Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3117–3119

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Inhibition of HIV-1 Nuclear Import via Schiff Base Formation with Arylene Bis(methylketone) Compounds

Yousef Al-Abed,[a,*] Larisa Dubrovsky,[a] Bela Ruzsicska,[a] Mohindra Seepersaud[a] and Michael Bukrinsky[b]

[a]*Laboratory of Medicinal Chemistry, North Shore-LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030, USA*
[b]*Department of Microbiology and Tropical Medicine, The George Washington University, Washington, DC 20037, USA*

Received 31 January 2002; accepted 31 July 2002

Abstract—Arylene bis(methylketone) compounds specifically block nuclear translocation of the HIV-1 pre-integration complex by forming Schiff-base adducts with contiguous lysines within nuclear localization signal.

Over the last several years, significant progress has been achieved in treating human immunodeficiency virus type 1 (HIV-1) infection. This progress is mostly due to the introduction into clinical practice of HIV protease inhibitors, a new class of potent antiviral agents. When used in combination with inhibitors of viral reverse transcriptase (RT), they reduce the viral load in HIV-infected patients to undetectable levels and stop the depletion of $CD4^+$ T cells in patients with an AIDS-compromised immune system.[1,2] However, major problems in fighting this virus remain. They include viral resistance to drugs, low-level replication of drug-sensitive virus due to incomplete antiviral effect of the drugs, and pronounced toxic side effects associated with prolonged administration of the drugs. These considerations clearly justify the need to develop novel highly specific anti-HIV compounds.

Establishment of integrated HIV provirus and productive viral infection depend critically on the successful translocation of the HIV-1 genome from the cytoplasm into the nucleus of an infected cell following viral entry.[3] This step becomes especially critical for HIV-1 infection of non-dividing cells, such as terminally differentiated macrophages, where the nuclear envelope stays intact throughout the life span of the cell. The viral genome is transported into the nucleus as part of a large pre-integration complex comprising viral DNA and proteins, including integrase (IN), reverse transcriptase (RT), viral protein R (Vpr), and matrix antigen (MA).[4–6] Within MA, there are two clusters of basic amino acids (residues 26–32 and 110–114) that are highly homologous to the nuclear localization sequence (NLS) of simian virus 40 (SV40) large T antigen[7] and participate in targeting the HIV-1 pre-integration complexe to the nucleus.[8] Contiguous lysine residues in the NLS are essential to confer its nuclear targeting activity, which is mediated by NLS interaction with a cellular transport protein, karyopherin α (reviewed in ref 9).

A class of arylene bis(methylketone) compounds was designed to specifically inactivate basic-type HIV-1 NLSs by forming Schiff base adducts with contiguous lysines in the signal (Fig. 1).[9] The compounds were shown to be potent inhibitors of nuclear import of the HIV-1 genome and of virus replication in cultures of primary monocytes and T cells in vitro[10,11] and in lymphoid tissue cultured ex vivo.[12] The selectivity and low cytotoxicity of these compounds were determined by their specific targeting to HIV-1 pre-integration complexes due to interaction between the pyrimidine side chain of the compounds and the RT protein.[10] However, the compounds of this class are poor inhibitors of RT, and their mechanism of anti-HIV action does not involve RT inhibition.[11]

While the mechanism of activity of arylene bis (methylketone) compounds was hypothesized to involve formation of Schiff bases between the carbonyl groups

*Corresponding author. Tel.: +1-516-562-9461; fax: +1-516-365-5090; e-mail: yalabed@nshs.edu

0960-894X/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0960-894X(02)00642-X

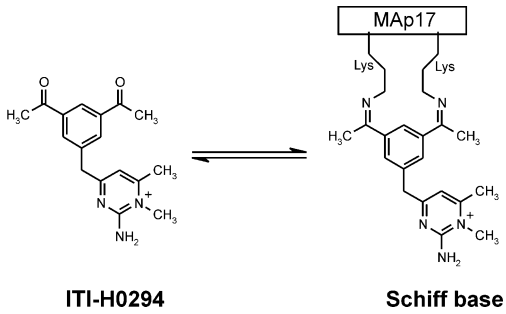


Figure 1. Postulated interaction of ITI-0294 with the NLS of HIV-1 matrix antigen (MA).

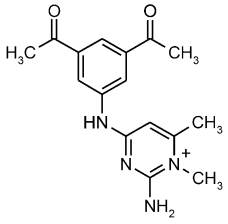
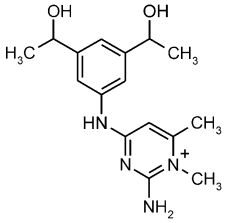
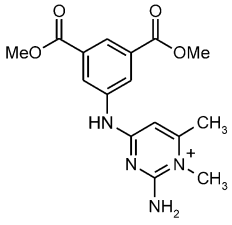
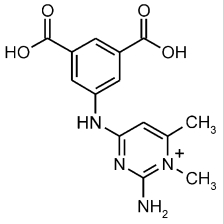
of the compounds and the ε-amino groups of lysine residues in the NLSs of the HIV-1 matrix antigen[9,10] no direct experimental support for this hypothesis has been provided. Here, we report results of structure-function analysis of arylene bis(methylketone) compounds, which support the role of Schiff-base formation as a mechanism of compounds' anti-HIV activity.

To investigate the mechanism of anti-HIV activity of arylene bis(methylketone) compounds, we synthesized several analogues of ITI-0294, where the keto groups were the focus of the study (Table 1). Reduction of ITI-0294 bis-keto groups by $NaBH_4$ quantitatively yielded the bis-alcohol product (ITI-0299). The structural confirmation of the alcohol compound was achieved by NMR and mass spectrometry.[13] Another methyl ester derivative (ITI-0499) was synthesized by coupling dimethyl 5-aminoisophthalate with 2-amino-4-chloro-6-methylpyrimidine as described previously.[14] The acid product (ITI-0599) was generated from the methyl ester by base treatment. These compounds were tested in vitro in parallel with a prototypic active compound ITI-0294 for anti-HIV activity and for the ability to inhibit nuclear import of the HIV-1 PIC.

As shown in Figure 2a, among the new derivatives only compound ITI-0599 demonstrated significant anti-HIV activity in macrophage cultures at a concentration 1 μM, however, its effect was much weaker than that of the prototypic compound ITI-0294 at 0.1 μM. To measure directly the effect of these compounds on HIV-1 nuclear import, we employed a recently developed cell-free nuclear import assay.[15] In this assay, nuclear import of the HIV-1 pre-integration complex is measured in digitonin-permeabilized HeLa cells by PCR using primers specific for 2-LTR forms of viral DNA, which are produced exclusively within the nucleus. Using this assay, we determined that the inhibitory effect of ITI-0559 on HIV-1 nuclear import is at least 10-fold weaker than that of ITI-0294 (Fig. 2b).

Previously, we hypothesized that a direct reaction between the lysine residues within MA p17 NLS results in the formation of a Schiff base. Formation of two Schiff bases after reaction with adjacent lysines characteristic of nuclear localization signals would stabilize the biomolecular complex, shifting the equilibrium toward product formation. This assumption was indirectly supported by

Table 1. Structure–function analysis of anti-HIV activity of CNI compounds

Compd	Structure	IC_{50} (nM)
ITI-H0294		100
ITI-H0299		NA
ITI-H0499		NA
ITI-H0599		100

IC_{50} concentration of the drug giving 50% reduction in viral replication, measured by RT activity in the supernatant of HIV-1_{ADA}-infected macrophage cultures.

incorporation of tritium into MA upon reducing a reaction mixture of MA and ITI-0294 with $NaBT_4$.[10] This evidence, however, was weakened by the possibility of non-specific (i.e., outside of the NLS regions) interactions between the compound and lysine-rich MA protein. Our present findings confirm the essential role of the keto-groups of ITI compounds in inhibition of HIV-1 nuclear import and also negate any critical role for a hydrogen bond formation between MA NLS and ITI-0294. Of note, a possible formation of salt bridges between NLS and ITI-0599 (Table 1) may explain the weak inhibitory effect of this compound.

Taken together, our results demonstrate that the ketone groups are required for anti-HIV activity of the ITI compounds. This requirement is consistent with the proposed Schiff base formation between the compounds and the lysine residues in MA NLSs. Therefore, these groups should be preserved in the design of future analogues.

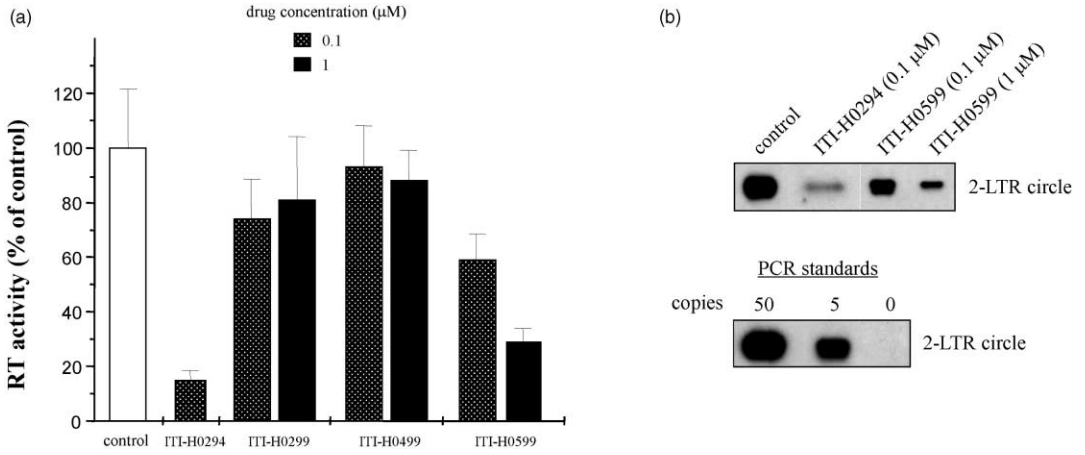


Figure 2. Analysis of anti-HIV activity of anti-NLS compounds. (a) Analysis of compounds' activity in macrophage cultures. Triplicate cultures of primary monocyte-derived macrophages were infected with HIV-1_{ADA} in the absence (control) or presence of indicated concentrations of the compounds. Half of the culture medium was changed every 3 days maintaining the same concentration of the compounds. On day 14 after infection, reverse transcriptase (RT) activity in the culture supernatant was determined as described.[16] Results are presented as percentage of RT activity in drug-treated versus control (untreated) cultures, and error bars show SD of the mean. (b) Analysis of ITI-0599 activity in the nuclear import assay. Nuclear import assay was performed with digitonin-treated HeLa cells and HIV-1 pre-integration complexes derived from HIV-1_{LAV}-infected H9 cells in the absence (negative control) or presence of indicated concentrations of ITI-0599 or ITI-0294 (positive control) compounds as described previously.[15] Results were analyzed by PCR using primers specific for 2-LTR circle forms of HIV-1 DNA, which are detected exclusively in the nucleus. PCR was performed in the linear range of amplification, as verified by amplification of PCR standards (dilutions of p2LTR, a plasmid containing cloned fragment of circularized HIV-1 genome).

Acknowledgements

This work was supported in part by the NIH grant R01 AI 40386 and by the funds from The Picower Institute for Medical Research. We are grateful to Dr. S. Popov for help with nuclear import experiments.

References and Notes

1. Collier, A. C.; Coombs, R. W.; Schoenfeld, D. A.; Bassett, R. L.; Timpone, J.; Baruch, A.; Jones, M.; Facey, K.; Whitacre, C.; McAuliffe, V. J.; Friedman, H. M.; Merigan, T. C.; Reichman, R. C.; Hooper, C.; Corey, L. *N. Engl. J. Med.* **1996**, *334*, 1011.
2. Collier, A. C.; Coombs, R. W.; Schoenfeld, D. A.; Bassett, R.; Baruch, A.; Corey, L. *Antiviral Res.* **1996**, *29*, 99.
3. Bukrinsky, M. I.; Haffar, O. K. *Front. Biosci.* **1999**, *4*, D772.
4. Bukrinsky, M. I.; Haggerty, S.; Dempsey, M. P.; Sharova, N.; Adzhubel, A.; Spitz, L.; Lewis, P.; Goldfarb, D.; Emerman, M.; Stevenson, M. *Nature* **1993**, *365*, 666.
5. Heinzinger, N. K.; Bukrinsky, M. I.; Haggerty, S. A.; Ragland, A. M.; Kewalramani, V.; Lee, M. A.; Gendelman, H. E.; Ratner, L.; Stevenson, M.; Emerman, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7311.
6. Bouyac-Bertoia, M.; Dvorin, J. D.; Fouchier, R. A.; Jenkins, Y.; Meyer, B. E.; Wu, L. I.; Emerman, M.; Malim, M. H. *Mol. Cell* **2001**, *7*, 1025.
7. Mattaj, I. W.; Englmeier, L. *Annu. Rev. Biochem.* **1998**, *67*, 265.
8. Haffar, O. K.; Popov, S.; Dubrovsky, L.; Agostini, I.; Tang, H.; Pushkarsky, T.; Nadler, S. G.; Bukrinsky, M. *J. Mol. Biol.* **2000**, *299*, 359.
9. Dubrovsky, L.; Ulrich, P.; Nuovo, G. J.; Manogue, K. R.; Cerami, A.; Bukrinsky, M. *Mol. Med.* **1995**, *1*, 217.
10. Popov, S.; Dubrovsky, L.; Lee, M. A.; Pennathur, S.; Haffar, O.; Al-Abed, Y.; Tonge, P.; Ulrich, P.; Rexach, M.; Blobel, G.; Cerami, A.; Bukrinsky, M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11859.
11. Haffar, O. K.; Smithgall, M. D.; Popov, S.; Ulrich, P.; Bruce, A. G.; Nadler, S. G.; Cerami, A.; Bukrinsky, M. I. *Antimicrob. Agents Chemother.* **1998**, *42*, 1133.
12. Glushakova, S.; Dubrovsky, L.; Grivel, J. C.; Haffar, O.; Bukrinsky, M. *Antiviral Res.* **2000**, *47*, 89.
13. The ITI-H0294 (110 mg, 0.29 mmol) was dissolved in methanol (5 mL) and treated with $NaBH_4$ (23 mg, 0.60 mmol) at 0 °C. The mixture was allowed to warm to room temperature and the reaction was quenched by neutralization with methanolic HCl (HCl/MeOH; 1:10). The excess solvent was evaporated under reduced pressure to give the crude product, which was purified by Flash column chromatography (10–60% MeOH/$CHCl_3$) to give the diol ITI-0299 (106 mg, 96%): R_f 0.5 (40% MeOH/$CHCl_3$). ^1H NMR (CD_3OD) δ 1.45 (d, 6H), 2.41 (s, 2H), 3.33 (m, 2H), 3.51 s, 3H), 6.31 (s, 1H), 7.10 (s, 1H), 7.80 (s, 2H); ^{13}C NMR (CD_3OD) δ 19.0, 24.5, 33.2, 69.4, 100.1, 116.4, 118.5, 138.1, 147.3, 154.3, 156.8, 159.7.
14. Ulrich, P.; Cerami, A. *J. Med. Chem.* **1984**, *27*, 35.
15. Popov, S.; Rexach, M.; Zybarth, G.; Reiling, N.; Lee, M. A.; Ratner, L.; Lane, C. M.; Moore, M. S.; Blobel, G.; Bukrinsky, M. *EMBO J.* **1998**, *17*, 909.
16. Schmidtmayerova, H.; Nuovo, G. J.; Bukrinsky, M. *Virology* **1997**, *232*, 379.