individuals with latent TB infection. Also, people living with HIV are 17–22 times more likely to develop TB than people without HIV. Apparently, *M. tuberculosis* and HIV act synergistically, resulting in a decline of immunological functions and death if untreated [3].

The synergy between TB and HIV makes the search for new combined dual action drugs extremely important. In general, combining two active compounds into one molecule has a long history [4–8]. In particular, we reported earlier on the synthesis of hybrid antivirals by linking two nucleoside reverse transcriptase inhibitors (NRTI) of HIV [9].

Here, we describe new anti-TB/anti-HIV compounds that are based on heterodimerization of our recently designed anti-TB 5-arylamino-uracil derivatives [10] (as well as of 2'-deoxyuridine monophosphates and their analogs [11] with the anti-HIV NRTI, 3'-azido-3'-deoxythymidine (AZT)). We report here on the synthesis of five heterodimers. These compounds inhibit the growth of *M. tuberculosis* and suppress the replication of HIV in human cell cultures and human lymphoid tissues *ex vivo*. Four of the synthesized compounds proved to be of low cytotoxicity in these systems.

2. Materials and methods

2.1. Chemical synthesis

Chemical synthesis and spectroscopic characterizations are described in the supplementary data file. Briefly, in this work, five new hybrid molecules were synthesized; three of them (1–3) are the conjugates of AZT and 5-(4-decyl-1,2,3-triazol-1-yl-methyl)-2'-deoxyuridine (dU^R) (Scheme 1) and the other two (4 and 5) were conjugates of AZT and modified 5'-norcarbocyclic analogues of 2',3'-dideoxy-2',3'-dideoxyuridine (Scheme 2).

A solution of dU^R and 1,1'-carbonyldiimidazole in DMF was heated for 18 h at 37°C, and then AZT was added to give a mixture of products 1–3. The reaction mixture was then evaporated and chromatographed on silica gel with a gradient concentration of ethanol in chloroform (Scheme 1).

For the synthesis of heterodimers 4 and 5, the previously described method of selective N-3 alkylation was used [5]. The reaction of azidothymidine and dibromopropane in the presence of potassium carbonate gave intermediate 6 (15% yield), to which the second key component 7 or 8 was further added (Scheme 2). The yields of the heterodimers 4 and 5 on the second stage were 60% and 65%, respectively. Structures and purities of the synthesized compounds 1–5 were confirmed from ¹H and ¹³C NMR spectroscopy, high-resolution mass spectroscopy, and TLC.

5'-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (1), 3'-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (2), and 3',5'-di-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (3)—N,N'-carbonyldiimidazole (CDI, 95 mg, 0.59 mmol) was added to a solution of 5-(4-decyl-1,2,3-triazol-1-yl)-2'-deoxyuridine (dU^R, 130 mg, 0.28 mmol) in

DMF (3 mL), and the mixture was kept for 18 h at 37° C; then 3'-azido-3'-deoxythymidine (AZT, 309 mg, 1.16 mmol) was added and the mixture was kept for 18 h at 37° C. The mixture containing products **1**, **2**, and **3** was evaporated under vacuum; the residue was dissolved in chloroform (2 mL), applied onto a silica gel column (20×250 mm), and eluted with a gradient of ethanol in chloroform (0 → 7%) to give crude products **1–3**. We performed final purification of the isolated products with preparative TLC on silica gel plates (20×20×2 mm) using ethyl acetate/ethanol/hexane (9:1:1, v/v) as an eluent to provide the desired compounds.

5'-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (1)—

5'-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (1) was obtained as a white solid (31 mg, 15%). UV: $\lambda_{\max}$ 264.2 nm (19 215). ¹H-NMR (DMSO-*d*₆): 11.53 (1H, s, 3NH_{dUR}), 11.30 (1H, s, 3NH_{AZT}), 7.89 (1H, s, CH_{dUR}), 7.72 (1H, s, H6_{dUR}), 7.43 (1H, s, H6_{AZT}), 6.16 (1H, dd, *J*_{6,7}, 6.8, H1'_{dUR}), 6.11 (1H, t, *J*_{6,5}, H1'_{AZT}), 5.45 (1H, d, *J*_{4,6}, 3'OH_{dUR}), 5.12 (2H, s, 5CH₂O_{dUR}), 4.48–4.24 (6H, m, H3'_{AZT} + H3'_{dUR} + H5'_{AZT} + H5'_{dUR}), 3.97 (1H, m, H4'_{AZT} + H4'_{dUR}), 2.55 (2H, m, CH₂C₉H₁₉ dUR), 2.46–2.19 (4H, m, H2'_{AZT} + H2'_{dUR}), 1.75 (3H, s, 5CH₃ AZT), 1.55 (2H, m, CH₂CH₂C₆H₁₇ dUR), 1.23 (14H, m, C₂H₄(CH₂)₇CH₃ dUR), 0.85 (3H, dd, *J*_{6,7}, 6.8, CH₃ dUR). ¹³C NMR (DMSO-*d*₆): δ 163.52, 162.41, 154.04, 150.25, 150.023, 146.69, 140.77, 135.86, 121, 55, 109.86, 108.19, 84.67, 84.61, 83.70, 80.39, 80.31, 69.95, 67.75, 67.06, 59.84, 45.67, 38.77, 35.52, 31.19, 28.90, 28.68, 28.64, 28.59, 24.92, 22.00, 13.85, 11.93. MS (ESI): calculated for C₃₃H₄₆N₁₀O₁₀ 743.3471 [M+H]⁺, found 743.3467, calculated for C₃₃H₄₆N₁₀O₁₀ 765.3291.4040 [M+Na]⁺, found 765.3274.

3'-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (2)—

3'-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (2) was obtained as a white solid (25 mg, 12%). UV: $\lambda_{\max}$ 264.2 nm (19 220). ¹H-NMR (DMSO-*d*₆): 11.57 (1H, s, 3NH_{dUR}), 11.32 (1H, s, 3NH_{AZT}), 8.12 (1H, s, CH_{dUR}), 7.74 (1H, s, H6_{dUR}), 7.48 (1H, s, H6_{AZT}), 6.13 (2H, m, H1'_{AZT} + H-1'_{dUR}), 5.19 (2H, m, H3'_{dUR} + 5'OH_{dUR}), 5.12 (2H, m, 5CH₂O_{dUR}), 4.48 (1H, m, H3'_{AZT}), 4.35 (2H, m, H5'_{AZT}), 4.09 (1H, m, H4'_{AZT}), 4.02 (1H, m, H4'_{dUR}), 3.63 (2H, m, H-5'_{dUR}), 2.56 (2H, m, CH₂C₉H₁₉ dUR), 2.46–2.34 (4H, m, H2'_{AZT} + H2'_{dUR}), 1.80 (3H s, 5CH₃ AZT), 1.55 (2H, m, CH₂CH₂C₆H₁₇ dUR), 1.24 (14H, m, C₂H₄(CH₂)₇CH₃ dUR), 0.85 (3H, dd, *J*_{6,7}, 7.0, CH₃ dUR). ¹³C NMR (DMSO-*d*₆): δ 163.62, 162.48, 153.56, 150.33, 150.13, 146.80, 140.49, 135.99, 121, 63, 109.89, 108.24, 84.67, 84.44, 83.79, 80.39, 78.76, 66.94, 61.22, 59.72, 45.83, 36.86, 35.66, 31.25, 28.94, 28.74, 28.64, 28.62, 24.98, 22.0513.87, 12.07. MS (ESI): calculated for C₃₃H₄₆N₁₀O₁₀ 743.3471 [M+H]⁺, found 743.3481, calculated for C₃₃H₄₆N₁₀O₁₀ 765.3291.4040 [M+Na]⁺ found 765.3271.

3',5'-di-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (3)—

3',5'-di-O-[AZT-carbonyl]-5-[(4-decyl-1,2,3-triazol-1-yl)methyl]-2'-deoxyuridine (3) was obtained as a white solid (61 mg, 21%). UV: $\lambda_{\max}$ 264.2 nm (28 800). ¹H-NMR (DMSO-*d*₆): 11.61, 11.32, 11.31 (3H, 3s, 3NH_{dUR} + (3NH_{AZT})₂), 7.97 (1H, s, CH_{dUR}), 7.73 (1H, s, H6_{dUR}), 7.48, 7.43 (2H, 2s, (H6_{AZT})₂), 6.13 (3H, m, (H1'_{AZT})₂ + H1'_{dUR}), 5.12 (2H, s, CH₂O_{dUR}), 4.47 (2H, m, (H3'_{AZT})₂), 4.35 (5H, m, (H5'_{AZT})₂ + H4'_{dUR}), 4.01 (2H, m,

(H-4' AZT)₂, 3.31 (2H, M, H5' dUR), 2.57 (2H, T, J_{6,2}, CH₂C₉H₁₉ dUR), 2.12 (2H, M, H2' dUR), 1.80–1.78 (6H, 2s, (5CH₃ AZT)₂), 1.52 (2H, m, CH₂CH₂C₆H₁₇ dUR), 1.23 (14H, m, C₂H₄(CH₂)₇CH₃ dUR), 0.85 (3H, T, J_{6,9}, CH₃ dUR). ¹³C NMR (DMSO-*d*₆): δ 164.07, 162.938, 154.44, 153.99, 151.37, 150.80, 150.53, 147.26, 141.45, 138.12, 136.52, 136.39, 122.09, 110.39, 109.969, 109.92, 85.53, 85.16, 84.46, 84.28, 83.89, 80.84, 78.07, 67.86, 67.70, 66.82, 60.64, 60.41, 60.22, 46.20, 36.11, 31.74, 2945, 2923, 29.13, 25.47, 22.54, 14.36, 12.59, 12.45. MS (ESI): calculated for C₄₄H₅₇N₁₅O₁₅ 1036.4221 [M+H]⁺, found 1036.4231, calculated for C₄₄H₅₇N₁₅O₁₅ 1058.4040 [M+Na]⁺ found 1058.4051.

3'-Azido-3-N-(3-bromopropyl)-2',3'-dideoxythymidine (6)—To a solution of AZT (1 eq, 500 mg, 1.87 mM) in acetone were added K₂CO₃ (2 eq, 516 mg, 3.74 mM) and 3-bromopropane (2 eq, 378 mL, 3.74 mM). The reaction mixture was refluxed for 4 h. After evaporation, the residue was purified by column chromatography on silica gel and eluted with hexane: ethyl acetate (1:1), with a yield of **6** (83%) as an off-white powder. ¹H NMR (CDCl₃): 7.35–7.34 (1H, s, H₆), 6.06–6.03 (1H, m, H1'), 4.39–4.37 (1H, m, H4'), 4.08–4.05 (2H, t, 5'CH₂), 3.97–3.95 (2H, t, CH₂-N), 3.84–3.79 (1H, s, OH), 3.42–3.39 (2H, t, CH₂-Br), 2.54–2.52 (1H, m, H2'b), 2.44–2.41 (2 H, m, H2'a, H3'), 2.22–2.18 (2H, m, CH₂), 1.92 (1H, s, CH₃). ¹³C NMR (CDCl₃): 163.37, 150.99, 134.96, 110.66, 87.67, 84.68, 62.21, 60.11, 40.50, 37.52, 31.06, 30.45, 13.38.

[AZT]-N3-propyl-N3-[1-N-[4'-(*o,o*-dimethylbenzyl)cyclopent-2'-enyl]uracil] (4)—To a solution of **(6)** in dry acetonitrile (62 mg, 0.16 mM) were added K₂CO₃ (40 mg, 0.3 mM) and 1-N-[4'-(*o,o*-dimethylbenzyl)cyclopent-2'-enyl]uracil (48 mg, 0.14 mM). The reaction mixture was refluxed for 4 h. After evaporation, the residue was purified by column chromatography on silica gel and eluted with chloroform: methanol (98:2); yield of **4** (75%) as a yellow powder. ¹H NMR (CDCl₃): 7.59 (2 H, s, 2×H₆), 7.31, 7.27, 7.19 (3H, s, Ph), 6.39–6.38 (1H, m, H1'), 6.03–6.0 (2H, m, H1' AZT, H2'), 5.86–5.84 (1H, m, H3'), 5.73–5.71 (2H, m, H5_{Ura}, H4'), 4.39–4.37 (1H, m, H3' AZT), 4.01–4.00 (5H, m, 2×CH₂, H4' AZT), 3.94–3.92 (2H, m, 5'CH₂AZT), 3.09–3.05 (1H, m, H5'a), 2.76 (1H, s, OH), 2.59–2.56 (1H, m, H2'aAZT), 2.01 (6H, s, 2CH₃Bz), 2.03–2.00 (1H, m, H2'bAZT), 1.89 (1H, s, CH₃), 1.84–1.82 (1H, m, H5'b). ¹³C NMR (CDCl₃): 166.33, 163.41, 162.80, 151.68, 150.99, 138.69, 138.40, 136.61, 135.14, 135.02, 134.99, 134.47, 129.78, 127.44, 110.46, 102.44, 87.81, 84.74, 62.06, 59.99, 59.46, 39.34, 39.26, 37.77, 37.39, 29.84, 26.35, 21.31, 13.41. HRMS: found *m/z* 656.2436; calculated for C₃₁H₃₅N₇O₈ [M+Na]⁺ 656.2439.

[AZT]-N3-propyl-N3-[1-N-(4'-hydroxycyclopent-2'-enyl)-5-(*p*-butoxyphenylamino)]uracil (5)—[AZT]-N3-propyl-N3-[1-N-(4'-hydroxycyclopent-2'-enyl)-5-(*p*-butoxyphenylamino)]uracil (**5**) was obtained by the method described for **(4)** using 1-N-(4'-hydroxycyclopent-2'-enyl)-5-(*p*-butoxyphenylamino)uracil with 68% yield as a pale yellow powder. ¹H NMR (CDCl₃): 7.03 (H, s, H₆AZT), 6.91 (H, s, H₆Ura), 6.82–6.80 (4H, m, Ph), 6.15–6.14 (1H, m, H1'), 6.02–5.99 (H, m, H1' AZT), 5.81–5.80 (1H, m, H2'), 5.79–5.74 (1H, m, H3'), 5.33–5.32 (1H, m, H4'), 4.78–4.77 (1H, m, H4' AZT), 4.40–4.38 (1H, m, H3' AZT), 4.05–4.03 (5H, m, 2×CH₂), 3.97–3.92 (4H, m, 5'CH₂AZT, CH₂^a), 3.79–3.74 (2H, m, 2×OH), 2.82–2.79 (1H, m, H5'a), 2.59–2.56 (1H, m, H2'aAZT), 2.39–2.38 (1H, m, H2'bAZT), 2.05–2.03 (2H, m, CH₂), 1.90 (1H, s, CH₃), 1.85–1.82 (3H, m, CH₂^β,

H5'b), 1.76–1.74 (2H, m, CH₂'), 1.53–1.51 (1H, m, CH₃). ¹³C NMR (CDCl₃): 163.34, 160.54, 154.61, 151.04, 149.33, 138.95, 135.11, 131.93, 121.26, 120.66, 116.77, 115.65, 110.48, 87.95, 87.67, 84.68, 75.05, 68.27, 62.19, 62.04, 61.91, 59.90, 39.75, 39.63, 39.18, 37.22, 31.51, 29.78, 26.42, 19.34, 13.93, 13.34. HRMS:found *m/z* 687.2857; calculated for C₃₂H₄₀N₈O₈ [M+Na]⁺ 687.2861.

2.2. Stability of compounds synthesized

Chemical stability at pH 2.2, 7.4, and 9.0 as well as stability in human blood serum were evaluated according to the standard procedures [12, 13]. We performed HPLC-HRMS analysis of the samples using an Agilent 1260 chromatograph equipped with an Agilent Poroshell-120 column (3.0 × 50 mm; 2.7 μm) and a Phenomenex SecurityGuard Ultra C-18 precolumn and used a Bruker Daltonics micrOTOF-Q II mass-spectrometer for detection. The column was eluted in a gradient of acetonitrile (A) in water (B). Gradient parameters: 100% B for 2 min; 10% → 20% A for 2 min, 20% → 30% A for 2 min, 30% → 40% A for 2 min, 40% → 50% A for 2 min, 50% → 60% A for 2 min, 60% → 70% A for 2 min, 70% → 80% A for 2 min, 80% → 90% A for 2 min, 90% → 100% A for 2 min; 100% A for 5 min, 100% → 0% A for 5 min. Flow rate was set at 0.130 ml/min.

2.3. Anti-HIV-1 activity

HIV-1 inhibition by compounds (1–5) was assessed in MT-4 cell cultures and in human tissue *ex vivo*.

2.3.1. MT-4 cell cultures—Human T lymphocytes MT-4 (obtained from American Type Culture Collection) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. We performed HIV-1 infection of MT-4 by incubating 100 μL of MT-4 at 10⁷ cells/mL with 100 μL of X4LAI.04 containing 5 ng of p24_{gag} in a vial for 1 h 30 min at 37°C. After incubation, infected cell suspensions were washed with PBS and resuspended in 1 mL of medium. We first transferred 100 μL of infected MT-4 cells to a 12-well plate and then mixed them with 900 μL of medium containing the test compound at an appropriate dilution. MT-4 cells were incubated at 37°C and cultured for 3 days.

2.3.2. Human tissue culture ex vivo—Human tonsillar tissues were obtained from patients undergoing routine tonsillectomy at the Children's National Medical Center (Washington DC) under an IRB-approved protocol. Tissues were dissected into 2- to 3-mm³ blocks and placed onto collagen sponge gels in culture medium at the air–liquid interface as described earlier [14]. Human tonsillar tissues (27 blocks of tissue from each of *n* donors for each experimental condition) were pretreated with compounds (1–5) overnight and then infected with a prototypical X4 variant of HIV-1 (HIV-1 X4_{LAI.04}) (Rush University Virology Quality Assurance Laboratory, Chicago, IL). The tissue culture was kept for 12 days, and drugs were replenished after each medium change (every 3 days).

2.3.3. Evaluation of antiviral activity of compounds (1–5)—We measured p24_{gag} using a bead-based Luminex assay as described earlier [15]. We then evaluated the antiviral activity of each compound by measuring inhibition of human HIV-1 replication in MT-4 cell cultures and in human lymphoid tissues. For each compound, in MT-4 cell cultures or in

lymphoid tissue *ex vivo*, HIV-1 inhibition at each single concentration was defined by the following formula: inhibition = $(1 - R_{\text{compounds}}/R_{\text{Ctl}}) \times 100$, where $R_{\text{compounds}}$ and R_{Ctl} are the amounts of p24 accumulated in the medium in compound-treated cultures and in untreated cultures, respectively. We calculated the EC₅₀ values (with 95% confidence interval (CI)) by fitting the data points to a sigmoidal dose–response curve, using Prism software, (version 6.0; GraphPad). The EC₅₀ (50% effective concentration) is defined as the compound concentration required for inhibition of viral replication by 50%.

2.4 Cell toxicity

Cell toxicity was assessed both in MT-4 cell cultures and in human lymphoid tissue.

2.4.1. Viability assay in MT-4 cell cultures—We performed viability assays (cytotoxicity and cytopathicity) in MT-4 cell cultures with the Nucleocounter automated cell counting system (ChemoMetec). We determined the numbers of total cells and dead cells in control (untreated) cultures and in cultures treated with heterodimers (**1–5**) using a propidium iodide–based assay according to the manufacturer’s protocol. We collected and analyzed data using Nucleoview software (version 1.0; ChemoMetec).

2.4.2. Viability assay in human lymphoid tissue—To assess the cytotoxicity of heterodimers in human tonsillar tissues, we isolated cells from the compound-treated and untreated tissue blocks and stained them for surface markers. Each cell suspension was divided in two fractions and used for two different stainings. The first fraction was stained with a combination of the following fluorescence-labeled monoclonal antibodies: anti–CD3-QD655, anti–CD4-QD605, anti–CD8-QD800, anti–CD45RA-FITC, and anti–CCR7-PE-Cy7. The second fraction was stained with the following antibodies: anti–CD3-QD655, anti–CD4-QD605, anti–CD8-QD800, anti–CD25-APC, anti–CD38-PE, anti–CD95-PE-Cy7, and anti–HLA-DR-BV570 (Caltag Laboratories, CA; B&D Biosciences, CA). Data were acquired and analyzed on a NovoCyt flow cytometer (Acetabiosciences, Inc). We quantified cell depletion using TruCount beads (Becton Dickinson) for volumetric control and normalized cell numbers by tissue-block weights.

2.5. Anti-TB activity

Two strains of *M. tuberculosis*, H37Rv and MS-115, were used for testing anti-TB activity of the synthesized heterodimers. The former strain is sensitive to standard anti-TB drugs, while the latter was MDR-resistant [10, 16]. The cultures were checked for the absence of contamination by nonspecific microflora. Anti-TB activity was measured as described earlier [16] with each concentration of the test compounds, and the control samples were tested in duplicate.

3. Results

3.1. Heterodimer synthesis

In this work, five new hybrid molecules were synthesized; three of them (**1–3**) are the conjugates of AZT and 5-(4-decyl-1,2,3-triazol-1-yl-methyl)-2'-deoxyuridine (dU^R)

(Scheme 1) and the other two (**4** and **5**) were conjugates of AZT and modified 5'-norcarbocyclic analogues of 2',3'-dideoxy-2',3'-didehydrouridine (Scheme 2).

A solution of dU^R and 1,1'-carbonyldiimidazole in DMF was heated for 18 h at 37°C, and then AZT was added to give a mixture of products **1–3**. The reaction mixture was then evaporated and chromatographed on silica gel with a gradient concentration of ethanol in chloroform (Scheme 1).

For the synthesis of heterodimers **4** and **5**, the previously described method of selective N-3 alkylation was used [5]. The reaction of azidothymidine and dibromopropane in the presence of potassium carbonate gave intermediate **6** (15% yield), to which the second key component **7** or **8** was further added (Scheme 2). The yields of the heterodimers **4** and **5** on the second stage were 60% and 65%, respectively. Structures and purities of the synthesized compounds **1–5** were confirmed from ¹H and ¹³C NMR spectroscopy, high-resolution mass spectroscopy, and TLC.

3.2 Stability of compounds synthesized

All the tested compounds were stable to chemical hydrolysis at pH 2.2 (glycine+HCl) and at pH 7.4 (PBS). Compounds **4** and **5** were also stable at pH 9.0 (glycine+NaOH); T_{1/2} for compounds **1–3** at the same conditions was about 14–15 h.

Similar T_{1/2} values for compounds **1–3** were obtained in human blood serum. Serum activity was assessed with the standard compounds: T_{1/2} for 3',5'-diacetyl thymidine was 18 h; T_{1/2} for TMP was 20 min. For compounds **1** and **2**, both AZT and dU^R derivative were found as main products. For compound **3**, the entire set of hydrolysis products was detected: **1**, **2**, AZT, and dU^R. Compound **4** produced only slight debenzoylation at the carbocyclic fragment after 15 h in human blood serum (debenzoylated product retention time was 19.50 min); compound **5** was stable. Retention times were as follows: **1**, 22.10 min; **2**, 22.50 min; **3**, 23.05 min; **4**, 21.85 min; **5**, 21.10 min; AZT, 13.75 min; and dU^R, 20.80 min.

3.3. Heterodimers inhibited HIV-1 replication

The anti-HIV activities of all the heterodimers (**1–5**) were evaluated in MT-4 cell cultures and in *ex vivo* human lymphoid tissues. All the compounds inhibited X4_{Lai.04} replication in MT-4 cell cultures. HIV-1 inhibition was assessed at heterodimer concentrations ranging from 1 nM to 30 µM. HIV-1 inhibition was dose-dependent, with EC₅₀ for heterodimers **1–3** being around 2 logs lower than that of heterodimers **4–5** (Table 1).

Similarly, we found that all compounds inhibited X4_{Lai.04} replication in tissues *ex vivo* in a dose-dependent manner. For each heterodimer, the anti-HIV-1 activity in tissues was tested at concentrations ranging from 1 pM to 30 µM. As described earlier (reviewed in [14]), the absolute levels of HIV replication, evaluated from measurements of p24gag accumulation in culture medium, were donor-dependent. Therefore for each donor, HIV-inhibition in the treated experimental condition was expressed as a percent of that in the donor-matched untreated condition. Although EC₅₀s were different from those in MT-4 cell cultures (Table 1), heterodimers **1–3** were more potent than heterodimers **4–5** as observed in MT-4 cultures.

EC₅₀s in human tissues ranged from 0.14 to 0.5 μ M for heterodimers **1–3** and from 1 to 6 μ M for heterodimers **4–5**, respectively (Table 1).

3.4. Heterodimers inhibited *Mycobacterium tuberculosis*

We studied anti-mycobacterial activities of the synthesized compounds using two *M. tuberculosis* strains: H37Rv, a laboratory strain sensitive to the five first-line anti-TB drugs (isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide), and MS-115, a clinical MDR-resistant strain. We evaluated the anti-mycobacterial effects of the compounds as bacterial growth in enriched Middlebrook 7H9 liquid medium, using BACTEC MGIT960 automated system registration in the presence of different concentrations of compounds. The resistance of MS-115 was confirmed in the experiments in which this strain was cultured in media containing rifampicin (1 mg/mL) and isoniazid (0.1 mg/mL). We found that its growth was similar to that in the media without these compounds. Levofloxacin (1.5 mg/mL) was used as a positive control.

We found that compounds **1–3** and **5** exhibit anti-mycobacterial activity in the *in vitro* system. The minimal concentrations at which inhibition of bacterial growth was not less than 99% for both strains (MIC (99)) were determined (Table 2).

Thus, the anti- *M. tuberculosis* activity was highest for compounds **2** and **3**. For these compounds, the MIC (99) values against the sensitive laboratory strain of *M. tuberculosis*, H37Rv, were 10 μ g/mL and 20 μ g/mL, respectively, while those for the clinical MDR strain of *M. tuberculosis*, MS-115, were equal to 20 μ g/mL and 10 μ g/mL, respectively.

3.5. Heterodimers were not toxic for isolated cells or for tissue cultures

First, we tested the potential cell toxicity of our newly-synthesized heterodimers in human tissue *ex vivo*, which remains the model of choice to evaluate the potency and cytotoxicity of newly synthesized compounds [14] (Figure 2). Here, we tested the toxicity of each compound at two concentrations, 10 and 50 μ M, with each experimental condition performed with 27 tissue blocks from each donor. After 12 days of culture, we isolated cells and assessed the viability of various lymphocyte subsets using flow cytometry. We evaluated the depletion of B cells (CD3[−]) and of various sets of T cells (CD3⁺): CD3⁺CD4⁺, CD3⁺CD8⁺, CD45RA⁺CCR7⁺ (naïve), CD45RA[−]CCR7⁺ (central memory, T_{CM}), CD45RA[−]CCR7[−] (effector memory, T_{EM}) and different state of their activation (CD25⁺, CD38⁺, CD95⁺, HLA-DR⁺). We assessed the cytotoxicity of each heterodimer by comparing the numbers of cells of each of the above-listed subsets in treated tissue blocks with those in donor-matched untreated tissue blocks. To account for size differences between tissue blocks, data were normalized by the tissue weight (Figure 2).

On day 12 of culture, the numbers of lymphocytes in the CD3[−], CD3⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, T_{naïve}, T_{CM}, T_{EM}, and activated T cell subsets were not statistically different ($P > 0.05$) in untreated tissues compared with donor-matched tissues treated with heterodimers **1–5** at a concentration of 10 μ M. In contrast, when heterodimers were used at a concentration of 50 μ M, mild cell depletion was observed (10–20% cell loss in treated versus untreated conditions). No particular heterodimer was more cytotoxic than others.

Next, heterodimers were used in the MT-4 cell culture, allowing the evaluation of both their cytotoxic and cytostatic effects. For each compound, the CC₅₀ (concentration that reduced the viability of MT-4 by 50%) and the IC₅₀ (concentration that inhibited MT-4 cell growth by 50%) were evaluated. As with what was observed in tissues, no significant cytotoxicity was observed for any of the five newly developed heterodimers (the CC₅₀s ranged from 74 to >300 µM) (Table 3). Although heterodimers were not toxic, some of them slowed down cell growth, with IC₅₀s ranging from 18 to 150 µM). Heterodimer **3** was the least toxic and the least cytostatic, with both CC₅₀>300 µM and IC₅₀ = 150 µM.

4. Discussion

TB remains a major cause of morbidity and mortality globally, with an estimated ten million people developing the disease in 2015 [1]. Although the great majority of individuals infected with *M. tuberculosis* do not develop active TB [17], this scenario is distinct for individuals who are coinfecting with HIV [3]. HIV coinfection is the most important risk factor for developing active TB for at least two reasons: (i) HIV coinfection increases the susceptibility to primary infection or reinfection with *M. tuberculosis* and (ii) it also increases the risk of TB reactivation for patients with latent TB. The risk of TB reactivation rises as the CD4 T cell count declines [18–20].

Reciprocally, *M. tuberculosis* infection has a negative impact on the immune response to HIV, accelerating the progression from HIV infection to AIDS [21–23] (reviewed in [3, 17]). This explains, in part, why TB remains the leading cause of death among people living with HIV. The effect of TB on HIV disease progression is linked to increased immune activation [24] (reviewed in [3, 17]) and to increased expression of the CCR5 and CXCR4 coreceptors on CD4 T cells [25].

Therefore, in HIV/TB-coinfecting individuals, *M. tuberculosis* and HIV potentiate one another by accelerating the deterioration of immunity. Without treatment, HIV and TB considerably shorten lifespan. Paradoxically, initiation of antiretroviral therapy (ART) to treat HIV infection in HIV/TB-coinfecting individuals may also result in a clinical deterioration known as IRIS (immune reconstitution syndrome) [26]. TB-IRIS, which happens against the background of increased CD4 T-cell count and suppression of HIV-1 RNA level in persons receiving ART, is estimated to occur in 11 to 45% of patients coinfecting with TB and HIV. Moreover, despite the consistent decrease in TB risk among persons who receive ART, this risk remains higher than that in HIV-uninfected persons, even after several years of treatment, suggesting that immune restoration is not complete (reviewed in [26]).

The immense burden placed by TB and HIV on coinfecting individuals and on health care systems in general necessitates a search for new active compounds. Here, we report on the designing of dual-targeted compounds, hybrid molecules (heterodimers) made of two compounds respectively active against HIV and TB. Such a strategy to design heterodimer compounds was already implemented in the late 1990s by Imbach and his associates, who synthesized the first bis-nucleoside phosphates [4]. However, those heterodimers were relatively resistant to enzymatic hydrolysis by phosphodiesterases and thus too stable to

release the active compounds. Other compounds combining two different anti-HIV agents were later synthesized [5–8]. Recently, we reported on the design and synthesis of 3TC-AZT phosphonate, which potentially inhibited HIV replication in human tissue *ex vivo* [9].

Here, we synthesized five dual-targeted HIV/TB heterodimer compounds. Four of them (**1–3, 5**) inhibit both HIV and TB because these molecules are dimers of the anti-HIV agent AZT and, as the anti-TB agent, either 2'-deoxyuridine analogue (**1–3**) or 5'-norcarbocyclic uracil derivative (**5**). Compound (**4**) is a heterodimer of an anti-TB and two anti-HIV reverse transcriptase inhibitors of different types. Using *Mycobacteria* growth indicator tubes, we showed that the newly-synthesized compounds expressed anti-TB activity (compounds **1–3, 5**).

The anti-HIV activity was assessed in two different models: MT-4 cell cultures and human tissues *ex vivo*. MT-4 cell cultures were used because of their typical high level of HIV replication. Human tissue blocks *ex vivo* (histocultures) were used because they uniquely reflect many important functional aspects of cell–cell interactions *in vivo*. The system of human lymphoid tissue *ex vivo* was used earlier for preclinical evaluation of microbicides [27, 28].

Using these two systems, we showed that all the tested compounds inhibited HIV replication. In particular, we found that compounds **1–3** were generally more potent than compounds **4–5**. Since AZT was the anti-HIV agent in all five heterodimers, this suggests that the breakage of the heterodimers into active molecules was dependent on the nature of the linker as well as on the position of the connection between two components of the hybrid. Thus, the activity of the heterodimers seemed to be directly determined by the hydrolytic stability in this system. Heterodimer **3** was the compound with the most potent anti-HIV activity altogether because of the two AZT molecules included. Compounds **4** and **5** were less active than **1–3** in the MT-4 cell system, but in the tissue system the difference between activity levels was not so dramatic. It can be assumed that there are additional mechanisms for the hydrolysis of heterodimers **4–5** in lymphoid tissue.

Drug resistance is a major issue in the treatment of TB. Multidrug-resistant TB (MDR TB) is resistant to at least two of the best anti-TB drugs, isoniazid and rifampin. Commonly, HIV patients with drug-resistant TB have a reduced survival rate compared with those with drug-susceptible TB [29, 30]. Therefore, it is of critical importance that our dual-targeted compounds active against HIV were active not only against a sensitive TB strain but also against a clinical MDR strain, TB MS-115, that is resistant to the five first-line anti-TB drugs isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide.

The concurrent treatment of TB and HIV is generally associated with a higher risk of adverse reactions than treatment of either infection alone, because of overlapping drug toxicities and drug–drug interactions between antiretroviral therapy and anti-TB therapy [26, 31]. Therefore, an efficient dual-targeted drug has obvious advantages. Nevertheless, we carefully addressed the potential toxicity of our dual-targeted compounds. We addressed this issue in human tissues *ex vivo* first, as this model does not require exogenous activation, retains the natural cytoarchitecture of tissue, and contains all cell types found in tissue *in*

vivo, thus making this model an adequate tool to approximate cell toxicity *in vivo* [14]. To exclude potential depletion of even a small but important subset of cells, we addressed cytotoxicity in 12 different cell subsets, and in none of them did we find cell loss at concentrations of compounds that suppressed both HIV and TB. Even when the compounds were used at 50 μ M (i.e., between 10 and 350 times the EC₅₀ depending on the compound), mild cell loss was only noticed for some heterodimers and for some selected subsets. The lack of general toxicity of the tested heterodimers was confirmed in MT-4 cell cultures. In these cultures, we also addressed the cytostaticity of the heterodimers. We found that none of the compounds at concentrations that suppress HIV replication arrested cell growth.

In conclusion, we successfully designed and synthesized five dual-targeted compounds that inhibit HIV and TB *in vitro* and *ex vivo*. The compounds described here are the first examples of nucleoside derivatives possessing both anti-HIV and anti-tuberculosis activities. Heterodimers **1–5** are depot drugs comprising two active components and having different rates of release: they are either rapidly (**1–3**) or slowly (**4–5**) hydrolyzable compounds. To optimize the combination of the active components and their binding linker, further research is needed, in particular the evaluation of the pharmacokinetic parameters of anti-HIV and anti-TB agents released in the blood of laboratory animals. We found that one particular heterodimer, compound **3**, was the most potent in inhibiting HIV and the drug-resistant TB strain MS -115. This compound **3** was also not toxic and had no cytopathic effect on cell cultures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work of CV, SZ, and LM was supported by the NIH office of AIDS Research – Intramural-to-Russian (I-to-R) Program and the NICHD Intramural Program. The synthetic work of LA and EM was supported by the joint NIH–RFBR grant NIZ_aNo13-04-91441. The work of AK and SK, as well as all physico-chemical analysis, was supported by Russian Science Foundation project No14-50-00060. We thank the entire staff of the Department of Pathology of Children’s National Medical Center for their generous assistance in obtaining human tonsillar tissues.

References

1. Organization WH. Global tuberculosis report 2016. Geneva, Switzerland: World Health Organization; 2016.
2. Suchindran S, Brouwer ES, Van Rie A. Is HIV infection a risk factor for multi-drug resistant tuberculosis? A systematic review. PLoS One. 2009; 4:e5561. [PubMed: 19440304]
3. Pawlowski A, Jansson M, Skold M, Rottenberg ME, Kallenius G. Tuberculosis and HIV co-infection. PLoS Pathog. 2012; 8:e1002464. [PubMed: 22363214]
4. Puech F, Gosselin G, Balzarini J, Good SS, Rideout JL, De Clercq E, et al. Synthesis and biological evaluation of dinucleoside methylphosphonates of 3'-azido-3'-deoxythymidine and 2', 3'-dideoxycytidine. Antiviral Res. 1990; 14:11–23. [PubMed: 1964371]
5. Velazquez S, Alvarez R, San-Felix A, Jimeno ML, De Clercq E, Balzarini J, et al. Synthesis and anti-HIV activity of [AZT]-[TSAO-T] and [AZT]-[HEPT] dimers as potential multifunctional inhibitors of HIV-1 reverse transcriptase. J Med Chem. 1995; 38:1641–1649. [PubMed: 7538589]
6. Velazquez S, Tunon V, Jimeno ML, Chamorro C, De Clercq E, Balzarini J, et al. Potential multifunctional inhibitors of HIV-1 reverse transcriptase. Novel [AZT]-[TSAO-T] and [d4T]-

- [TSAO-T] heterodimers modified in the linker and in the dideoxynucleoside region. *J Med Chem.* 1999; 42:5188–5196. [PubMed: 10602704]
7. Pontikis R, Dolle V, Guillaumel J, Dechaux E, Note R, Nguyen CH, et al. Synthesis and evaluation of “AZT-HEPT”, “AZT-pyridinone”, and “ddC-HEPT” conjugates as inhibitors of HIV reverse transcriptase. *J Med Chem.* 2000; 43:1927–1939. [PubMed: 10821705]
 8. Laduree D, Sugeac E, Fossey C, Schmidt S, Laumond G, Aubertin AM. Synthesis of certain heterodimers expected as HIV-1 reverse transcriptase inhibitors. *Nucleosides Nucleotides Nucleic Acids.* 2003; 22:873–875. [PubMed: 14565300]
 9. Vanpouille C, Khandazhinskaya A, Karpenko I, Zicari S, Barreto-de-Souza V, Frolova S, et al. A new antiviral: chimeric 3TC-AZT phosphonate efficiently inhibits HIV-1 in human tissues ex vivo. *Antiviral Res.* 2014; 109:125–131. [PubMed: 25010891]
 10. Matyugina E, Novikov M, Babkov D, Ozerov A, Chernousova L, Andreevskaya S, et al. 5-Arylaminoouracil Derivatives: New Inhibitors of *Mycobacterium tuberculosis*. *Chem Biol Drug Des.* 2015; 86:1387–1396. [PubMed: 26061192]
 11. Shmalenyuk ER, Chernousova LN, Karpenko IL, Kochetkov SN, Smirnova TG, Andreevskaya SN, et al. Inhibition of *Mycobacterium tuberculosis* strains H37Rv and MDR MS-115 by a new set of C5 modified pyrimidine nucleosides. *Bioorg Med Chem.* 2013; 21:4874–4884. [PubMed: 23891229]
 12. Shirokova EA, Jasko MV, Khandazhinskaya AL, Ivanov AV, Yanvarev DV, Skoblov YS, et al. Uncharged AZT and D4T derivatives of phosphonoformic and phosphonoacetic acids as anti-HIV pronucleosides. *J Med Chem.* 2004; 47:3606–3614. [PubMed: 15214788]
 13. Khandazhinskaya AL, Yanvarev DV, Jasko MV, Shipitsin AV, Khalizev VA, Shram SI, et al. 5'-aminocarbonyl phosphonates as new zidovudine depot forms: antiviral properties, intracellular transformations, and pharmacokinetic parameters. *Drug Metab Dispos.* 2009; 37:494–501. [PubMed: 19106116]
 14. Grivel JC, Margolis L. Use of human tissue explants to study human infectious agents. *Nat Protoc.* 2009; 4:256–269. [PubMed: 19197269]
 15. Biancotto A, Brichacek B, Chen SS, Fitzgerald W, Lisco A, Vanpouille C, et al. A highly sensitive and dynamic immunofluorescent cytometric bead assay for the detection of HIV-1 p24. *J Virol Methods.* 2009; 157:98–101. [PubMed: 19100289]
 16. Matyugina ES, Andreevskaya SN, Smirnova TG, Khandazhinskaya AL. Carbocyclic analogues of inosine-5'-monophosphate: synthesis and biological activity. *Acta Naturae.* 2012; 4:73–77. [PubMed: 23346382]
 17. Bruchfeld J, Correia-Neves M, Kallén G. Tuberculosis and HIV Coinfection. *Cold Spring Harb Perspect Med.* 2015; 5:a017871. [PubMed: 25722472]
 18. Moreno S, Baraia-Etxaburu J, Bouza E, Parras F, Perez-Tascon M, Miralles P, et al. Risk for developing tuberculosis among anergic patients infected with HIV. *Ann Intern Med.* 1993; 119:194–198. [PubMed: 8100693]
 19. Antonucci G, Girardi E, Raviglione MC, Ippolito G. Risk factors for tuberculosis in HIV-infected persons. A prospective cohort study. The Gruppo Italiano di Studio Tubercolosi e AIDS (GISTA). *JAMA.* 1995; 274:143–148. [PubMed: 7596002]
 20. Holmes CB, Wood R, Badri M, Zilber S, Wang B, Maartens G, et al. CD4 decline and incidence of opportunistic infections in Cape Town, South Africa: implications for prophylaxis and treatment. *J Acquir Immune Defic Syndr.* 2006; 42:464–469. [PubMed: 16810113]
 21. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, Ellner J. Accelerated course of human immunodeficiency virus infection after tuberculosis. *Am J Respir Crit Care Med.* 1995; 151:129–135. [PubMed: 7812542]
 22. Badri M, Ehrlich R, Wood R, Maartens G. Initiating co-trimoxazole prophylaxis in HIV-infected patients in Africa: an evaluation of the provisional WHO/UNAIDS recommendations. *AIDS.* 2001; 15:1143–1148. [PubMed: 11416716]
 23. Lopez-Gatell H, Cole SR, Hessol NA, French AL, Greenblatt RM, Landesman S, et al. Effect of tuberculosis on the survival of women infected with human immunodeficiency virus. *Am J Epidemiol.* 2007; 165:1134–1142. [PubMed: 17339383]

24. Vanham G, Edmonds K, Qing L, Hom D, Toossi Z, Jones B, et al. Generalized immune activation in pulmonary tuberculosis: co-activation with HIV infection. *Clin Exp Immunol.* 1996; 103:30–34. [PubMed: 8565282]
25. Wolday D, Tegbaru B, Kassu A, Messele T, Coutinho R, van Baarle D, et al. Expression of chemokine receptors CCR5 and CXCR4 on CD4+ T cells and plasma chemokine levels during treatment of active tuberculosis in HIV-1-coinfected patients. *J Acquir Immune Defic Syndr.* 2005; 39:265–271. [PubMed: 15980685]
26. Piggott DA, Karakousis PC. Timing of antiretroviral therapy for HIV in the setting of TB treatment. *Clin Dev Immunol.* 2011; 2011:103917. [PubMed: 21234380]
27. Lisco A, Vanpouille C, Tchesnokov EP, Grivel JC, Biancotto A, Brichacek B, et al. Acyclovir is activated into a HIV-1 reverse transcriptase inhibitor in herpesvirus-infected human tissues. *Cell Host Microbe.* 2008; 4:260–270. [PubMed: 18779052]
28. Andrei G, Lisco A, Vanpouille C, Introvini A, Balestra E, van den Oord J, et al. Topical tenofovir, a microbicide effective against HIV, inhibits herpes simplex virus-2 replication. *Cell Host Microbe.* 2011; 10:379–389. [PubMed: 22018238]
29. Chan ED, Iseman MD. Multidrug-resistant and extensively drug-resistant tuberculosis: a review. *Curr Opin Infect Dis.* 2008; 21:587–595. [PubMed: 18978526]
30. Gunther G, Gomez GB, Lange C, Rupert S, van Leth F, Tbnnet. Availability, price and affordability of anti-tuberculosis drugs in Europe: a TBNET survey. *Eur Respir J.* 2015; 45:1081–1088. [PubMed: 25395035]
31. Sterling TR, Pham PA, Chaisson RE. HIV infection-related tuberculosis: clinical manifestations and treatment. *Clin Infect Dis.* 2010; 50(Suppl 3):S223–230. [PubMed: 20397952]

Highlights

- We synthesized five heterodimers of an anti-TB molecule and an anti-HIV molecule.
- These compounds inhibited growth of *M. tuberculosis* and replication of HIV in a cell line and in human lymphoid tissue.
- One compound that combined the strongest anti-bacterial and anti-viral effects was neither cytopathic nor cytostatic.
- This compound is a leading candidate for future development and *in vivo* testing.

Figure 1. Scheme of synthesis of heterodimers

A. Scheme 1: synthesis of heterodimers of AZT and 5-(4-decyl-1,2,3-triazol-1-yl-methyl)-2'-deoxyuridine.

B. Scheme 2: Synthesis of heterodimers of AZT and the 5'-modified norcarbocyclic analogues of 2', 3'-dideoxy-2', 3'-didehydrouridine.

Figure 2. Cell toxicity of heterodimer-inoculated *ex vivo*-cultured human lymphoid tissues
 Tissue blocks (27 from each individual human tonsil donor) were treated with heterodimers 1–5 at 10 and 50 μ M. At day 12, cells were stained with anti-CD3-QD655, anti-CD4-QD605, anti-CD8-QD800, anti-CD45RA-FITC, and anti-CCR7-PE-Cy7 (stain 1) or stained with anti-CD3-QD655, anti-CD4-QD605, anti-CD8-QD800, CD25-APC, CD38-PE, CD95-PE-Cy7, and HLA-DR-BV570 (stain 2). We evaluated the cytopathicity of the heterodimers for different cell subsets by comparing the numbers of cells in treated tissue blocks with those in matched untreated control tissue blocks. To account for size differences

in tissue blocks, we normalized the data by the weight of the tissues. Presented are means $\pm$ SEM (error bars) of six independent experiments performed with tissues from six different donors.

Figure 3.

Scheme 1.

Scheme 2.

Table 1Anti-HIV activity of compounds **1–5** in MT-4 cell cultures and in human tissues *ex vivo*

	Tonsil <i>ex vivo</i>		MT-4	
	EC ₅₀ (μM)	95% confidence interval	EC ₅₀ (μM)	95% confidence interval
1	1.32	0.37–4.73	0.39	0.24–0.65
2	1.28	0.10–15.95	0.07	0.05–0.10
3	0.14	0.08–0.25	0.08	0.04–0.15
4	20.14	1.10–368.3	19.77	12.51–31.22
5	1.29	0.35–4.77	12.94	10.29–16.26
AZT	0.003	0.001–0.16	0.03	0.021–0.043

Table 2MIC (99) values for the compounds tested against *M. tuberculosis* strains

Compounds	MIC (99), µg/mL	
	<i>M.tuberculosis</i> H37Rv	<i>M.tuberculosis</i> MS-115
1	50 (67µM)	50 (67µM)
2	10 (13.5µM)	20 (27µM)
3	20 (19.3µM)	10 (9.7 µM)
5	>50 (75µM)	50 (75µM)

Table 3

Evaluation of cell toxicity in MT-4 cell cultures

	MT-4		MT-4	
	CC ₅₀	95% confidence interval	IC ₅₀ (μM)	95% confidence interval
1	89.87	43.63–185.1	29.19	22.18–38.41
2	112.6	59.32–213.7	33.19	20.48–53.80
3	>300		472.1	very wide
4	53.62	30.32–94.82	14.25	12.67–160.2
5	46.96	39.59–55.69	17.5	15.2–20.14
AZT	> 100		115.5	69.83–191.1

CC₅₀s (concentrations that reduce the viability of the MT-4 by 50%) and IC₅₀s (concentrations that inhibit MT-4 or CEM cell growth by 50%)