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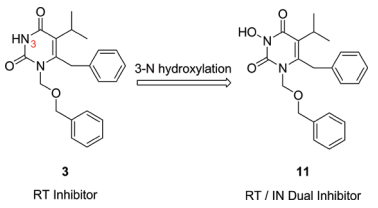
N-3 Hydroxylation of Pyrimidine-2,4-diones Yields Dual Inhibitors of HIV Reverse Transcriptase and Integrase

Jing Tang,[†] Kasthuraiah Maddali,[‡] Christine D. Dreis,[†] Yuk Y. Sham,[†] Robert Vince,[†] Yves Pommier,[‡] and Zhengqiang Wang*[,†]

[†]Center for Drug Design, Academic Health Center, University of Minnesota, 516 Delaware Street SE, Minneapolis, Minnesota 55455, United States, and [‡]Laboratory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, United States

ABSTRACT A new molecular scaffold featuring an *N*-hydroxyimide functionality and capable of inhibiting both reverse transcriptase (RT) and integrase (IN) of human immunodeficiency virus (HIV) was rationally designed based on 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) non-nucleoside RT inhibitors (NNRTIs). The design involves a minimal 3-N hydroxylation of the pyrimidine ring of HEPT compound to yield a chelating triad which, along with the existing benzyl group, appeared to satisfy major structural requirements for IN binding. In the mean time, this chemical modification did not severely compromise the compound's ability to inhibit RT. A preliminary structure−activity relationship (SAR) study reveals that this N-3 OH is essential for IN inhibition and that the benzyl group on N-1 side chain is more important for IN binding than the one on C-6.

KEYWORDS HIV, integrase, reverse transcriptase, dual inhibitor, rational design



HEPT (**1**)[1] and its analogues constitute an important class of HIV NNRTIs,[2] with the best ones being MKC-442 (emivirine, **2**)[3−5] and TNK-651 (**3**)[6] (Figure 1). The clinical development of these compounds was halted as they were found to stimulate liver enzyme CYP 3A4 and cause prohibitively low bioavailability of protease inhibitors used in combination therapy.[7] Drug−drug interactions of this type pose a major barrier to successful highly active antiretroviral therapy (HAART)against HIV/AIDS.[8,9] Simplifying regimens by reducing the number of drugs used in HAART could alleviate this issue. Toward this end, we have long been interested in identifying molecular scaffolds with dually inhibitory activities, particularly against HIV RT and IN.[10−12]

The design of inhibitors that can accommodate binding to two distinct biological targets remains an intriguing yet challenging scientific endeavor.[13−15] Our previous work has demonstrated the viability of constructing RT/IN dual inhibitors from NNRTIs using a design-in strategy. Significantly, IN binding requires minimally a two metal ion chelating functionality and a hydrophobic benzyl group (Figure 2). Certain NNRTI scaffolds, including HEPT[16] and BHAP[17] (e.g., delavirdine, **9**), have structural moieties that are sitting on the P236 loop of the NNRTI binding pocket and are not directly involved in RT binding. These moieties provide valuable handles (highlighted, Figure 3) for incorporating a relatively hydrophilic chelating functionality (boxed) to satisfy binding to the IN (Figure 3).[10,12] Most other NNRTIs are deeply buried in the highly hydrophobic binding pocket, thus are less amenable to hydrophilic modifications. In these cases, designing in structural components for IN binding requires careful considerations of all binding interactions of NNRTIs.

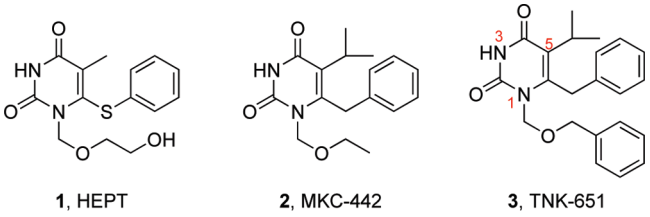


Figure 1. Representative structures of HEPT type NNRTIs.

Notably the NNRTI binding pocket is largely hydrophobic and few H-bonds are accommodated. For the HEPT type of NNRTIs, the H-bond between the N(3)H of the inhibitor (e.g., inhibitor **3**, Figure 4) and the backbone carbonyl group of K101 represents the major hydrophilic interaction.[18] This H-bond contributes to the NNRTI binding affinity and, more importantly, serves as an anchor for the inhibitor to adopt optimized conformation so that hydrophobic interactions can be maximized throughout the binding pocket. Additional water mediated H-bonds may be present, albeit of much less significance.[3] Therefore, the N(3)H group is generally considered too important to be modified. In our design, we hypothesize that hydroxylation of the N-3 of compound **3** would not significantly compromise its binding affinity to RT. This hypothesis is based on two observations: (1) the newly incorporated OH would retain H-bonding ability; and (2) the NNRTI binding pocket has four consecutive lysine residues (K101−K104) that could potentially H-bond to the

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newly introduced OH. Since this minimal modification only results in an insertion of an oxygen atom to NH, we expect that the key H-bond can be formed between the OH and one of these lysine residues, and the overall conformation of the inhibitor should be largely preserved. On the other hand, such a simple hydroxylation yields a C(2)O−N(3)OH−C(4)O chelating triad (**11**, Figure 4) capable of chelating two magnesium ions, which along with an existing hydrophobic benzyl group at N-1 or C-6 will essentially provide the minimal pharmacophore for IN binding (Figure 4). Therefore, through a simple N-3 hydroxylation we could convert these HEPT type NNRTIs into RT/IN dual inhibitors.

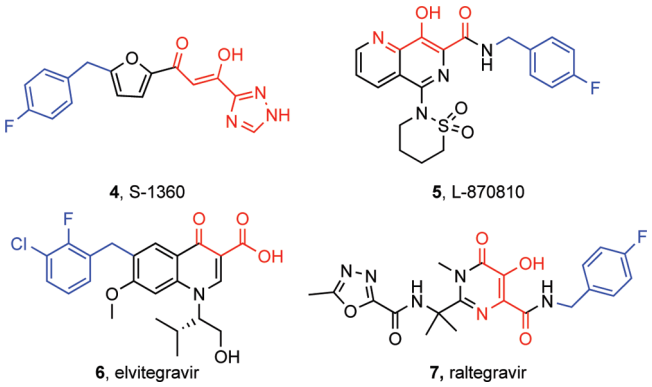

Figure 2. Structures of important IN inhibitors: the chelating functionality (red) and hydrophobic benzyl group (blue) represent the minimal pharmacophore.

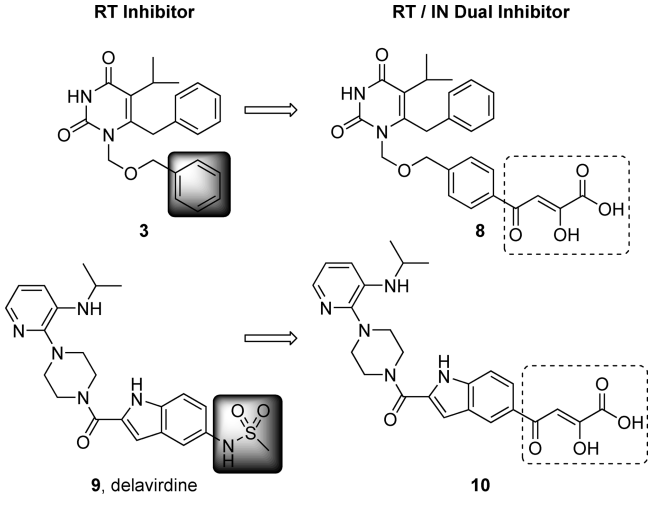

Figure 3. Previously designed RT/IN dual inhibitors based on NNRTIs.

This design is verified in silico by molecular modeling. All modeling was carried out using the Schrödinger modeling suite package.[19] Docking of all compounds was carried out using Glide v2.5 at Standard Precision.[20] For docking into HIV-1 IN, both Mg^{2+} ions and the interfacial hydrophobic pocket between the HIV-1 IN and DNA were defined as required constraints. Docking of **3** and **11** reveals their mode of binding in the NNRTI binding pocket almost identically (Figure 5A). Interestingly the OH of **11** and NH of **3** are both situated in close proximity to the K101 backbone (1.7 Å and 1.6 Å respectively). Accordingly, the H-bonding allows both inhibitors to adopt a nearly identical conformation in the binding pocket, suggesting that N-3 hydroxylation should not significantly compromise RT binding. Docking of **11** into our recent homology model[21] of HIV-1 IN catalytic core domain (CCD) in complex with Mg^{2+} and DNA indicated the newly designed inhibitor **11** also showed reasonable IN binding (Figure 5B). The 3-N hydroxylation of the pyrimidine ring yields a chelating triad for Mg^{2+} binding while allowing the placement of the benzyl group into the protein−DNA interfacial hydrophobic pocket, a crucial pharmacophore requirement for potent HIV-1 ST inhibition.[22,23]

The designed inhibitors are synthetically accessed through a route outlined in Scheme 1. The synthesis of advanced intermediates **2**, **3** and **29**−**33** is well-established in the literature[24] whereas the key transformation, the N-3 hydroxylation, is rarely known. This was achieved through deprotonation of HEPT intermediates and the subsequent treatment with an oxidizing agent. After screening of a wide array of oxidants it was found that *m*-CPBA can effect this N-3 hydroxylation efficiently (Scheme 1). Adaptation of this method also allowed us to successfully prepare an N-3 amino analogue (**16**), wherein mesityl sulfonyl-*O*-hydroxyl amine (MSH)[25] was employed as the aminating agent.

Testing of these compounds against recombinant HIV IN provided the first validation of our design. In this assay both

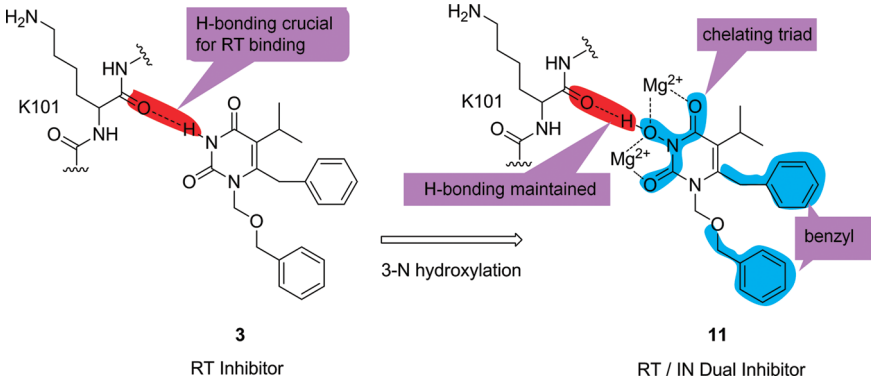

Figure 4. The design of novel RT/IN dual inhibitor **11** from NNRTI **3**. The H-bond crucial to RT binding is maintained (red) and the conformation of the molecule remains unchanged. The N-3 hydroxylation yields a chelating triad (blue), a critical structural component for IN binding.

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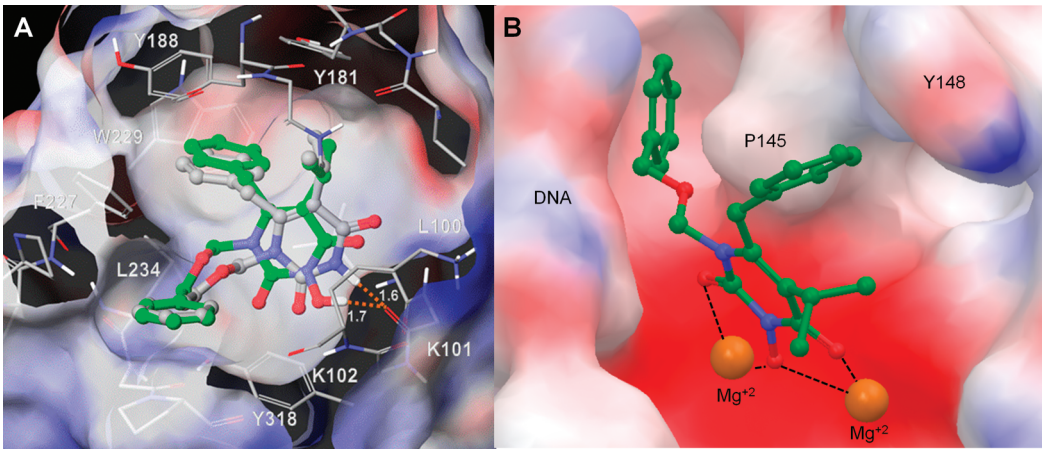
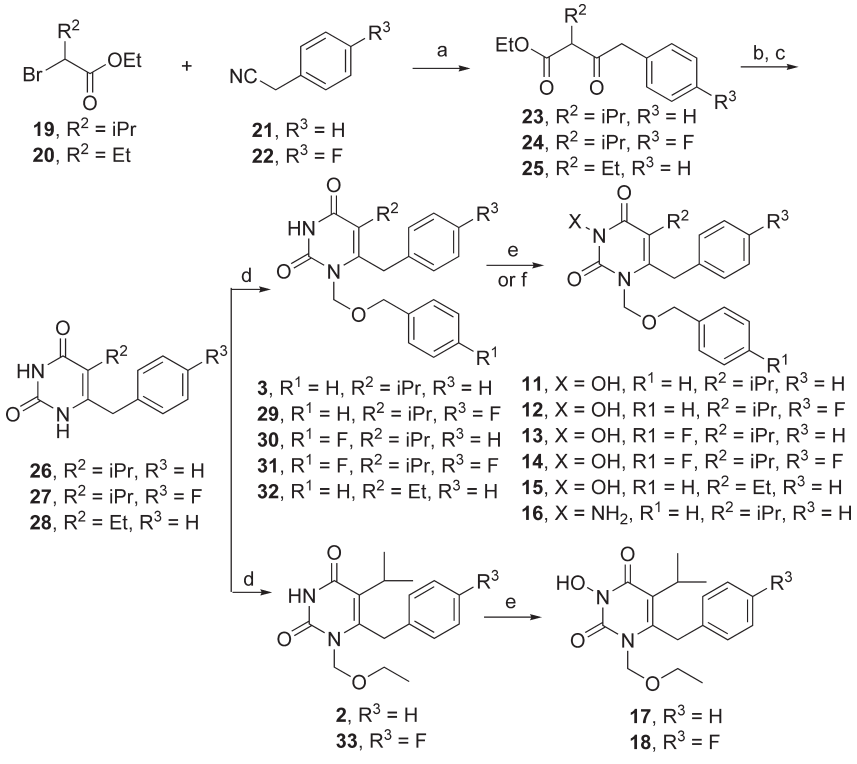


Figure 5. (A) Mode of binding of **3** (gray) and **11** (green) in the HIV NNRTI binding pocket. Both compounds hydrogen bonded to the backbone carbonyl oxygen atom of K101. (B) Mode of binding of **11** (green) in HIV-1 IN CCD in complex with Mg^{2+} and DNA. The 3-N hydroxyl group simultaneously chelates to both Mg^{2+} ions while allowing the placement of the benzyl group into the protein−DNA interfacial hydrophobic pocket. The electrostatic potential surface of the binding pocket is shown to highlight the hydrophobic regions (white) in both enzymes.

Scheme 1[a]



[a] Reagents and conditions: (a) Zn, I_2 (cat.), THF, 89−90%; (b) thiourea, KOtBu, *i*-PrOH, 40−71%; (c) $ClCH_2CO_2H$, AcOH, H_2O, 38−78%; (d) $CH_3C(OTMS)$═NTMS (BSA), chloromethyl ether, TBAI (cat.), CH_2Cl_2, rt, 51−89%; (e) NaH, *m*-CPBA, THF, rt, 50−73%; (f) NaH, MSH, THF, rt, 54%.

the 3′ processing (3′P) and the strand transfer (ST) activities, the two primary functions of IN, were assessed. As shown in Table 1, all N-3 hydroxylated compounds (**11**−**15**, **17**, **18**) selectively inhibit ST over 3′P at low micromolar concentrations, whereas the unmodified compound (**3**) did not show any inhibitory activity against HIV IN. This observation strongly suggests that the N-3 hydroxyl group, partially forming the chelating triad (Figure 4, **11**), is crucial to IN binding. Interestingly, the N-3 amino compound (**16**) also turned out inactive in IN assay. It is not surprising that the N-3 hydroxylation yields a better chelating triad than the N-3 amination as O tends to have a higher affinity for Mg^{2+} than N. In addition, compounds **17** and **18** demonstrate significantly reduced (ca. 10-fold) inhibition against ST when compared with compounds **11**−**15**, indicating

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Table 1. Inhibitory Activities of Compounds **11**−**18** against HIV IN

	IC_{50}[a] (μM)	
compd	3′P	ST
11	> 333	21
12	> 333	7.3
13	> 333	3.5
14	> 333	5.5
15	> 333	8.2
16	> 333	> 333
17	> 333	85
18	> 333	105
3	> 333	> 333

[a] Concentration inhibiting enzyme activity by 50 %.

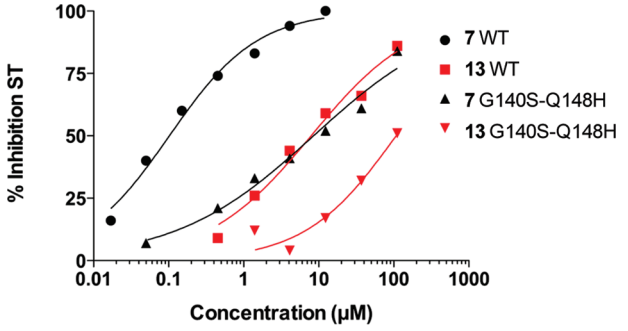

Figure 6. Comparison of newly designed inhibitor **13** with raltegravir **7** in recombinant HIV IN with G140S and Q148H mutants.

that the benzyl group at the N-1 side chain is far more important for IN binding than the one at the C-6 position of the pyrimidine-2,4-dione ring (Figure 4, compound **11**).

To further characterize the mechanism of action of our newly designed compounds, a representative compound **13** was tested head-to-head with raltegravir (**7**, Figure 2) against a recombinant HIV IN containing major resistant mutations G140S and Q148H (Figure 6).[26,27] This assay revealed that, although inhibiting IN at a higher concentration when compared with **7**, compound **13** does show a similar resistance profile to **7**, further proving that our new inhibitors are targeting IN ST.

Having established these new inhibitors as valid HIV IN inhibitors, we then studied their ability to inhibit HIV RT. Through a quick biochemical assay using a commercial RT assay kit it was found that N-3 hydroxylated compounds (e.g., **13**) and aminated compound (**16**) all show inhibitory activity at low or sub-micromolar range (Table 2). Apparently an N-3 OH or NH_2 allows these compound to virtually maintain the key H-bonding ability as expected, though the unmodified N(3)H group of compound **3** appears to form better H-bonding as compound **3** inhibits RT at a considerably lower concentration. Meanwhile, a dramatic fluorine effect was also observed with compounds **12**, **14** and **18**, where a fluorine substitution at the para position of the C-6 benzyl completely disables RT binding. By contrast, the para position of the other benzyl group in N-1 side chain appears to be highly tolerant toward fluorine substitution (compound **13**).

Table 2. Anti-RT and Anti-HIV Activities of Inhibitors **11**−**18**

		HIV-1		
compd	RT IC_{50}[a] (μM)	EC_{50}[b] (μM)	CC_{50}[c] (μM)	TI[d]
11	6.2	0.0080	> 20	> 2500
12	> 100	> 10		
13	0.17	0.024	> 20	> 830
14	> 100	> 10		
15	9.4	1.1	> 20	> 18
16	1.5	> 10		
17	13	4.3	> 20	> 4.7
18	> 100	> 10		
3	0.040	0.013	> 20	> 1500

[a] Concentration inhibiting enzyme activity by 50 %. [b] Concentration inhibiting virus replication by 50 %. [c] Concentration resulting in 50 % cell death. [d] Therapeutic index, defined by CC_{50}/EC_{50}.

Finally these compounds were tested in cell-based antiviral assay against HIV-1. With the exception of compounds with a terminal fluorine atom on the C-6 benzyl group (**12**, **14** and **18**), and the one with N-3 amino group (**16**), these inhibitors generally show excellent antiviral potency and safety (Table 2). The best compounds in this series, compounds **11** and **13**, demonstrate low nanomolar anti-HIV activity that is comparable to or even better than that of reference compound **3**.

In summary, through rational design we have generated a novel series of anti-HIV compounds that are dually active against IN and RT at low and sub-micromolar concentrations. Our design involves a minimal structural modification on a well-known NNRTI inhibitor scaffold (HEPT) to introduce inhibitory activity against IN. Major structural determinants for IN binding as well as a dramatic fluorine effect for RT binding were identified through preliminary SAR efforts. In the end, two compounds (**11** and **13**) were found to have low nanomolar inhibitory activities against HIV-1 in cell culture. Further studies of this newly designed chemotype as a novel IN inhibitor scaffold are currently underway and will be reported in due course.

SUPPORTING INFORMATION AVAILABLE Synthesis of **11**−**18**, experimental details, compound characterization (^1H, ^{13}C NMR, HRMS), HPLC analysis for final products and assay methods. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *E-mail: wangx472@umn.edu. Phone: +1 (612) 626-7025. Fax: +1 (612) 625-8154.

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ABBREVIATIONS RT, reverse transcriptase; IN, integrase; HIV, human immunodeficiency virus; HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine; NNRTI, non-nucleoside RT inhibitor; SAR, structure−activity relationship; HAART, highly active antiretroviral therapy; CCD, catalytic core domain; MSH, mesityl sulfonyl-*O*-hydroxyl amine; TBAI, tetrabutylammonium iodide; 3′-P, 3′ processing; ST, strand transfer.

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