Biochemical and Biophysical Research Communications 375 (2008) 139–144

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

D77, one benzoic acid derivative, functions as a novel anti-HIV-1 inhibitor targeting the interaction between integrase and cellular LEDGF/p75

Li Du [a,1], Yaxue Zhao [b,1], Jing Chen [a], Liumeng Yang [c], Yongtang Zheng [c], Yun Tang [b,*], Xu Shen [a,b,*], Hualiang Jiang [a,b]

[a] Drug Discovery and Design Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China
[b] School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China
[c] Laboratory of Molecular Immunopharmacology, Key Laboratory of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

ARTICLE INFO

Article history:
Received 25 July 2008
Available online 6 August 2008

Keywords:
HIV-1 integrase
Lens epithelium-derived growth factor (LEDGF/p75)
Yeast two-hybrid assay
Surface plasmon resonance (SPR)
Molecular docking
Site-directed mutagenesis

ABSTRACT

Integration of viral-DNA into host chromosome mediated by the viral protein HIV-1 integrase (IN) is an essential step in the HIV-1 life cycle. In this process, Lens epithelium-derived growth factor (LEDGF/p75) is discovered to function as a cellular co-factor for integration. Since LEDGF/p75 plays an important role in HIV integration, disruption of the LEDGF/p75 interaction with IN has provided a special interest for anti-HIV agent discovery. In this work, we reported that a benzoic acid derivative, 4-[(5-bromo-4-{[2,4-dioxo-3-(2-oxo-2-phenylethyl)-1,3-thiazolidin-5-ylidene]methyl}-2-ethoxyphenoxy)methyl]benzoic acid (D77) could potently inhibit the IN-LEDGF/p75 interaction and affect the HIV-1 IN nuclear distribution thus exhibiting antiretroviral activity. Molecular docking with site-directed mutagenesis analysis and surface plasmon resonance (SPR) binding assays has clarified possible binding mode of D77 against HIV-1 integrase. As the firstly discovered small molecular compound targeting HIV-1 integrase interaction with LEDGF/p75, D77 might supply useful structural information for further anti-HIV agent discovery.

Introduction

Human immunodeficiency virus type 1 (HIV-1) takes advantage of cellular proteins and cellular pathways for completing different steps in its life cycle, and to achieve productive infection, the reverse transcribed cDNA of HIV-1 is inserted in the host cell genome. HIV-1 integrase (IN) is responsible for the integration step [1]. Integration occurs in two catalytic reactions, referred to as 3′-processing and strand transfer, completed in the cytoplasm and nucleus, respectively [2–6].

A variety of cellular proteins have been put forward as important partners in the integration, among which Lens epithelium-derived growth factor (LEDGF/p75) is the first co-factor shown to function as a cellular co-factor for integration *in vivo* [7], and binding to LEDGF/p75 appears to account for the characteristic intracellular distribution of IN [8].

LEDGF/p75 was originally isolated and characterized as a general transcriptional co-activator that plays a protective role during stress-induced apoptosis [9–11], and was then identified by several laboratories for its ability to bind tightly to HIV IN by solution methods or cellular assays[12,13].

The C-terminus consisting of residues 347–429 for LEDGF/p75 was identified as integrase binding domain (IBD) and the crystal structure of the catalytic domain of HIV-1 IN in complex with LEDGF/p75 IBD supported much structural information about the interaction [13–16]. Further studies showed that LEDGF/p75 acts through a tethering mechanism as a potent co-factor for HIV-1 integration with the N-terminal Pro- Trp- Trp- Pro (PWWP) and A/T-hook elements binding to chromatin, and a C-terminal integrase-binding domain (IBD) binding to IN [17–20].

As has been discovered, LEDGF/p75 plays an important role in HIV integration, disruption of the LEDGF/p75-IN interaction may thus demonstrate potent therapeutic potential [19]. In this work, one small molecular compound D77 (Fig. 1A) was discovered that showed strong inhibition activity against IN-LEDGF/p75 interaction and affected the HIV-1 IN nuclear distribution by exhibiting antiretroviral activity. Molecular docking with site-directed mutagenesis investigation and SPR assays provided a possible

* Corresponding authors. Fax: +86 21 50806918.
E-mail addresses: ytang234@ecust.edu.cn (Y. Tang), xshen@mail.shcnc.ac.cn (X. Shen).
[1] These authors contributed equally.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.07.139

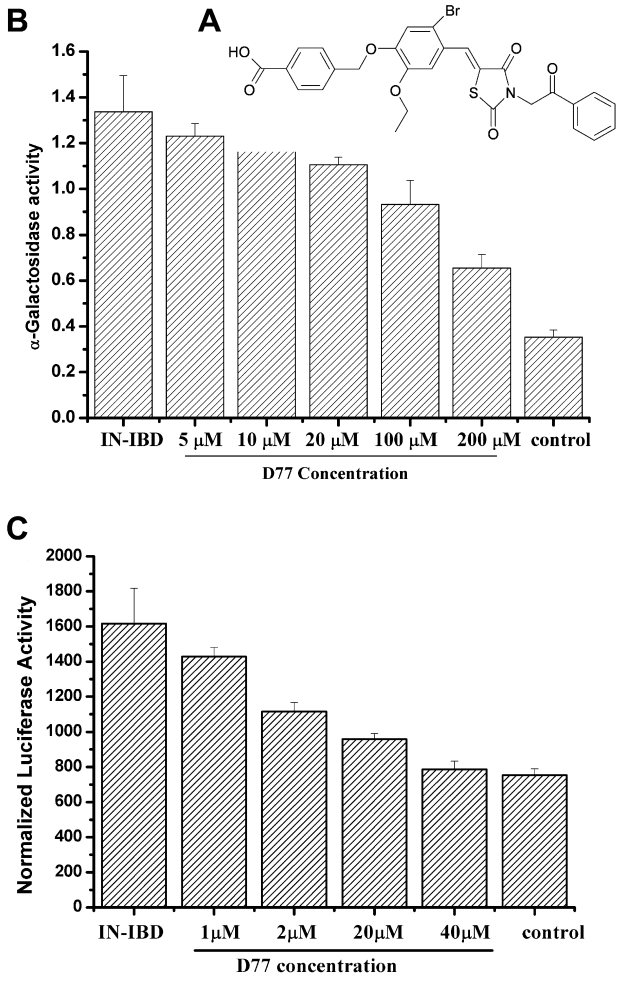

Fig. 1. Structure of D77 (A) and the inhibition of D77 against IN/IBD interaction as evaluated by yeast two-hybrid technology based assay (B) and mammalian two-hybrid assay (C). (B) IN-IBD, yeast cells co-transformed with pGBKT7-IN and pGADT7-IBD, 5–200 μM, yeast cells co-transformed with pGBKT7-IN and pGADT7-IBD treated with different concentrations of D77, control, yeast cells co-transformed with empty vectors pGBKT7 and pGADT7. (C) IN-IBD, CHO-K1 cells co-transfected with pBIND-IN: pACT-IBD: pG5*luc* plasmids, 1–40 μM, the CHO-K1 cells co-transfected with pBIND-IN: pACT-IBD: pG5*luc* plasmids treated with different concentrations of D77, control, CHO-K1 cells co-transfected with empty vectors pBIND, pACT and pG5*luc* plasmids. All the experiments were repeated for three times and the data were obtained by the average.

binding mode of D77 against HIV-1 integrase. It is expected that D77, as the first small molecular compound targeting IN-LEDGF/p75 interaction, might provide useful information for the discovery and development of new anti-HIV agents.

Materials and methods

Chemistry. Compound D77 was purchased from SPECS bank.

Protein preparation. The wild type HIV-1 IN DNA coding for HIV-1 integrase (GenBank No. AF 040373) was synthesized with an Applied Biosystems DNA synthesizer (Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd.) and cloned into glutathione *S*-transferase (GST) expression vector pGEX-4T-1. F185K substitution was introduced to construct the mutant plasmid pGEX-4T-1-IN (F185K) to increase the solubility [21]. The plasmid pGEX-4T-1-IN (F185K) was used as the template DNA to construct the deletion mutant pGEX-4T-1-IN$^{52-210}$ and IN mutants. The plasmid pCPNat p75 was a gift from Prof. Zeger Debyser, Katholieke Universiteit Leuven, Belgium. The integrase binding domain (IBD) (residues 347–442) was cloned into pGEX-4T-1 for expression and purification. IN, IN$^{52-210}$, and IBD were expressed and purified according to the GST gene fusion system handbook (Amersham Biosicence). IN and IN mutants IN(Q95A), IN(T125A), IN(W131A) and IN(T174A) were purified in GST-fusion form. The purity of all proteins was confirmed by SDS–PAGE.

Yeast two-hybrid assay. IN and IBD were cloned into yeast vectors pGBKT7 and pGADT7 (Clontech, Palo Alto, CA). Yeast transformation was performed according to the protocol of manufacturer. Quantitative α-galactosidase activity assays were carried out by using *p*-nitrophenyl α-D-galactopyranoside (PNP-α-Gal) as substrate according to the Clontech manual. The α-galactosidase activity was calculated with the following formula:

$$\alpha\text{-galactosidase activity [milliunits/(ml} \times \text{cell)]} = \mathrm{OD}_{410} \times V_f \times 1000/(\varepsilon \times b \times t \times V_i \times \mathrm{OD}_{600})$$

where t is elapsed time (in min) of incubation, V_f is the final volume of assay (200 μl), V_i is the volume of culture medium supernatant added (16 μl), and OD_{600} is the cell density at the start of assay, $\varepsilon \times b$ is *p*-nitrophenol molar absorptivity at 410 nm × the light path (cm) = 10.5 (ml/mol) here (Yeast Protocols Handbook).

Mammalian two-hybrid assay. The CheckMate™ Mammalian Two-Hybrid Assay Kit was obtained from Promega. CHO-K1 cells were grown in 24-well tissue culture plates to 70% confluency and transfected with a total of 0.45 μg plasmid DNA (1:1:1 mix of the pBIND-IN: pACT-IBD: pG5*luc* plasmids) using the Lipofectamine™ 2000 transfection reagent (Invitrogen). Growth medium

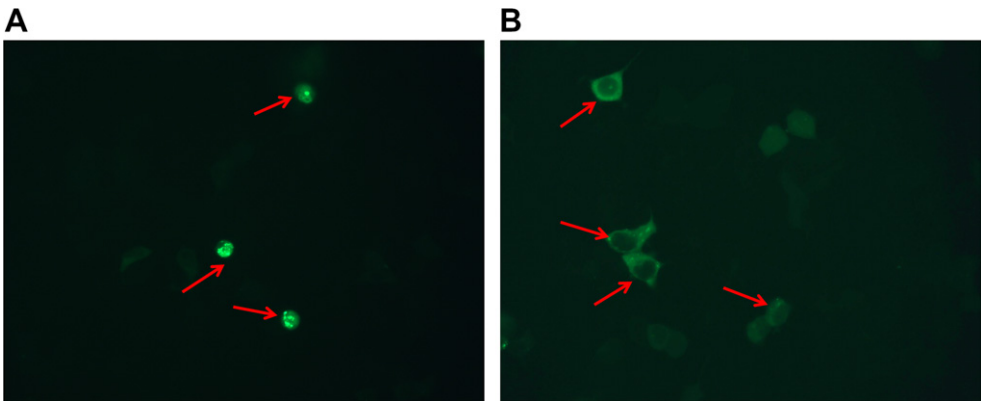

Fig. 2. Effects of D77 on EYFP-IN nuclear distribution. (A) 293T cells transformed with pEYFP-IN plasmid, EYFP-IN was mainly distributed in the nucleus. (B) 293T cells transformed with pEYFP-IN plasmid treated with 5 μM of D77, EYFP-IN was mainly distributed in the cytoplasm. Red arrowheads show the EYFP-IN distribution.

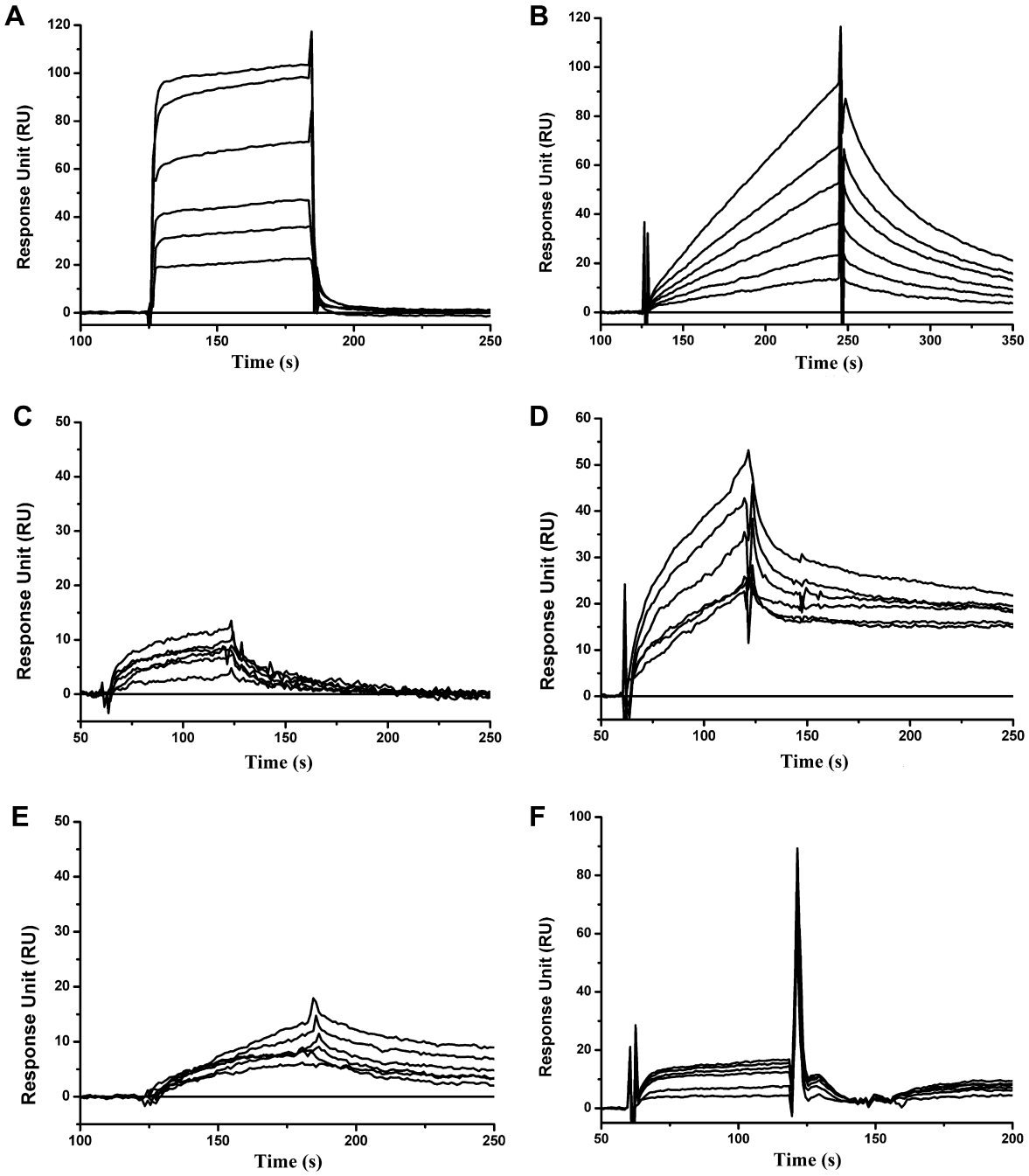

Fig. 3. Binding analysis of D77 to HIV-1 IN (A), IN catalytic domain (B), HIV-1 IN(Q95A) (C), IN(T125A) (D), IN(W131A) (E) and IN(T174A) (F) as determined by SPR assay. Representative sensorgrams obtained with D77 at concentrations of 10, 7.0, 4.9, 3.43, 2.40, 1.68, 0 μM (curves from top to bottom) are shown. D77 was injected for 60 s, and dissociation was monitored for 120 s.

was replaced with DMSO or test compound diluted F-12 after 4–6 h. At 24 h post-transfection, both firefly luciferase and *Renilla* luciferase enzyme activities were measured from the same cell lysate sample on TECAN microplate reader using the Dual-Luciferase Reporter Assay System (Promega).

EYFP-IN intracellular distribution assay. IN was cloned to pEYFP-C1 with XhoI and BamHI for EYFP-fusion IN expression. 293T cells transiently transfected with pEYFP-IN were treated with different concentrations of D77 for 10–12 h. Hoechst was used for nuclear staining. Cells were examined with an Olympus IX51 microscope equipped with a CCD-digital camera (Olympus U-TV0.5XC-3). Fluorescence was analyzed with a combination filter cube for YFP and Hoechst 33342/bound DNA (excitation/emission for these fluorophores, 475/535 and 360/460 nm). In the assay, the 293T cells transformed with the EYFP-C1 vector were set as control.

Antiretroviral activity assay. MT-4 cells were grown and maintained in RPMI medium 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 0.1% sodium bicarbonate, and 20 g of gentamicin per ml. HIV-1(III_B) was obtained from Medical Research Council, AIDS Reagent Project (UK). The inhibitory effects of the compound on HIV-1 replication were monitored by the inhibition of virus-induced cytopathicity in MT-4 cells at 5 days after infection as described. Cytotoxicity of the compounds against MT-4 cells was determined by measuring the viability after 5 days

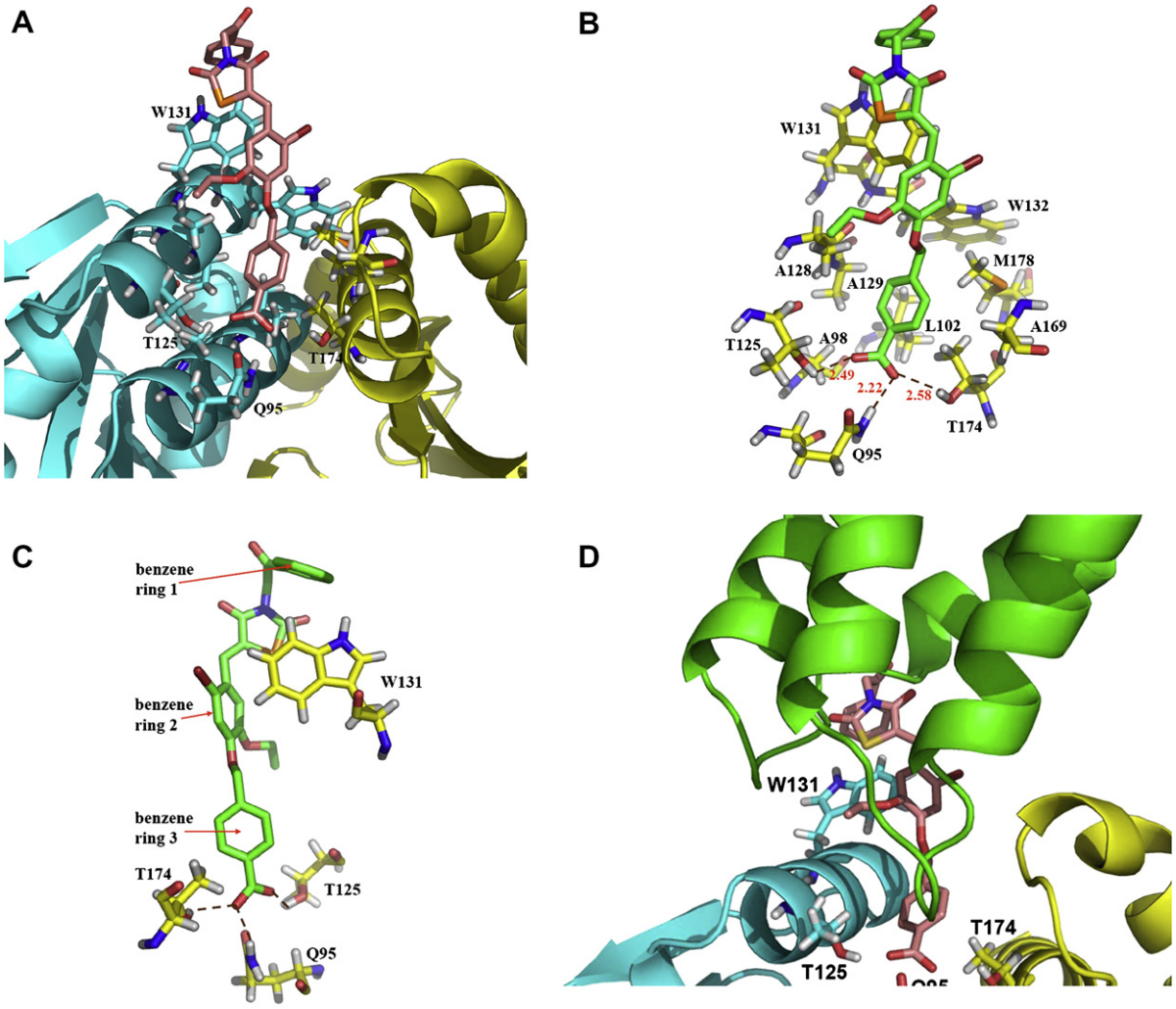

Fig. 4. Molecular docking of D77 into HIV-1 IN CCD. (A) The binding site of D77 on HIV-1 IN CCD dimer interface. Yellow and cyan-IN chains, salmon-D77. (B, C) The interaction between D77 and HIV-1 IN CCD in detail. (B-front view, C-side view) D77 was shown in green and IN residues were colored in yellow. Hydrogen bond was signed in red. (D) Molecular docking of D77 with CCD/IBD complex. Yellow and cyan-IN chains, green-IBD, salmon-D77.

of incubation [22]. C8166 cells were also employed in the anti-HIV-1 activity assay as previously reported [23].

SPR binding assay. The binding affinity of the compound to HIV-1 IN, IN^{52-210}, IN(Q95A), IN(T125A), IN(W131A) and IN(T174A) *in vitro* was determined by using SPR biosensor technology. The measurement was performed on the dual flow cell Biocore 3000 instrument (Biacore AB, Uppsala, Sweden). Immobilization of wild-type and mutant IN proteins to sensor chip CM5 (Biacore) was carried out by the standard primary amine coupling method. For the GST fusion protein IN, IN(Q95A), IN(T125A), IN(W131A) and IN(T174A) binding assays, the reference flow cell surface was immobilized at a parallel level using GST as a control. All the sensorgrams were processed by using automatic correction for non-specific bulk refractive index effects. The specific binding profiles of compound to immobilized protein were obtained after subtracting response signal from control flow cell. All Biacore data were collected at 25C with HBS-EP as running buffer at a constant flow of 30 μL/min. The equilibrium dissociation constants (K_D) evaluating the protein–ligand binding affinity were determined using 1:1 binding model (Langmuir) and the curve fitting efficiency was checked by residual plots and χ^2.

Site-directed mutagenesis. Site-directed mutagenesis was performed based on the plasmid pGEX-4T-1-IN using QuikChange site-directed mutagenesis system (Stratagene, La Jolla, Calif.). Glu95, Thr125, Trp131 and Thr174 were mutated to alanine. All clones were verified by sequencing.

Molecular modeling. Molecular modeling was carried out with SYBYL version 7.0 [24] on a Dell Precision 670 workstation running Linux Redhat WS 3.0. Compound structure was manually sketched and energetically minimized with Gasteiger–Hückel charges [25]

Table 1
Binding affinities [a] of D77 to IN, IN CCD (catalytic core domain) and IN mutants (IN(Q95A), IN(T125A), IN(W131A), IN(T174A)) determined by surface plasmon resonance (SPR) technology based Biacore 3000

Protein	IN	IN CCD	IN(Q95A)	IN(T125A)	IN(W131A)	IN(T174A)
K_D (μM)	5.81 ± 0.32	6.83 ± 0.56	—[b]	15.2 ± 0.98	—[b]	—[b]

[a] Equilibrium dissociation constant, K_D values (μM).
[b] No binding was detected.

and Tripos force field [26]. Crystal structures of HIV-1 IN CCD at free state and in complex with LEDGF/p75 IBD were obtained from Protein Data Bank (PDB) with entry codes 1QS4 and 2B4J, respectively [15,27]. All hydrogen atoms were minimized using Kollman all-atom force field and Kollman all-atom charges [28,29].

Molecular docking was performed with program GOLD version 3.0.1 on a Dell PowerEdge 1850 cluster running Linux Redhat AS 3.0 [30]. During GOLD docking, the default parameters of GA were applied to search reasonable binding conformation of the compound and ChemScore function was used to evaluate docking results [31].

Results

Yeast and mammalian two-hybrid assay of D77 inhibition against HIV-1 integrase interaction with LEDGF/p75 IBD

To investigate the inhibition of D77 against HIV-1 IN interaction with LEDGF/p75 IBD, a yeast two-hybrid system was constructed. In this system, yeast cells expressing HIV-1 IN fused to the Gal4 DNA-binding domain and LEDGF/p75 IBD fused to the Gal4 activation domain constitutively interact, resulting in the activation of the LacZ reporter gene expression. To test the effects of compounds on this interaction, about 300 compounds were added to culture medium during yeast growth and α-galactosidase activity was monitored. It is discovered that compound D77 induced a dramatic concentration-dependent decrease of α-galactosidase activity compared to the compound-untreated cells. As shown in Fig. 1B, D77 revealed a significant inhibition activity against the interaction of IN with IBD.

To further verify the inhibition activity of D77 against IN/IBD interaction at cellular level, we examined the effect of D77 on IN/IBD interaction in a mammalian two-hybrid system. During the assay, CHO-K1 cells were transfected with Gal4 fused IN, VP16 fused IBD and pG5*luc* plasmids, which resulted in a strong luciferase activity compared to the blank control cells transfected with pBIND, pACT and pG5*luc* plasmids. The transfected cells were treated with different concentrations of D77 for 24 h, and then harvested for luciferase assay. As shown in Fig. 1C, D77 was obviously able to reduce the expression of reporter gene in a dose-dependent manner, further indicating that D77 could inhibit HIV-1 integrase interaction with LEDGF/p75 IBD.

Effect of D77 on EYFP-IN intracellular distribution

In order to identify the effects of D77 on IN intracellular distribution, EYFP-fused IN was transfected into 293T cells. Transient expression of EYFP-IN resulted in a significant nuclear localization (Fig. 2A). To test the effect of D77, 5 μM of D77 was added at 5 h post-transfection and cells were observed 12 h later. As shown in Fig. 2, the addition of D77 obviously affected nuclear accumulation of EYFP-IN without changing cell morphology or influencing the growth of the transfected cells (data not shown). Compared with the EYFP-IN transfected cells untreated with D77, EYFP-IN appeared diffusely distributed in cytoplasm and almost no fluorescence could be observed in nucleus. It could be speculated that D77 might disrupt the IN nuclear distribution through interrupting transfected IN binding to endogenous LEDGF/p75.

Antiretroviral activity

The antiviral activity of D77 against HIV-induced CPE in MT-4 cell culture was determined by MTT assay in MT-4 and C8166 cells [22,23]. The results showed that compound D77 could inhibit HIV-1(III_B) replication by EC_{50} value of 23.8 μg/ml in MT-4 cell (5.03 μg/ml for C8166 cells) and cytotoxicity was observed with 50% cytotoxic concentration (CC_{50}) value of 76.82 μg/ml in mock-infected MT-4 cells (26.36 μg/ml for C8166 cells) (Fig S1).

D77 exhibited a highly specific binding affinity to HIV-1 integrase (IN) catalytic core domain (CCD)

To investigate the binding of D77 to HIV-1 IN, SPR-based Biacore 3000 biosensor was used. In the SPR assay, purified IN and IN^{52-210} were separately immobilized on CM5 chip. Binding response was continuously recorded in the response unit (RU) and presented graphically as a function of time (Fig. 3A and B). The 1:1 Langmuir binding fit model was used to determine equilibrium dissociation constant (K_D). The SPR binding assay results determined that compound D77 could bind to HIV-1 IN and IN^{52-210} by K_D value at 5.81 and 6.83 μM, respectively, which strongly supported that D77 interacted with HIV-1 IN at the catalytic core domain.

Molecular docking

Molecular docking was employed to investigate IN CCD/D77 interaction at atomic level. The 3D structural model was based on the crystal structure of IN CCD in complex with IBD (PDB entry code 2B4J).

As shown in Fig. 4A, D77 was located at the LEDGF/p75 binding site in HIV-1 IN CCD dimer interface, and was docked into the hydrophobic pocket enclosed by IN B-chain residues Ala98, Leu102, Ala128, Ala129, Trp131, and Trp132 and IN A-chain residues Ala169 and Met178 (Fig. 4B). By using hydroxyl oxygen atom, D77 formed O...H—O and O...H—N hydrogen bonds with the side chain of IN residue Thr174 in chain A and the side chain of IN residue Gln95 in chain B, respectively. The oxygen atom of carboxyl group of D77 formed an O...H—O hydrogen bond with the side chain of IN residue Thr125 in chain B. There were two C—H...π hydrogen bonds between indole group of Trp131 in chain B and benzene ring 1 and the benzene ring 2 (Fig. 4C). From the docking results, residues Trp131, Thr125, Gln95 in chain B and Thr174 in chain A were discovered to play important roles for the binding of D77 to IN CCD.

Site-directed mutagenesis analysis

To validate the binding sites of D77 against HIV-1 IN, site-directed mutagenesis technique with SPR assays were preformed. During the assays, four important residues Gln95, Thr125, Trp131 and Thr174 suggested by molecular docking results were substituted by alanine, respectively. As indicated in Table 1 and Fig. 3, substitutions of alanine for Thr125 could significantly reduce D77 binding to IN, and the mutation of Gln95Ala, Trp131Ala and Thr174Ala could almost abolish D77 binding to IN. Obviously, such mutagenesis experiments have clearly implied that residues Gln95, Thr125, Trp131 and Thr174 of IN might be exactly involved in the interaction of HIV-1 CCD with D77. Such binding assay of D77 with IN mutants also supplied the reliability of our molecular docking prediction.

Discussion

In the present work, LEDGF/p75 plays an important role in HIV integration, disruption of the LEDGF/p75-IN interaction may thus demonstrate potent therapeutic potential [19]. In our current study, one small molecular compound D77 (Fig 1A) was discovered that showed strong inhibition activity against IN-LEDGF/p75 interaction in yeast two-hybrid assay and mammalian two-hybrid

assay. HIV-1 IN nuclear distribution was affected by D77 observed in EYFP-IN cell assay. D77 also exhibited antiretroviral activity. Molecular docking with site-directed mutagenesis investigation and SPR technology based assays provided a possible binding mode of D77 against HIV-1 integrase and key residues involved in the binding were identified as Gln95, Thr125, Trp131 and Thr174.

In order to further investigate whether D77 could inhibit IN/IBD binding directly, the competitive assay was carried out by an SPR assay. Purified IN^{52-210} was immobilized on the CM5 chip surface, and IBD pre-incubated with different concentrations of D77 was injected and the response unit (RU) was monitored. The sensorgrams after subtracting the control showed a significant dose-dependent decrease in RU value with the increase of the D77 concentration (Fig S2), which indicated that D77 could directly inhibit IN CCD binding to IBD.

Molecular docking result revealed that D77 located at the interface of IN CCD dimer with IBD (Fig. 4D), which clewed that D77 might influence IN dimerization as it inserted into IN CCD dimer interface. To exclude this possibility, yeast two-hybrid assay was carried out. In brief, IN was cloned into pGADT7 and pGBKT7 vector and transformed into yeast cells for high expression of report gene due to IN dimerization. D77 rendered no influence on IN dimerization (Fig S3). It could be thus deduced that the inhibition of D77 against IN/IBD interaction was not caused by the disruption of IN dimerization.

Taken together, our study identified a compound, D77, which could directly inhibit IN-LEDGF/p75 binding, disturb the nuclear distribution of IN and show antiretroviral activity. The IN-binding sites were identified as Gln95, Thr125, Trp131 and Thr174 by molecular docking and site-directed mutagenesis. Our work provided valuable information for the new anti-HIV agents development targeting IN-LEDGF/p75 interaction. Some modification on D77 has been carried on to increase the solubility and inhibition activity.

Acknowledgments

We thank Prof. Zeger Debyser (Katholieke Universiteit Leuven) for kindly offering the plasmid pCPNat p75. This work was financially supported by the National Natural Science Foundation of China (Grants Nos. 30525024, 20472095, 20572023), the National "863" project of China 2006AA609Z447 and 2006AA609Z41), Shanghai Pujiang Program No. 05PJ14034), Shanghai Key Basic Research Project (Grants Nos. 06JC14080, 05JC14092), the State Key Program of Basic Research of China (Grants Nos. 2004CB58905, 2006AA09Z447), and the grant from CAS (Grant No. KSCX2-YW-R-18).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.07.139.

References

[1] E. Asante-Appiah, A.M. Skalka, HIV-1 integrase: structural organization, conformational changes, and catalysis, Adv. Virus Res. 52 (1999) 351–369.
[2] A. Engelman, K. Mizuuchi, R. Craigie, HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer, Cell 67 (1991) 1211–1221.
[3] C.M. Farnet, W.A. Haseltine, Integration of human immunodeficiency virus type 1 DNA in vitro, Proc. Natl. Acad. Sci. USA 87 (1990) 4164–4168.
[4] P.A. Sherman, J.A. Fyfe, Human immunodeficiency virus integration protein expressed in *Escherichia coli* possesses selective DNA cleaving activity, Proc. Natl. Acad. Sci. USA 87 (1990) 5119–5123.
[5] R.L. LaFemina, P.L. Callahan, M.G. Cordingley, Substrate specificity of recombinant human immunodeficiency virus integrase protein, J. Virol. 65 (1991) 5624–5630.
[6] F.D. Bushman, R. Craigie, Activities of human immunodeficiency virus (HIV) integration protein in vitro: specific cleavage and integration of HIV DNA, Proc. Natl. Acad. Sci. USA 88 (1991) 1339–1343.
[7] P. Cherepanov, G. Maertens, P. Proost, B. Devreese, J. Van Beeumen, Y. Engelborghs, E. De Clercq, Z. Debyser, HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells, J. Biol. Chem. 278 (2003) 372–381.
[8] M. Llano, M. Vanegas, O. Fregoso, D. Saenz, S. Chung, M. Peretz, E.M. Poeschla, LEDGF/p75 determines cellular trafficking of diverse lentiviral but not murine oncoretroviral integrase proteins and is a component of functional lentiviral preintegration complexes, J. Virol. 78 (2004) 9524–9537.
[9] F. Dietz, S. Franken, K. Yoshida, H. Nakamura, J. Kappler, V. Gieselmann, The family of hepatoma-derived growth factor proteins: characterization of a new member HRP-4 and classification of its subfamilies, Biochem. J. 366 (2002) 491–500.
[10] H. Ge, Y. Si, R.G. Roeder, Isolation of cDNAs encoding novel transcription coactivators p52 and p75 reveals an alternate regulatory mechanism of transcriptional activation, EMBO J. 17 (1998) 6723–6729.
[11] D.P. Singh, N. Ohguro, T. Kikuchi, T. Sueno, V.N. Reddy, K. Yuge, L.T. Chylack Jr., T. Shinohara, Lens epithelium-derived growth factor: effects on growth and survival of lens epithelial cells, keratinocytes, and fibroblasts, Biochem. Biophys. Res. Commun. 267 (2000) 373–381.
[12] G. Maertens, P. Cherepanov, W. Pluymers, K. Busschots, E. De Clercq, Z. Debyser, Y. Engelborghs, LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells, J. Biol. Chem. 278 (2003) 33528–33539.
[13] P. Cherepanov, E. Devroe, P.A. Silver, A. Engelman, Identification of an evolutionarily conserved domain in human lens epithelium-derived growth factor/transcriptional co-activator p75 (LEDGF/p75) that binds HIV-1 integrase, J. Biol. Chem. 279 (2004) 48883–48892.
[14] P. Cherepanov, Z.Y. Sun, S. Rahman, G. Maertens, G. Wagner, A. Engelman, Solution structure of the HIV-1 integrase-binding domain in LEDGF/p75, Nat. Struct. Mol. Biol. 12 (2005) 526–532.
[15] C.M. Bradley, R. Craigie, Seeing is believing: structure of the catalytic domain of HIV-1 integrase in complex with human LEDGF/p75, Proc. Natl. Acad. Sci. USA 102 (2005) 17543–17544.
[16] P. Cherepanov, A.L. Ambrosio, S. Rahman, T. Ellenberger, A. Engelman, Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75, Proc. Natl. Acad. Sci. USA 102 (2005) 17308–17313.
[17] M. Llano, S. Delgado, M. Vanegas, E.M. Poeschla, Lens epithelium-derived growth factor/p75 prevents proteasomal degradation of HIV-1 integrase, J. Biol. Chem. 279 (2004) 55570–55577.
[18] S. Emiliani, A. Mousnier, K. Busschots, M. Maroun, B. Van Maele, D. Tempe, L. Vandekerckhove, F. Moisant, L. Ben-Slama, M. Witvrouw, F. Christ, J.C. Rain, C. Dargemont, Z. Debyser, R. Benarous, Integrase mutants defective for interaction with LEDGF/p75 are impaired in chromosome tethering and HIV-1 replication, J. Biol. Chem. 280 (2005) 25517–25523.
[19] M. Llano, D.T. Saenz, A. Meehan, P. Wongthida, M. Peretz, W.H. Walker, W. Teo, E.M. Poeschla, An essential role for LEDGF/p75 in HIV integration, Science 314 (2006) 461–464.
[20] M. Llano, M. Vanegas, N. Hutchins, D. Thompson, S. Delgado, E.M. Poeschla, Identification and characterization of the chromatin-binding domains of the HIV-1 integrase interactor LEDGF/p75, J. Mol. Biol. 360 (2006) 760–773.
[21] T.M. Jenkins, A. Engelman, R. Ghirlando, R. Craigie, A soluble active mutant of HIV-1 integrase: involvement of both the core and carboxyl-terminal domains in multimerization, J. Biol. Chem. 271 (1996) 7712–7718.
[22] V.A. Johnson, R.E. Byington, Quantitative Assays for Virus Infectivity, Srockton Press, New York, 1990.
[23] Q. Wang, Y. Wang, S. Pu, Y. Zheng, Zinc coupling potentiates anti-HIV-1 activity of baicalin, Biochem. Biophys. Res. Commun. 324 (2004) 610–615.
[24] Sybyl Version 7.0, Tripos Associates Inc., St. Louis, MO, 2004.
[25] J. Gsateiger, M. Marsili, Iterative partial equalization of orbital electronegativity: a rapid access to atomic charges, Tetrahedron 36 (1980) 3219–3228.
[26] M. Clark, R. Cramer, N. van Opdebosch, Validation of the general purpose tripos 5.2 force field, J. Comput. Chem. 10 (1989) 982–1012.
[27] Y. Goldgur, R. Craigie, G.H. Cohen, T. Fujiwara, T. Yoshinaga, T. Fujishita, H. Sugimoto, T. Endo, H. Murai, D.R. Davies, Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design, Proc. Natl. Acad. Sci. USA 96 (1999) 13040–13043.
[28] S.J. Weiner, P.A. Kollman, D.T. Nguyen, D.A. Case, An all atom force field for simulations of proteins and nucleic acid, J. Comput. Chem. 11 (1986) 431–439.
[29] S.J. Weiner, P.A. Kollman, D.A. Case, C. Singh, G. Ghio, S. Alagona, P. Profeta, P.A. Weiner, New force field for molecular mechanical simulation of nucleic acids and proteins, J. Am. Chem. Soc. 106 (1984) 765–784.
[30] G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, Development and validation of a genetic algorithm for flexible docking, J. Mol. Biol. 267 (1997) 727–748.
[31] M.L. Verdonk, J.C. Cole, M.J. Hartshorn, C.W. Murray, R.D. Taylor, Improved protein–ligand docking using GOLD, Proteins 52 (2003) 609–623.