Screening of Non-catalytic Integrase Inhibitors for Anti-HIV Drug Development

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Research

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Abstract

Background: Integrase (IN) is an essential protein for HIV replication that catalyzes insertion of the reverse-transcribed viral genome into the host chromosome during the early steps of viral infection. Highly active anti-retroviral therapy (HAART) is a HIV/AIDS treatment method that combines three or more antiviral drugs often formulated from compounds that inhibit the activities of viral reverse transcriptase and protease enzymes. Early IN inhibitors (INIs) mainly serve as integrase strand transfer inhibitors (INSTI) that disrupt strand transfer by binding the catalytic core domain (CCD) of IN. However, mutations of IN can confer resistance to INSTI. Therefore, non-catalytic integrase inhibitors (NCINI) have been developed as next-generation INIs.

Methods: In this study, we evaluated and compared the activity of INSTI and NCINI according to the analysis method. Antiviral activity was compared using p24 ELISA with MT2 cell and TZM-bl luciferase system with TZM-bl cell. Each drug was serially diluted and treated to MT2 and TZM-b1 cells, infected with HIV-1 AD8 strain and incubated for 5 and 2 days, respectively. Additionally, to analyze properties of INSTI and NCINI, transfer inhibition assay and 3’-processing inhibition assay were performed.

Results: During screening of INIs using the p24 ELISA and TZM-bl luciferase systems, we found an inconsistent result with INSTI and NCINI drugs. Following infection of MT2 and TZM-bl cells with T-tropic HIV-1 strain, both INSTI and NCINI treatments induced significant p24 reduction in MT2 cells. However, NCINI showed no antiviral activity in the TZM-bl luciferase system, indicating that this widely used and convenient antiretroviral assay is not suitable for screening of NCINI compounds that target the second round of HIV-1 replication.

Conclusion: Accordingly, we recommend application of other assay procedures, such as p24 ELISA or reverse transcription activity, in lieu of the TZM-bl luciferase system for preliminary NCINI drug screening. Utilization of appropriate analytical methods based on underlying mechanisms is necessary for accurate assessment of drug efficacy.

Background

Antiretroviral drugs are divided into four classes: entry/fusion inhibitor, reverse transcription inhibitor (RTI), integrase inhibitor (INI), and protease inhibitor (PI). [5, 6] Highly active antiretroviral therapy (HAART), a standard HIV/AIDS treatment method, is a cocktail therapy combining three or more antiretroviral drugs that act on different targets. HAART generally comprises three drugs, specifically, two nucleoside reverse transcription inhibitors (NRTIs) with one non-nucleoside reverse transcriptase inhibitor (NNRTI) or PI (2 NRTIs + NNRTI or 2NRTIs + PI). RTI and PI or entry inhibitor are occasionally used as alternative treatment options (RTI + PI + INI or RTI + entry inhibitor). [7, 8] HAART effectively reduces the viral load and facilitates significant recovery of immune functions in HIV/AIDS patients, increasing the survival period by more than 7 to 10 years or even longer compared to single drug-treated patients. [7–9] However, continued efforts to design more effective novel anti-HIV drugs are urgently required to combat the emergence of highly mutagenic HIV strains and continuous drug resistance. [10] Following the initial approval of zidovudine as a therapeutic NNRTI by the US Food and Drug Administration (FDA) in 1987, various anti-HIV drugs have been developed as INI, starting with raltegravir (RAL) in 2007 followed by elvitegravir (EVG) and dolutegravir (DTG). [9, 11, 12]

The integrase (IN) protein plays an important role in transferring viral DNA to the host nucleus in the HIV replication process and serves as an important pharmacological target for next-generation anti-HIV drugs. [13, 14] Integration can be classified into two steps: (1) ‘3’ processing’ that refers to the process of cutting dinucleotides at both ends of viral DNA for formation of a pre-integration complex (PIC) by combining with IN and (2) the strand transfer step whereby PIC is transported into the host nucleus by IN and combined with lens epithelial-derived growth factor/p75 (LEDGF/p75) in chromosomal DNA to integrate viral DNA. [14–17] RAL, EVG, and DTG are strand transfer inhibitors (INSTI) that recognize and bind the catalytic core domain (CCD) of integrase and block this process. [12, 13, 17, 18]

In 2014, BI 224436, the first approved non-catalytic site integrase inhibitor (NCINI), was developed by Boehringer Ingelheim (Canada) Ltd. In contrast to INSTIs, BI 224436 binds a highly conserved allosteric site in CCD of IN to induce conformational changes in the catalytic site, thus disrupting interactions of IN with long-terminal repeat (LTR) DNA, and is additionally reported to inhibit 3’-processing. [13, 19, 20] NCINIs, a new class of INI drugs, can overcome the problems of INSTI-resistant viruses based on its different inhibition mechanism. Specifically, INSTIs directly inhibit IN-LEDGF/p75 interactions while NCINIs bind CCD to inhibit IN-LTR DNA generation. [13, 20]

We were commissioned by a domestic pharmaceutical company to evaluate the efficacy of a novel NCINI candidate but were unable to verify antiviral activity using the TZM-bl luciferase system. In view of this finding, we attempted to develop a suitable method for accurately assessing the antiviral efficacy of NCINI.

To achieve optimal results, a drug efficacy test should always take into account the biological mechanism. Comparative evaluation of the efficacy of INSTI and NCINI compounds using various in vitro methods in this study facilitated the identification of a novel system appropriate for screening of NCINIs.

Methods

Cells and viruses

MT2 cells were maintained in Roswell Park Memorial Institute 1640 medium (RPMI1640, HyClone, Logan, UT) containing 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA) and 1% penicillin/streptomycin (P/S, gibco, Waltham, MA). TZM-bl and HeLa cells were maintained in high-glucose Dulbecco’s
infectious HIV-1 AD8 strain, 20 µg pNL4.3(AD8) clone was transfected into HeLa cells using iN-fect™ (iNtRON Biotechnology, Seongnam, Korea). After 48 h of culture, culture media were harvested and centrifuged for 2,000 rpm (1,344 rcf) at 5 min for removal of cell debris. Harvested viruses were stored at -80°C. Infectious virus titers were determined based on 50% tissue culture infectious dose (TCID<sub>50</sub>) according to the endpoint method of Reed and Muench (1938).

Drugs

RAL, DTG and EVG were kindly provided by the New Drug development team, R&D center, ST Pharm (Seoul, Korea) and BI 224436 by Professor Baek Kim (School of Medicine Health Science Research Building, Emory University, Atlanta, GA).

Measurement of cell cytotoxicity of anti-HIV drugs

To determine the cytotoxicity of anti-HIV drugs, cell viability was assessed via the water-soluble tetrazolium salt (WST) method using an EZ-Cytox kit (Daeil Lab Service, Seoul, Korea) according to the manufacturer's instructions. Briefly, TZM-bl and MT2 cells were seeded on 96-well cell culture plates at a density of 1×10<sup>4</sup> cells/well and cultured overnight. Cells were treated with serial dilutions of each drug (two-fold dilutions from 50,000 nM to 2.54 nM). On days 2 and 5 of incubation, 10 µl EZ-Cytox solution was added to each well and incubated for 2 h, followed by spectrophotometric measurement of absorbance at 540 nm. The CC<sub>50</sub> value of samples was defined as the concentration inducing 50% cell death.

Enzyme-linked immunosorbent assay for p24

ELISA was conducted to detect HIV-1 p24 for assessment of antiviral activity using a HIV-1 p24 ELISA kit (XpressBio, Frederick, MD). To this end, MT2 cells seeded on a 96-well cell culture plate at a density of 1×10<sup>4</sup> cells/well were infected with 500 TCID<sub>50</sub> of HIV-1 AD8 strain. Each drug was serially diluted 3-fold (from 10,000 nM to 0.51 nM) for treatment of cells. After five days of culture at 37°C with 5% CO<sub>2</sub>, 100 µl of culture medium was harvested and supernatant obtained by centrifugation for 5 min at 5,000 rpm (8,400 rcf), with storage at -80°C. Cell culture media of non-infected and infected cells without INI treatment were set as the negative and positive control, respectively. The ELISA procedure was conducted according to the manufacturer's instructions. Briefly, samples were mixed with 20 µl lysis buffer, 200 µl aliquots pipetted into a microplate, and incubated for 1 h at 37°C. After incubation, the contents of the wells were aspirated and microtitration plates washed six times with 350 µl wash buffer. Each well was treated with 100 µl zymogram detection antibody for 1 h at 37°C, which was subsequently removed by washing under the same conditions. An aliquot (100 µl) of streptavidin-HRP conjugate was added into each well, followed by incubation at room temperature for 30 min. After washing under the same conditions, 100 µl substrate solution was immediately dispensed into each well and incubated for 30 min at room temperature with protection from direct sunlight. For termination of the reaction, 100 µl stop solution was added to each well and absorbance values at 450 nm immediately read using a microplate reader.

Antiviral activity test using the TZM-bl cell system

TZM-bl cells were seeded on 96-well cell culture plates at a density of 1×10<sup>4</sup> cells/well and cultured overnight. Each drug was 2-fold serially diluted from 10,000 nM to 0.15 nM and treated to cells. After a 30 min incubation period, viral infection was performed with 500 TCID<sub>50</sub> of HIV-1 AD8. Cells were cultured for 48 h after infection and luciferase activity measured using Beetle-Lysis Juice (PJK GmbH, Kleinblittersdorf, Germany) according to the manufacturer's instructions. Briefly, the medium was removed, and cells washed three times with 200 µl PBS. Next, 100 µl Beetle-Lysis Juice containing luciferin and ATP were added to each well and incubated for 5 min with protection from sunlight. Subsequently, luciferase activity was measured using a micro beta counter (PerkinElmer, Waltham, MA) after transferring solutions to a white 96-well plate. Anti-HIV efficacy of drugs was determined based on reduced expression of luciferase relative to the virus-only treatment group.

Strand transfer inhibition assay

The strand transfer assay was performed using the HIV-1 integrase assay kit (XpressBio, Frederick, MD) according to the manufacturer's instructions. Briefly, 100 µl of 1X donor substrate DNA (DS DNA) solution was added to each well and incubated for 30 min at 37°C. The liquid was aspirated from the wells and washed 5 times with 300 µl wash buffer, followed by incubation with 200 µl blocking buffer per well for 30 min at 37°C. Following aspiration of the liquid, wells were washed three times with 200 µl reaction buffer. Next, 100 µl IN enzyme solution was added to each well and incubated under similar conditions followed by removal of liquid and three washes with 200 µl reaction buffer. Each test sample was 5-fold serially diluted (from 1,000 µM to 8 µM) in reaction buffer and 50 µl aliquots added per well. After 5 min incubation at room temperature, 50 µl 1X target substrate DNA (TS DNA) solution was directly added to the 50 µl test sample within the wells. Reactions were mixed by tapping the plate gently 3-5 times and incubating for 30 min at 37°C, washed 5 times with 300 µl wash solution and incubated with 100 µl HRP antibody for 30 min at 37°C. After washing the plate under the same conditions, 100 µl TMB peroxidase substrate solution was added per well and incubated for 10 min at room temperature. To terminate the reaction, 100 µl TMB stop solution was directly added to wells and absorbance read using a plate reader at 450 nm.

Confirmation of inhibition of 3`-processing activity of NCINI

To confirm the 3`-processing inhibition activity NCINI, the strand transfer inhibition assay was modified. The plate coating process with DS DNA was changed to treatment with LTR DS DNA, aliquots of 5-fold serially diluted INI (from 2,000 µM to 3.2 µM) were incubated
with 20 nM IN for 30 min. Reaction of integrase first with the drug before its reaction with DS DNA is a necessary step to validate the 3’-processing inhibitory activity of the compound. The integrase-inhibitor mixture was added to LTR DS DNA-conjugated 96-well plates. Subsequent steps were conducted in a similar manner as the strand transfer assay.

**Statistical analysis**

All measures of variance are presented as standard error of mean (SEM). Data were analyzed via two-way analysis of variance (two-way ANOVA) with Tukey post-hoc test using Prism8 (GraphPad Software, San Diego, CA). Differences were considered significant at \( p \)-values < 0.05.

**Results**

**Comparison of INSTI and NCINI efficacy in the TZM-bl system**

TZM-bl cell lines expressing luciferase are commonly used for *in vitro* evaluation HIV associated.[21] We were commissioned to evaluate the effectiveness of NCINI candidates, but observed no activity using the TZM-bl system. BI 22436 was employed as the positive control *in vitro* to ascertain whether the TZM-bl system could be applied to assess the efficacy of NCINIs. Efficacy of candidates against HIV-1 AD8 was measured in MT2 cells (Fig. 1). EC\(_{50}\) values of 10.38 and 1.6 nM were obtained for RAL and DTG, respectively. EVG showed the greatest anti-HIV efficacy with an EC\(_{50}\) value < 0.15 nM. On the other hand, an EC\(_{50}\) value of 566.4 nM was obtained for BI 224436, suggestive of extremely low efficacy. In contrast to our data, BI 224436 is reported to have EC\(_{50}\) values in the range of 11–27 nM against HxB2, NL4.3 and a recombinant NL4.3 strain.[19] The extremely low antiviral activity of BI224436 evaluated using the TZM-bl system suggests that this assay may not be suitable for assessment of NCINI efficacy.

**Differential INSTI and NCINI activities using the TZM-bl and p24 ELISA assay systems**

The capsid protein, p24, is the most abundant protein of HIV[22, 23] and commonly used in early detection and diagnosis of AIDS/HIV.[23–26] Given the low anti-HIV efficacy of BI22436 in our TZM-bl system, we developed a novel p24 ELISA assay to validate its antiviral activity and compared the results (Fig. 2). Notably, EC\(_{50}\) values of BI 224436, RAL, ELG, and DTG determined with p24 ELISA were 28.72, 0.58, > 0.51, and > 0.51 nM, respectively, distinct from data obtained with the TZM-b1 system.

The cytotoxicity evaluation test was performed to calculate the selectivity index (SI) of each drug in both TZM-b1 (Table 1) and MT2 cell lines (Table 2). BI 224436 displayed low cytotoxicity with high CC\(_{50}\) values > 50,000 nM in both MT2 and TZM-bl cell lines. In the TZM-bl system, BI 224436 showed high EC\(_{50}\) and low SI values, it looks like unsuitable for further development (Table 1). On the other hand, in MT2 cells, BI 224436 showed a high SI value compared to other INSTIs, indicating that the newly developed system is more appropriate for evaluating antiviral efficacy of NCINIs (Table 2).

| Cytotoxicity and effective concentrations of integrase inhibitors in the TZM-bl cell system |
|-----------------------------------------------|
| **CC\(_{50}\) (nM)** | **EC\(_{50}\) (nM)** | **SI value** |
|-------------------------|-------------------|-------------|
| BI 224436 | >50000.00 | 566.40 | >88.28 |
| EVG | 26041.67 | <0.15 | 173611.13 |
| RAL | >50000.00 | 10.38 | 4816.96 |
| DTG | >50000.00 | 1.60 | 31250.00 |

*CC\(_{50}\); Half maximal cell cytotoxicity concentration, EC\(_{50}\); Half maximal effective concentration. SI value; Selectivity index, CC\(_{50}/EC\(_{50}\)*

Table 2

| Cytotoxicity and effective concentrations of integrase inhibitors in the MT2 cell system |
|-----------------------------------------------|
| **CC\(_{50}\) (nM)** | **EC\(_{50}\) (nM)** | **SI value** |
|-------------------------|-------------------|-------------|
| BI 224436 | >50000.00 | 566.40 | >88.28 |
| EVG | 26041.67 | <0.15 | 173611.13 |
| RAL | >50000.00 | 10.38 | 4816.96 |
| DTG | >50000.00 | 1.60 | 31250.00 |

*CC\(_{50}\); Half maximal cell cytotoxicity concentration, EC\(_{50}\); Half maximal effective concentration. SI value; Selectivity index, CC\(_{50}/EC\(_{50}\)*
Strand Transfer Inhibition Test

To assess the differences in activities between NCINIs and INSTIs according to mechanism, an IN inhibition assay evaluating the effectiveness of strand transfer inhibition was performed. EVG, RAL, and DTG exerted >50% inhibitory activity at 8 µM, with EVG exerting the greatest effect (up to 94% inhibition). In contrast, BI 224436 showed low inhibitory activity (36% at 1000 µM and 20% at 8 µM; Fig. 3). Our data are consistent with previous reports that BI 224436 has an EC$_{50}$ value > 50 µM and does not inhibit strand transfer.19, 20

Evaluation of differences in INSTI and NCINI activities according to inhibition mechanism

The experimental assay used in this study measured integrase inhibitor activity as follows: first, donor DNA was reacted with integrase that caused 3'-processing, followed by addition of inhibitor. Next, strand transfer was induced by adding target DNA. An HRP-conjugated antibody recognizing the 3`-modified target DNA was added to the mixture, and absorbance measured to determine the strand transfer inhibition rate. To assess 3'-processing inhibition by BI 224436 using this kit, the protocol was modified to allow initial reaction of the inhibitor with IN for 30 minutes, and the inhibitor-integrase mixture subsequently added to DS DNA. The subsequent steps were the same as the strand transfer assay protocol (Fig. 4).

EVG showing the highest anti-HIV activity in the previous experiment was used as a control. Using the modified experimental protocol, BI 224436 showed significant IN inhibition activity at both high and low concentrations of 2000 and 3.2 µM (87.14% and 80.65%, respectively). Additionally, high inhibitory activity of EVG (76.58–97.01%) was detected. Interestingly, BI 224436 was twice as effective as an inhibitory agent in the modified system (especially at 3.2 µM) with higher efficiency than EVG (Fig. 5).

Discussion

Following the earliest isolation and identification of HIV, extensive research has focused on effective treatments for the disease. The rapid development of drugs that inhibit HIV through various mechanisms, such as suppression of integration, reverse transcription and virus entry/fusion, has led to a steady decline in HIV-associated deaths worldwide. Evaluation of the efficacy of various types of drug candidates requires accurate methods based on their specific characteristics and mechanisms of action. Inappropriate analysis of candidates may lead to false or no results, and consequently, considerable losses to the drug development industry. We were commissioned to evaluate the efficacy of the novel NCINI candidates. Initially, efficacy assessment was conducted with the TZM-bl system, which we concluded was unsuitable for assessment of NCINI compounds in view of the low efficacy of BI 224436. Therefore, we developed an alternative method to evaluate NCINI activity. In previous experiments, BI 224436 efficacy was evaluated using human peripheral blood mononuclear cells (hPBMCs), C8166,[19, 20] and T lymphocyte cell lines. However, separation of hPBMCs from blood is difficult. In our experiment, the lymphocyte cell line, MT2, was employed to facilitate evaluation of the process. We developed a novel p24 ELISA method by infecting the cell suspension with the virus for longer periods of time, which yielded distinct results from the TZM-bl system. p24 ELISA data obtained using MT2 cells were nearly 20-fold higher than those with the TZM-bl system, consistent with earlier findings on BI 224436.[19, 20] INSTI compounds used as controls showed effective anti-HIV activity in both TZM-b1 and p24 ELISA assays while accurate anti-HIV activity of BI 224436 was only determined via p24 ELISA. The key difference between the two systems is the possibility of long-term incubation after virus infection or drug treatment, which is attributable to the different cell lines used.27

NCINI is classified as an allosteric integrase inhibitor (ALLINI).[13] ALLINI directly inhibits not only HIV integration but also binding of viral RNA with integrase in virions. The compound causes damage in the morphogenesis process, creating a non-infectious virion. NCINI exerts its inhibitory activity under conditions of more than two cycles of virus replication.[27] Thus, incubation time of cells in efficacy analysis of NCINI in vitro may be an important factor for accurate activity measurement. TZM-bl is an adherent cell line and due to spatial constraints, long-term monitoring is not possible owing to cell overgrowth problems following incubation periods of more than three days. However, the cell suspension can be cultured for longer periods than adherent cell lines due to less restriction of space. Therefore, when assessing the efficacy of NCINI and other ALLINI candidates in cells, the precise effects may be measurable only under long-term incubation conditions. Some ALLINIs bind directly to CCD of IN to exert their effects but others are reported to inhibit protein-protein

|          | CC50 (nM)* | EC50 (nM)* | SI value* |
|----------|------------|------------|-----------|
| BI 224436| >50000.00  | 28.72      | >1740.95  |
| EVG      | 1263.50    | <0.51      | >2477.46  |
| RAL      | 6423.61    | 0.58       | 11075.19  |
| DTG      | 1740.93    | <0.51      | >3413.60  |

*CC50; Half maximal cell cytotoxicity concentration, EC50; Half maximal effective concentration. SI value; Selectivity index, CC50/EC50
interactions by combining it into a distinct site.\cite{13, 19, 27} BI 224436, the first identified NCINI, prevents integration by blocking 3'-processing as well as structural deformation of CCD. Therefore, for evaluation of the effectiveness of new ALLINI candidates, co-execution of cell and mechanism-based activity assays, such as the 3'-processing inhibition assay, should be considered.

**Conclusion**

Herein, we have demonstrated differences in NCINI efficacy based the evaluation method and developed a novel protocol to facilitate accurate evaluation of antiviral activity. For development of NCINI as a new class of drug, accurate drug efficacy assessment is urgently required to allow drug regulators to make informed decisions.\cite{28–30} Our study presents a novel assay method for NCINI activity through simple modification of the existing protocol and may serve as a guideline for clinical efficacy assessment of NCINI compounds in the future.

**Abbreviations**

AIDS
acquired immune deficiency syndrome
ALLINI
allosteric integrase inhibitor
CCD
catalytic core domain
DTG
dolutegravir
EVG
elvitegravir
FDA
US Food and Drug Administration
HAART
highly active anti-retroviral therapy
hPBMC
human peripheral blood mononuclear cell
IN
integrase
INI
integrase inhibitor
INSTI
integrase strand transfer inhibitor
LEDGF/p75
lens epithelial-derived growth factor/p75
LTR
long-terminal repeat
NCINI
non-catalytic integrase inhibitor
NNRTI
non-nucleoside reverse transcriptase inhibitor
NRTI
reverse transcription inhibitor
PI
protease inhibitor
PIC
pre-integration complex
RAL
raltegravir
RTI
reverse transcription inhibitor

**Declarations**

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The authors thank SP Pharm for supporting the raw materials.
Authors’ contribution
KP conducted experiment and constructed screening method, wrote the manuscript. MK interpreted data, performed statistical analysis and edited the final version manuscript. SB and HL helped constructing the experiment. KK and BC reconfirmed about uncertain result. YK contributed to design of the study and controlled overall project planning.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
Not applicable

Consent to publication
Not applicable

Competing interests
No competing financial interests exist.

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Figures

Figure 1

Determination of antiviral activities of integrase inhibitors in the TZM-bl cell line system. Integrase inhibitors were serially diluted and incubated with 1x104 TZM-bl cells per well during 30 min, followed by infection with 500 TCID50 of HIV-1 A8 strain. For determination of antiviral efficacy, luciferase activity was measured after 48 h of incubation. Cell culture media of non-infected and infected cells without HIV drug treatment were set as the negative and positive control, respectively. EVG: Elvitegravir, RAL: Raltegravir, DTG: Dolutegravir.
Figure 2

Comparison of antiviral activities of HIV drugs determined with p24 ELISA. MT2 cells infected with 500 TCID50 of HIV-1 AD8 strain were treated with a range of concentrations of each drug. At 5 days post-infection, cell culture media were harvested for p24 ELISA. Media of non-infected and infected cells without HIV drug treatment were set as the negative or positive control, respectively. EVG: Elvitegravir; RAL: Raltegravir; DTG: Dolutegravir.

Figure 3

Strand transfer inhibition efficiency of catalytic and non-catalytic integrase inhibitors. Integrase inhibitor compounds at a range of concentrations generated from 5-fold serial dilutions were used to treat 3’-end cleaved LTR DS DNA by integrase. After incubation, TS DNA was added to each well for the strand transfer reaction. The reaction products were detected colorimetrically using a HRP-labeled antibody against modified target DNA. "No integrase" and "Integrase only" wells were set as the negative and positive control, respectively.
Figure 4

Schematic diagram of the strand transfer and 3'-processing inhibition assay. The strand transfer assay protocol was modified to perform the 3'-processing inhibition assay as illustrated in the green box.

Figure 5

3'-Processing inhibition efficiency of catalytic and non-catalytic integrase was assessed using a commercial INhibitor assay kit. The figures in the yellow boxes illustrate the non-catalytic and catalytic integrase inhibitors. Before addition of LTR dsDNA, 5-fold serially diluted integrase inhibitors were incubated with 20 nM integrase for 30 min. The integrase-inhibitor mixtures were treated with LTR dsDNA-conjugated 96-well plates. After incubation, target DNA was added to each well for the strand transfer reaction, and the products were detected colorimetrically using a HRP-labeled antibody against 3'-modified target DNA. "No integrase" and "integrase-only" wells were set as the negative and positive control, respectively.