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Structure-based design, synthesis, and in vitro assay of novel nucleoside analog inhibitors against HIV-1 reverse transcriptase

Xianjun Liu, Wei Xie and Raven H. Huang*

Department of Biochemistry, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801, USA

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Abstract—Crystal structure of HIV-RT in complex with a DNA template:primer and a dTTP leads us to design and synthesize a new class of nucleoside analog inhibitors containing a branched 3′-group against HIV-RT. An in vitro primer extension assay indicates that three out of five compounds are effective HIV-RT inhibitors.

The human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS), which is responsible for the death of millions of people worldwide.[1,2] Proliferation of HIV requires that its RNA genome be converted into a double-stranded DNA copy that is then integrated into the host genome. Reverse transcriptase (RT) is responsible for catalyzing this conversion and therefore it is a primary target for therapeutic agents, such as AZT.[3,4] However, if an RT inhibitor is administered alone, drug resistance increases rapidly, compromising the effectiveness of treatment.[5,6] To circumvent this problem, a combination therapy that usually includes three inhibitors—two against RT and one against HIV protease—is employed, which substantially reduces the death rate of AIDS patients.[7] Despite this progress, the battle against the AIDS epidemic is far from over, as indicated by an ever-increasing population of people infected by HIV and the continuing death caused by the disease.[8] Clearly, more and better drugs are needed.

We have reported a crystal structure of RT in complex with a DNA template:primer and a dTTP.[9] Inspection of the structure surrounding the active site of RT indicates that the 3′-hydroxyl group of the resident dTTP, which is modified in the nucleoside analog drugs, projects into a pocket (Fig. 1). Surprisingly, this '3′-pocket' is large, certainly having enough space for a larger group, such as the azido group of AZT. This may

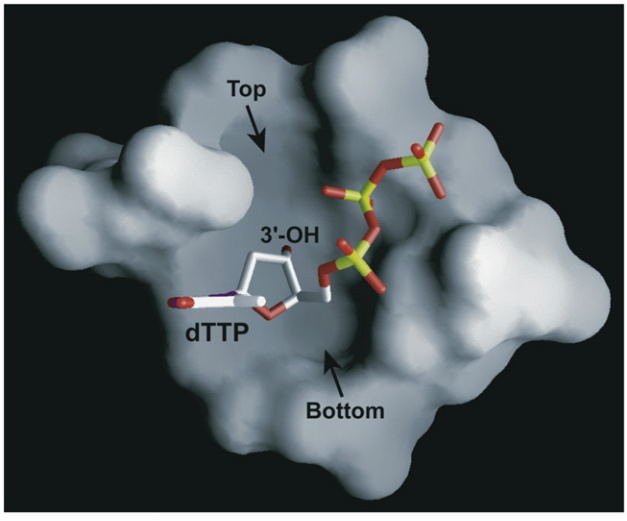

Figure 1. Surface representation of a structure surrounding the polymerase active site of RT, with emphasis on the 3′-pocket. The protein part is shown in gray. dTTP is shown as a stick model and is colored by individual atoms (oxygen, red; phosphate, yellow; nitrogen, blue; carbon, white). Arrows indicate the top and bottom portions of the 3′-pocket.

Keywords: RT inhibitors; Nucleoside analogs; Structure-based design; HIV-RT; Primer extension assay.
* Corresponding author. Tel.: +1 217 333 3967; fax: +1 217 244 5858; e-mail: huang@uiuc.edu

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explain the effectiveness of AZT as an inhibitor of HIV-RT but not human DNA polymerases, because human DNA polymerases may not possess such a large 3′-pocket. However, when we tried to model AZTTP, which is an active form of AZT in vivo, into the position of dTTP in the structure, we found that the azido group could be placed either in the top portion or in the bottom portion of the 3′-pocket, which are oriented roughly in the opposite direction (Fig. 1). We reasoned that a nucleoside analog that contains a branched 3′-group, with a sp^2 hybridized central atom to orient the two attached groups in the opposite direction, might fit the 3′-pocket better, and therefore could be a more selective RT inhibitor because it would accentuate further the structural differences between the RT and human DNA polymerases. This reasoning, together with the consideration of limited size of the 3′-pocket, led us to design and synthesize compounds **2**, **3**, and **4** (Fig. 2). In addition to these compounds, we included compounds **5** and **6**, which contain a 3′-group that is one methylene unit larger than that in **3** and **4**, in our synthesis for comparison. Our molecular modeling predicted that the 3′-pocket of HIV-RT should have enough room to accommodate a small branched 3′-group shown in **2**, **3**, or **4**, but not the ones in **5** or **6** without any substantial structural perturbation (data not shown).

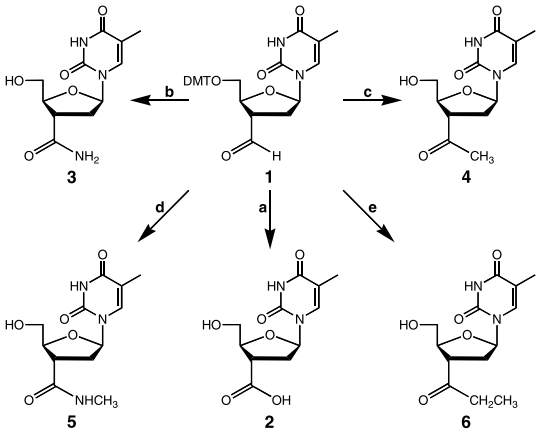

Figure 2. Chemical synthesis of designed nucleoside analogs. Reagents: (a) I_2, KOH, CH_3CN/H_2O, CCl_3COOH/H_2O; (b) NBS, AIBN, NH_3, CCl_3COOH/H_2O; (c) MeMgBr, $CeCl_3$, Dess–Martin reagent, CCl_3COOH/H_2O; (d) NBS, AIBN, CH_3NH_2, CCl_3COOH/H_2O; (e) CH_3CH_2MgBr, $CeCl_3$, Dess–Martin reagent, CCl_3COOH/H_2O.

To construct the desired nucleoside analogs with a branched 3′-group, the key intermediate aldehyde was synthesized through a known protocol (**1** in Fig. 2).[10] All the new derivatives of **1** were synthesized according to a general strategy illustrated in Figure 2. Oxidation of aldehyde **1** to the corresponding carboxylic acid with iodine in aqueous alkaline solution,[11] followed by detritylation with trichloroacetic acid, resulted in **2**. In an attempt to convert the aldehyde into the amides **3** and **5**, several methods were examined. The best result was obtained by utilizing radical-mediated oxidation.[12] Aldehyde **1** was first treated with NBS in the presence of a catalytic amount of AIBN. The reaction mixture was then cooled and treated with a slight excess of ammonia or methylamine to give **3** and **5**, respectively, after detritylation. Ketones **4** and **6** were prepared first by alkylation of the aldehyde with Grignard reagents in the presence of $CeCl_3$[13] and followed by oxidation. Specifically, the reaction of MeMgBr or CH_3CH_2MgBr in the presence of an equal-molar amount of $CeCl_3$ with **1** in THF delivered the corresponding alcohols, which were used directly in the next step reaction without further purification. Dess–Martin oxidation in CH_2Cl_2 and subsequent detritylation with trichloroacetic acid afforded **4** and **6**. In addition, compounds **2**–**6**, together with ddT and AZT, were converted into their corresponding triphosphate derivatives using a published procedure to carry out the in vitro assay (see supporting material for experimental details).[14]

A primer extension assay, analogous to the Sanger DNA sequencing,[15] was employed to analyze the efficacy of these compounds as RT inhibitors.[16] For this purpose, a DNA template:primer was designed such that there is only one **A** (colored in red in Fig. 3a) centered in the single-stranded region of the template. In the presence of dNTP (mixture of four nucleotides,

Figure 3. An in vitro primer extension assay for the efficacy of compounds **2**–**6** as possible RT inhibitors. (a) Nucleotide sequence of the DNA template:primer used in the assay. (b) Denaturing polyacrylamide gel electrophoresis analysis of reaction samples carried out under the conditions indicated. * designates the radiolabeled oligonucleotides. P, primer; SFP, synthetic full-length product; STP, synthetic terminated product.

Table 1. Quantitative data of primer extension assays

Reaction time (min)	Inhibitors				
	ddTTP	AZTTP	**2**TP	**3**TP	**4**TP
5	37.1	14.4	15.0	15.1	15.7
20	37.7	15.0	14.7	14.9	14.7
60	35.4	14.5	14.6	15.2	16.3

The number is the percentile of the intensity of the terminated product divided by that of the full-length product, averaged from three independent experiments.

dATPs, dCTP, dGTP, and dTTP) and HIV-RT, the primer was fully extended (Fig. 3b, lane 4, and compare it to lane 2). Addition of ddTTP or AZTTP to the reaction mixtures resulted in chain termination, as indicated by the appearance of a band with the same mobility as the molecular marker (Fig. 3b, lanes 5 and 6, and compare them to lane 3). This is consistent with the fact that both ddTTP and AZTTP are substrates of RT and are chain terminators. Addition of the triphosphate derivatives of compounds **2**, **3**, and **4** (named **2**TP, **3**TP, and **4**TP, respectively) also resulted in the appearance of chain-terminated band of the primer extension (Fig. 3b, lanes 7, 8, and 9). These results clearly indicate that **2**TP, **3**TP, and **4**TP are substrates of RT. As the amounts of AZTTP, **2**TP, **3**TP, and **4**TP used in these reactions were the same, the fact that the ratio of the full-length product to the terminated product was roughly the same in these lanes (Table 1) indicates that **2**TP, **3**TP, and **4**TP are as effective substrates of RT as AZTTP in vitro. Interestingly, no chain termination products were observed when **5**TP or **6**TP was added to the reaction mixture (Fig. 3b, lanes 10 and 11), indicating that they are not substrates of RT. Considering that **5** and **6** contain a 3′-group that is only one methylene unit larger than that in **3** and **4**, the selectivity of RT is remarkable.

In conclusion, we have designed and synthesized five nucleoside analogs containing a branched 3′-group. An in vitro primer extension assay indicates that the three analogs with smaller 3′-group are effective RT inhibitors. We are currently in the process of arranging for them to be tested in an HIV-infected cell line for their anti-HIV activity in vivo. The study described here provides a blueprint for the development of new classes of nucleoside analog inhibitors. As in the case of other RT inhibitors, it is likely that drug resistance will arise if one of the inhibitors described here is administered alone. However, the risk of drug resistance can be reduced with the adoption of the combination therapy described previously. More importantly, because of the change of chemical structures at the 3′-position in our inhibitors as compared to the current drugs, the resistance profile, if it were to develop, could very well be different because the profile of drug resistance has been shown to be correlated to the chemical structure of the drug.[9] Therefore, this new class of inhibitors may help circumvent the current drug resistance profiles and, together with currently available HIV-RT drugs, may allow for a broader spectrum of combinatorial therapies.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.05.099.

References and notes

1. Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868.
2. Gallo, R. C.; Salahuddin, S. Z.; Popovic, M.; Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safai, B., et al. *Science* **1984**, *224*, 500.
3. Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 1533.
4. Havlir, D. V.; Richman, D. D. *Ann. Intern. Med.* **1996**, *124*, 984.
5. Oxford, J. S.; al-Jabri, A. A.; Stein, C. A.; Levantis, P. *Methods Enzymol.* **1996**, *275*, 555.
6. Moyle, G. J. *J. Antimicrob. Chemother.* **1997**, *40*, 765.
7. Palella, F. J., Jr.; Delaney, K. M.; Moorman, A. C.; Loveless, M. O.; Fuhrer, J.; Satten, G. A.; Aschman, D. J.; Holmberg, S. D. *N. Engl. J. Med.* **1998**, *338*, 853.
8. UNAIDS *AIDS Epidemic Update*; UNAIDS: www.unaids.org, 2004.
9. Huang, H.; Chopra, R.; Verdine, G. L.; Harrison, S. C. *Science* **1998**, *282*, 1669.
10. Swyze, E. E.; Sanghvi, Y. S. *Syn. Lett.* **1997**, *7*, 859.
11. Yamada, S.; Morizono, D.; Yamamoto, K. *Tetrahedron Lett.* **1992**, *33*, 4329.
12. Marko, I. E.; Mekhalfia, A. *Tetrahedron Lett.* **1990**, *31*, 7237.
13. Imamoto, T.; Takiyama, N.; Nakamura, K.; Hatajima, T.; Kamiya, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4392.
14. Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1993**, *32*, 10489.
15. Church, G. M.; Kieffer-Higgins, S. *Science* **1988**, *240*, 185.
16. HIV-1 RT was overexpressed and purified according to a published procedure.[9] The DNA primer was radiolabeled at the 5′-end by T4 polynucleotide kinase and [γ-^{32}P]ATP. The radiolabeled DNA primer was annealed to the DNA template using a standard procedure. For a typical primer extension reaction, 15 μL of DNA duplex containing 45 nM DNA primer and 30 nM of DNA template was mixed with 6 μL of dNTP (containing 100 μM of each dGTP, dATP, dCTP, and dTTP), 6 μL of 200 μM XTP (X = ddT, AZT, **2**, **3**, **4**, **5**, or **6**), 6 μL of RT (0.3 mg/mL), and 3 μL of 0.1 mM DTT in a 1× DNA sequenase buffer. The extension reaction was allowed to proceed at 25 °C. Twelve microliters of reaction mixture was taken out at the reaction time points of 5, 20, and 60 min, and the reaction was terminated by addition of 5 μL of DPAGE loading buffer and heating of the sample at 90 °C for 5 min. Five microliters of the mixture was analyzed by denaturing PAGE.