Rationally Designed Dual Inhibitors of HIV Reverse Transcriptase and Integrase

Zhengqiang Wang, Eric M. Bennett, Daniel J. Wilson, Christine Salomon, and Robert Vince*

Center for Drug Design, Academic Health Center, University of Minnesota, 308 Harvard Street SE, Minneapolis, Minnesota 55455

Received May 2, 2007

Abstract: Bifunctional inhibitors were designed and synthesized based on 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)[a1] nonnucleoside reverse transcriptase (RT) inhibitors and diketoacid (DKA) integrase (IN) inhibitors. Biochemical studies revealed activity against RT and IN at low nanomolar and low micromolar concentrations, respectively. Exceptionally low IC_{50} values from a cell-based assay were achieved along with remarkably high therapeutic indices. Compound **7** was identified as the best compound of the series (IC_{50}: 24 nM against RT, 4.4 μM against IN, and 10 nM against HIV-1).

Inhibitors of HIV RT and protease (PR) constitute the core of chemotherapy for AIDS treatment.[1] The therapeutic effect of nucleoside RT inhibitors (NRTIs) is greatly hampered by their intrinsic toxicity,[2,3] whereas the less toxic protease inhibitors (PIs) and non-nucleoside RT inhibitors (NNRTIs) are severely compromised by the quick emergence of resistant viral strains.[4,5] In general, disease resistant to a single-target therapy can be mitigated by developing multitarget therapies. In the case of AIDS, highly active antiretroviral therapy (HAART)[6] combines NRTIs with NNRTIs or PIs and successfully suppresses HIV viral load to an undetectable level, dramatically improving the life quality of AIDS patients.[7] Unfortunately, the efficacy of this primary treatment for AIDS requires nearly perfect adherence,[8] which is extremely difficult to achieve because of complicated dosing and intolerable short- and long-term toxicities. Suboptimal adherence normally results in viral rebound and, even worse, multidrug resistance. In this context, we envision that multitarget therapy using a single drug to inhibit two viral enzymes could yield lower toxicity, simplified dosing, and improved patient adherence, thus reducing the likelihood of drug resistance.

HIV replication involves the synthesis and integration of proviral DNA into the host genome, a process catalyzed sequentially by RT and IN. IN is the third virally encoded enzyme that lacks a mammalian counterpart and represents a validated target for anti-HIV chemotherapeutics.[7−9] Intriguingly, active sites for RT and IN require one or two Mg^{2+} ions.[9] Furthermore, catalytic cooperation and even physical interaction between RT and IN are well-documented; certain peptide fragments from RT inhibit IN activity in vitro.[10−13] These relationships prompted efforts to search for common inhibitors, mainly through screening. As a result, a few compounds with activity against both enzymes have been found (Figure 1). Among these compounds, pyranodipyrimidines (PDP, **1**) are generally mentioned as IN inhibitors because time-of-addition (TOA) experiments support an inhibitory profile corroborating IN activity only.[14] 3,7-Dihydroxytropolones (**2**) primarily target the metal cofactors, and their therapeutic use is limited by poor selectivity.[9] Inhibitory activities against RT and IN were reported with naphthol-based urea derivatives (**3**), which were identified through screening.[15,16] The exploration of such multifunctional ligands has proven valuable for various diseases. However, many multifunctional scaffolds were first discovered by serendipity or screening; rational design by combining existing monofunctional scaffolds remains an enormous challenge.[17−19]

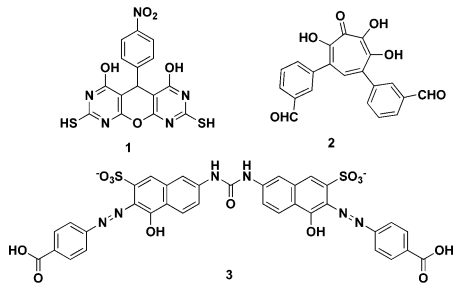


Figure 1. Structures of reported compounds active against RT and IN.

Morphy and Rankovic[18] reviewed designed multifunctional ligands and proposed nomenclature based on whether the scaffolds were combined by a linker (a "conjugate"), directly coupled with no linker ("fused"), or integrated into a single scaffold sharing common features ("merged"). However, classification based on the targeted binding sites is also possible. For example, the sites may (a) be adjacent pockets of a single protein,[20,21] (b) be located on different proteins but recognize similar endogenous ligands,[22,23] or (c) be located on different proteins and recognize dissimilar or no endogenous ligands.[24−26] We propose the term *portmanteau inhibitor*[27] to define what is arguably the most challenging design problem under these classifications: two scaffolds merged into one scaffold, which binds multiple sites that do not recognize similar endogenous ligands. The classification based on the types of binding sites differs from that of Morphy and Rankovic, which is based solely on the ligand structure. Here, we report a series of these inhibitors (Figure 2) targeting IN and the non-nucleoside RT site. The compounds show activity against live virus and have low cytotoxicity.

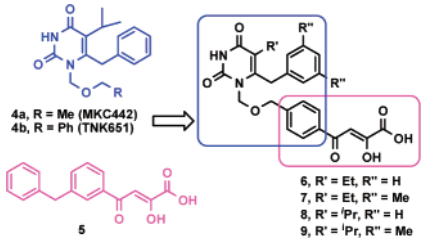


Figure 2. Design of portmanteau inhibitors against RT and IN, combining RT inhibitor **4b** with IN inhibitor **5**.

Our design began with the general knowledge that a DKA group and an immediately connected aromatic ring are the two indispensable structural features for the DKA class of IN inhibitors, as in **5**.[28,29] A portmanteau inhibitor would require incorporation of these two features into an RT inhibitor. Crystal structures of FDA-approved RT inhibitors nevirapine and

[a] Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine; RT, reverse transcriptase; IN, integrase; PR, protease; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; DKA, diketoacid; FDA, Food and Drug Administration; TI, therapeutic index.

* To whom correspondence should be addressed. Phone: 612-624-9911. Fax: 612-626-6891. E-mail: vince001@umn.edu.

10.1021/jm070512p CCC: $37.00
Published on Web 07/04/2007

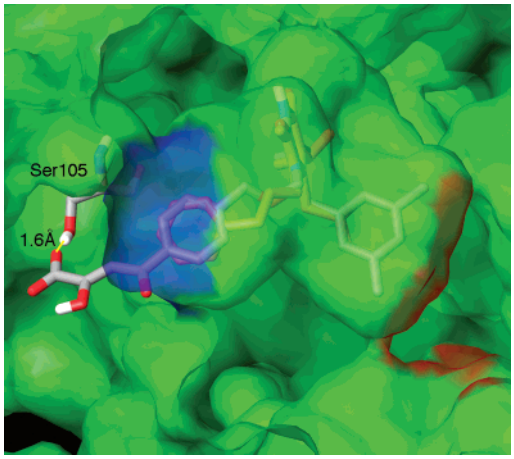
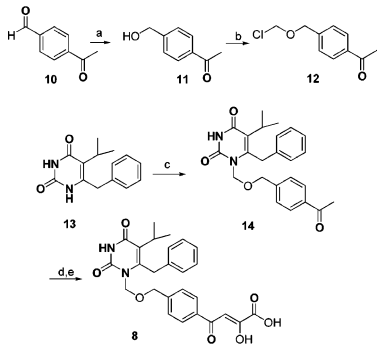


Figure 3. Docking of **7** (colored by element) in the NNRTI binding pocket, with a hydrogen bond (yellow) to the Ser105 side chain. This conformation is 0.3 kcal/mol above the global minimum, which is shown in Figure S1 of Supporting Information. The X-ray structure of **4b** is shown in orange. The protein surface is transparent green except for the surface of Pro236 (blue) and Trp229 (red). Except for the tail of the DKA, the inhibitor is behind the protein surface. The surface excludes Ser105.

efavirenz[30] show that these compounds are almost entirely enclosed by the NNRTI binding pocket, which is largely hydrophobic and not an appropriate environment for the DKA group. Other inhibitor families, such as bis(heteroaryl)piperazine (BHAP)[31] and HEPT,[32] contain members that extend from the NNRTI pocket toward solution and thus present a more attractive site for incorporating a DKA moiety. HEPT compounds are an important class of NNRTIs, even though the best compound of this family, 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (emivirine, **4a**),[32,40] was dropped after a phase III clinical trial.[33] We chose to incorporate a DKA moiety into 6-benzyl-1-(benzyloxymethyl)-5-isopropyluracil (**4b**),[34] a very potent second-generation HEPT RT inhibitor. Comprehensive SAR studies on **4b** have shown a general intolerance toward modifications at C2−C6 of the central pyrimidine ring.[34,35] X-ray crystallography of RT bound with **4b**, however, reveals that the N-1 substituent's phenyl group is positioned near the binding pocket opening controlled by the Pro236 loop, which adjusts position based on the size of the NNRTI inhibitor bound.[31,34] Because this phenyl group sits at the protein/solvent interface near a region of the protein known to be flexible, it might tolerate substitution by a DKA moiety as in **6**−**9**, satisfying both requirements for the DKA class of IN inhibitors. Docking of **7** to RT confirmed this design, showing that the DKA functionality is positioned at the protein/solvent interface and does not disturb the binding mode of the NNRTI core structure (Figures 3 and S1). Modeling showed possible interactions between the DKA group oxygens and the Ser105 side chain or the Val106 backbone nitrogen, but the entire functional group may also be solvated with no hydrogen bonds to the protein.

The chemistry involved in this study is represented by the synthesis of inhibitor **8** outlined in Scheme 1. 6-Benzyl-5-isopropylpyrimidine **13** was prepared according to known procedures.[36] The alkylating agent, chloromethyl ether **12**, was freshly prepared from benzyl alcohol **11** and paraformaldehyde suspended in TMSCl.[37] It is noteworthy that similar transformation under the action of dry HCl produced a significant amount of benzyl chloride byproduct. The benzyl alcohol intermediate **11** was obtained from aldehyde **10** via a chemoselective

Scheme 1[a]



[a] Reagents and conditions: (a) $NaHB(OAc)_3$, THF, 65 °C, 83%; (b) $(HCHO)_n$, TMSCl, room temp; (c) $CH_3C(OTMS)$═NTMS (BSA), CH_2Cl_2, room temp, then 12, tetrabutylammonium iodide (TBAI), 76%; (d) NaOEt/EtOH, diethyl oxalate; (e) NaOH (1 N), $EtOH/CH_2Cl_2$, room temp, 79% over two steps.

Table 1. Anti-RT, Anti-IN, and Antiviral Activities of Inhibitors **6**−**9**

inhibitor	RT IC_{50} (μM)	IN IC_{50} (μM)	HIV-1 EC_{50} (μM)	HIV-1 CC_{50} (μM)	TI
6	0.23	1.8	0.052	>10	>190
7	0.024	4.4	0.0097	>10	>1000
8	0.057	2.4	0.033	>10	>310
9	0.0092	7.7	0.017	>10	>600
1[14]	4.8	0.30	8.9	120	14
2[9]	1.2	1.1	3.0	6.0	2.0
3[39]	0.39	0.70			
4b[34,40]	0.016	>100	0.016	>10	>610
5[41]	>100	0.093	0.16	>10	>61

reduction with $NaHB(OAc)_3$. Preparation of the key intermediate ketone **14** was effected by a regioselective N-1 alkylation via a 2,4-oxo-bis-silylated intermediate.[25] A condensation between ketone **14** and diethyl oxalate provided a diketo ester, which was then hydrolyzed[39] to produce the desired DKA compound **8**. Compounds **6**, **7** and **9** were prepared similarly.

IC_{50} values against RT and IN were calculated using dose response curves and are summarized in Table 1. Significantly, the separate scaffolds are active against only one target; **4b** showed no activity against IN, while **5** had no activity against RT. However, all of the portmanteau inhibitors (**6**−**9**) show nanomolar IC_{50} values against RT, which are similar to the related RT inhibitor **4b**. The retention of high potency confirms our prediction that the open channel surrounding the N-1 phenyl would tolerate structural modifications. Conforming to the SAR of HEPT compounds,[38] we observed that the C-5 isopropyl group leads to better activity than an ethyl substituent. We also found that the *m*-dimethyl substitution on the phenyl ring benefits RT binding, presumably by providing additional hydrophobic interactions. These additional binding interactions have proved crucial for improving resistance profiles of HEPT inhibitors.[35] On the other hand, anti-IN assay of our compounds produced IC_{50} values in the low micromolar range, with the most potent about 1 order of magnitude lower than that of the known IN inhibitor **5**. Because only marginal differences in anti-IN activities were observed, there does not appear to be significant binding interference between the pyrimidine core and DKA moieties.

A cell-based antiviral assay was performed against HIV-1_{NL4-3} in human peripheral blood mononuclear cells. As shown in Table 1, the portmanteau compounds demonstrate magnificent anti-HIV activity and low toxicity. As a result, their therapeutic indices are far superior to those of **1**−**3**. With the exception of inhibitor **9**, all portmanteau inhibitors showed higher (2- to 5-fold) potency against HIV than RT. Although the contribution

of anti-IN activity to this increased potency is uncertain, there is clearly room for further improvement of the IN inhibition. Studies in this direction are currently underway. We are also exploring the applicability of this design strategy to other combinations of viral targets such as RT/PR and IN/PR.

Acknowledgment. This research was supported by the Center for Drug Design at the University of Minnesota. We thank Christine Dreis for RT assay data and Dr. Roger Ptak at Southern Research Institute for the cell-based assay. We also thank Dr. William M. Shannon for advice on the antiviral testing and interpretation of data, the Minnesota Supercomputing Institute for computer time, and Dr. Robert Craigie of NIH for the integrase plasmid.

Supporting Information Available: Synthesis of **6**−**9**, experimental details (^{1}H and ^{13}C NMR, HRMS, HPLC) for intermediates and final products, assay and molecular modeling methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) http://www.fda.gov/oashi/aids/virals.html.
(2) Schinazi, R. F.; Hernandez-Santiago, B. I.; Hurwitz, S. J. Pharmacology of current and promising nucleosides for the treatment of human immunodeficiency viruses. *Antiviral Res.* **2006**, *71*, 322−334.
(3) Fokunang, C. N.; Hitchcock, J.; Spence, F.; Tembe-Fokunang, E. A.; Burkhardt, J.; Levy, L.; George, C. An overview of mitochondrial toxicity of nucleoside reverse transcriptase inhibitors associated with HIV therapy. *Int. J. Pharmacol.* **2006**, *2*, 152−162.
(4) Young, B.; Kuritzkes, D. R. Resistance to HIV-1 protease inhibitors. *Infect. Dis. Ther.* **2002**, *25*, 257−282.
(5) Bacheler, L. T. Resistance to non-nucleoside inhibitors of HIV-I reverse transcriptase. *Drug Resist. Updates* **1999**, *2*, 56−67.
(6) Yeni, P. Update on HAART in HIV. *J. Hepatol.* **2006**, *44*, S100−S103.
(7) Palella, F. J. D. K.; Moorman, A.; et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N. Engl. J. Med.* **1998**, *338*, 853.
(8) Chesney, M. Adherence to HAART regimens. *AIDS Patient Care STDs.* **2003**, *17*, 169−177.
(9) Didierjean, J.; Isel, C.; Querre, F.; Mouscadet, J.-F.; Aubertin, A.-M.; Valnot, J.-Y.; Piettre, S. R.; Marquet, R. Inhibition of human immunodeficiency virus type 1 reverse transcriptase, RNase H, and integrase activities by hydroxytropolones. *Antimicrob. Agents Chemother.* **2005**, *49*, 4884−4894.
(10) Zawahir, Z.; Neamati, N. Inhibition of HIV-1 integrase activity by synthetic peptides derived from the HIV-1 HXB2 Pol region of the viral genome. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5199−5202.
(11) Hehl, E. A.; Joshi, P.; Kalpana, G. V.; Prasad, V. R. Interaction between human immunodeficiency virus type 1 reverse transcriptase and integrase proteins. *J. Virol.* **2004**, *78*, 5056−5067.
(12) Tasara, T.; Maga, G.; Hottiger, M. O.; Hubscher, U. HIV-1 reverse transcriptase and integrase enzymes physically interact and inhibit each other. *FEBS Lett.* **2001**, *507*, 39−44.
(13) Wilhelm, M.; Wilhelm, F. X. Cooperation between reverse transcriptase and integrase during reverse transcription and formation of the preintegrative complex of Ty1. *Eukaryotic Cell* **2006**, *5*, 1760−1769.
(14) Pannecouque, C.; Pluymers, W.; Van Maele, B.; Tetz, V.; Cherepanov, P.; De Clercq, E.; Witvrouw, M.; Debyser, Z. New class of HIV integrase inhibitors that block viral replication in cell culture. *Curr. Biol.* **2002**, *12*, 1169−1177.
(15) Maurer, K.; Tang, A. H.; Kenyon, G. L.; Leavitt, A. D. Carbonyl J derivatives: a new class of HIV-1 integrase inhibitors. *Bioorg. Chem.* **2000**, *28*, 140−155.
(16) Skillman, A. G.; Maurer, K. W.; Roe, D. C.; Stauber, M. J.; Eargle, D.; Ewing, T. J.; Muscate, A.; Davioud-Charvet, E.; Medaglia, M. V.; Fisher, R. J.; Arnold, E.; Gao, H. Q.; Buckheit, R.; Boyer, P. L.; Hughes, S. H.; Kuntz, I. D.; Kenyon, G. L. A novel mechanism for inhibition of HIV-1 reverse transcriptase. *Bioorg. Chem.* **2002**, *30*, 443−458.
(17) Morphy, R.; Kay, C.; Rankovic, Z. From magic bullets to designed multiple ligands. *Drug Discovery Today* **2004**, *9*, 641−651.
(18) Morphy, R.; Rankovic, Z. Designed multiple ligands. *J. Med. Chem.* **2005**, *48*, 6523−6543.
(19) Morphy, R.; Rankovic, Z. The physicochemical challenges of designing multiple ligands. *J. Med. Chem.* **2006**, *49*, 4961−4970.
(20) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. Click chemistry in situ: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 1053−1057.
(21) Munoz-Torrero, D.; Camps, P. Dimeric and hybrid anti-Alzheimer drug candidates. *Curr. Med. Chem.* **2006**, *13*, 399−422.
(22) Gemma, S.; Gabellieri, E.; Huleatt, P.; Fattorusso, C.; Borriello, M.; Catalanotti, B.; Butini, S.; De Angelis, M.; Novellino, E.; Nacci, V.; Belinskaya, T.; Saxena, A.; Campiani, G. Discovery of huperzine A-tacrine hybrids as potent inhibitors of human cholinesterases targeting their midgorge recognition sites. *J. Med. Chem.* **2006**, *49*, 3421−3425.
(23) Yamamoto, M.; Ikeda, S.; Kondo, H.; Inoue, S. Design and synthesis of dual inhibitors for matrix metalloproteinase and cathepsin. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 375−378.
(24) Kawanishi, Y.; Ishihara, S.; Tsushima, T.; Seno, K.; Miyagoshi, M.; Hagishita, S.; Ishikawa, M.; Shima, N.; Shimamura, M.; Ishihara, Y. Synthesis and pharmacological evaluation of highly potent dual histamine H2 and gastrin receptor antagonists. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1427−1430.
(25) Ryckmans, T.; Balancon, L.; Berton, O.; Genicot, C.; Lamberty, Y.; Lallemand, B.; Pasau, P.; Pirlot, N.; Quere, L.; Talaga, P. First dual NK(1) antagonists-serotonin reuptake inhibitors: synthesis and SAR of a new class of potential antidepressants. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 261−264.
(26) Groneberg, R. D.; Burns, C. J.; Morrissette, M. M.; Ullrich, J. W.; Morris, R. L.; Darnbrough, S.; Djuric, S. W.; Condon, S. M.; McGeehan, G. M.; Labaudiniere, R.; Neuenschwander, K.; Scotese, A. C.; Kline, J. A. Dual inhibition of phosphodiesterase 4 and matrix metalloproteinases by an (arylsulfonyl)hydroxamic acid template. *J. Med. Chem.* **1999**, *42*, 541−544.
(27) Carroll, L. *Through the Looking Glass*. First published in 1871.
(28) Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science (Washington, D.C.)* **2000**, *287*, 646−650.
(29) Dayam, R.; Sanchez, T.; Clement, O.; Shoemaker, R.; Sei, S.; Neamati, N. β-Diketo acid pharmacophore hypothesis. 1. Discovery of a novel class of HIV-1 integrase inhibitors. *J. Med. Chem.* **2005**, *48*, 111.
(30) PDB entries 1JKH and 1FKO, respectively. http://www.rcsb.org.
(31) Esnouf, R. M.; Ren, J.; Hopkins, A. L.; Ross, C. K.; Jones, E. Y.; Stammers, D. K.; Stuart, D. I. Unique features in the structure of the complex between HIV-1 reverse transcriptase and the bis(heteroaryl)-piperazine (BHAP) U-90152 explain resistance mutations for this nonnucleoside inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3984−3989.
(32) Tanaka, H.; Hayakawa, H.; Haraguchi, K.; Miyasaka, T.; Walker, R. T.; De Clercq, E.; Baba, M.; Stammers, D. K.; Stuart, D. I. HEPT: from an investigation of lithiation of nucleosides towards a rational design of non-nucleoside reverse transcriptase inhibitors of HIV-1. *Adv. Antiviral Drug Des.* **1999**, *3*, 93−144.
(33) http://www.aidsmeds.com/news/20020118rglt001.htm.
(34) Hopkins, A. L.; Ren, J.; Esnouf, R. M.; Willcox, B. E.; Jones, E. Y.; Ross, C.; Miyasaka, T.; Walker, R. T.; Tanaka, H.; Stammers, D. K.; Stuart, D. I. Complexes of HIV-1 reverse transcriptase with inhibitors of the HEPT series reveal conformational changes relevant to the design of potent non-nucleoside inhibitors. *J. Med. Chem.* **1996**, *39*, 1589.
(35) Hopkins, A. L.; Ren, J.; Tanaka, H.; Baba, M.; Okamato, M.; Stuart, D. I.; Stammers, D. K. Design of MKC-442 (emivirine) analogues with improved activity against drug-resistant HIV mutants. *J. Med. Chem.* **1999**, *42*, 4500.
(36) Adrian, G.; Mignonac, S.; Lecoutteux, F. Preparation of 5-(1-methylethyl)-6-(phenylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione. WO 2000003999, 2000.
(37) Shipov, A. G.; Savost'yanova, I. A.; Baukov, Y. I. Synthesis of alkyl chloromethyl ethers. *Zh. Obshch. Khim.* **1989**, *59*, 1204.
(38) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Inouye, N.; Baba, M.; Shigeta, S.; Walker, R. T.; Clercq, E. D.; Miyasaka, T. Synthesis and antiviral activity of 6-benzyl analogs of 1-[(2-hydroxyethoxy)methyl]-5-(phenylthio)thymine (HEPT) as potent and selective anti-HIV-1 agents. *J. Med. Chem.* **1995**, *38*, 2860.
(39) Kenyon, G. L.; Stauber, M. J.; Maurer, K.; Eargle, D.; Muscate, A.; Leavitt, A.; Roe, D. C.; Ewing, T. J. A.; Skillman, A. G., Jr.; Arnold,

E.; Kuntz, I. D.; Young, M. Preparation of *N*,*N*′-bis(hydroxysulfonaphthyl)ureas and analogs as HIV reverse transcriptase and integrase inhibitors. Patent 9850347, 1998.

(40) Baba, M.; Shigeta, S.; Yuasa, S.; Takashima, H.; Sekiya, K.; Ubasawa, M.; Tanaka, H.; Miyasaka, T.; Walker, R. T.; Clercq, E. D. Preclinical evaluation of MKC-442, a highly potent and specific inhibitor of human immunodeficiency virus type 1 in vitro. *Antimicrob. Agents Chemother*. **1994**, *38*, 688−692.

(41) Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Guare, J. P., Jr.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D. 4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. *J. Med. Chem*. **2000**, *43*, 4923−4926.

JM070512P