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A Novel Assay for Screening Inhibitors Targeting HIV Integrase LEDGF/p75 Interaction Based on Ni²⁺ Coated Magnetic Agarose Beads

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HIV-1 integrase (IN) plays an essential role in viral replication and thus serves as an important target for chemotherapeutic intervention against HIV-1 infection. However, the current three clinical IN inhibitors, raltegravir, elvitegravir and dolutegravir share the same inhibitory mechanism, resulting in a common clinical resistance profile which have emerged in infected patients receiving treatment. Therefore, it is important to develop small molecule inhibitors that impair IN function with distinct mechanisms of action. In this work, a magnetic-beads based biochemical assay targeting the protein-protein interaction (PPI) between HIV IN and the cellular cofactor LEDGF/p75 was developed for identification of HIV-1 IN inhibitors. Furthermore, a library containing 1000 US. Food and Drug Administration (FDA)-approved drugs currently used for human medication was screened to identify inhibitors targeting the PPI. The assay was proved to be quite robust and with the novel assay we successfully identified dexlansoprazole (IC₅₀ of 4.8 μM), a FDA-approved proton pump inhibitor, as a potential inhibitor for the PPI between IN and LEDGF/p75, which bound to the LEDGF/p75 partner with a kinetic dissociation (K_d) constant of 330 nM ± 2.6 nM.

Human immunodeficiency virus type 1 (HIV-1) integrase (IN) is a critical enzyme in the virus replication cycle as it is required for the covalent integration of a double-stranded DNA copy of the viral RNA genome into the infected host cell chromosomes¹. IN mainly mediates two spatially and temporally separated and energetically independent reactions, the 3' processing and the strand transfer reaction. IN has been validated as a therapeutic target for anti-retroviral drug development². Currently, there are three FDA-approved integrase inhibitors: raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG) in clinical usage. These inhibitors all impair strand transfer activity of IN and are collectively termed as IN strand transfer inhibitors (INSTIs)³. Recent study suggests that inhibition of integration will not only block viral replication, but should also enhance T cell survival⁴⁻⁶. Despite of the achievements in the development of the INSTI class, drug-resistant (and multidrug-resistant) HIV-1 strains to this inhibitor class can evolve readily in the clinic. In fact, drug-resistant mutations to all three clinically available INSTIs have emerged in infected patients receiving treatment⁷⁻¹⁰. Therefore, it is of great importance to develop novel inhibitors that target IN out of its active site while overcoming INSTI resistant mutants.

The viral replication of HIV-1 depends on cellular co-factors and cellular pathway¹¹. Host cell proteins that particularly assist the viral integration have been determined and termed as integration cofactors (INCFs). The INCFs have been proposed to play a role in one of the following steps: catalysis, nuclear import, target site selection, or repair of the single-stranded DNA gaps that occur at viral/chromosomal DNA junctions during

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integration¹². Among these identified INCFs, lens epithelium-derived growth factor (LEDGF/p75) is the first identified and the best characterized one to date^{13,14}. The interface of protein-protein interaction between LEDGF/p75 and HIV IN is primarily formed by HIV IN binding domain (LEDGF/p75 IBD) in the C-terminal region of LEDGF/p75 with the HIV IN catalytic core domain (IN CCD). The PPI has been validated for an effective drug target^{15,16}, which small molecules have recently been developed to block^{17–20}.

Up to date, assays for screening compounds that block PPI between IN and LEDGF/p75 could be classified into two main categories: (i) cellular-level screening method based on yeast two-hybrid assay²¹, and (ii) molecular-level screening methods based on Amplified Luminescence Proximity Homogeneous Assay (Alpha) or Homogeneous Time-Resolved Fluorescence^{22,23}. However, these above assays have limitations. Yeast two-hybrid assay is low-throughput, high false positive and time-consuming, while molecular-level screening methods need expensive and sophisticated instruments which are not available to all laboratories.

It has been suggested that novel therapeutics for many diseases including infectious disease may be found by exploiting medications that are already approved for use²⁴. Several precedents for compound “repurposing” exist, such as sildenafil, thalidomide and plerixafor²⁵. Moreover, many approved drugs have a well-established history of safe dosing in broad populations, novel repurposing indications can likely be rapidly tested directly in human subjects, with no need of extensive preliminary safety assessments. Therefore, drug repurposing has become a mainstream strategy in drug development.

In the present study, we developed a novel magnetic beads based assay to detect the PPI between IN and LEDGF/p75. Using the assay, we undertook a drug repositioning screen to identify inhibitors of the IN–LEDGF/p75 interaction from a library of US Food and Drug Administration (FDA)-approved drugs. We found that the assay developed was quite robust. It was also found that dexlansoprazole, which is clinically used for the treatment of erosive esophagitis and gastro-esophageal reflux disease, blocked the interaction of IN–LEDGF/p75 and bound to the LEDGF/p75 partner with a kinetic dissociation (Kd) constant of 330 nM ± 2.6 nM. These results indicated that the assay we developed was effective for drug screening targeting the PPI between IN and LEDGF/p75, and dexlansoprazole may be a potential IN-LEDGF/p75 interaction inhibitor.

Results and Discussion

A binding pocket presenting at the interface of two IN CCD monomers is an important structural feature required for PPI between HIV IN and LEDGF/p75 as a validated target for novel antivirals^{14,26}. Previous research has proved that compounds which inhibited the PPI between the truncated forms of these two target proteins could also block PPI between the full-length proteins²². In this study we designed an assay targeting this binding pocket by using the truncated forms of the two target proteins, IN CCD and LEDGF/p75 IBD. The IN CCD protein used contained HIV IN residues 50–212 fused with an C-terminal His₆-tag and incorporated the F185K mutation to improve protein solubility²⁷. IN (F185K) was previously shown to interact effectively with LEDGF/p75 IBD¹⁴. The LEDGF/p75 IBD component used for the assay was a fusion of LEDGF/p75 residues 347–471 with the N-terminal GST-tag. All of the recombinant proteins used for assay development were affinity purified to >90% as determined by 12% SDS-PAGE (results not shown). For the PPI assay, Ni-NTA magnetic agarose beads were used to bind the PPI complex with His₆-tag.

Principle of the assay. In the assay, GST-tagged LEDGF/p75 IBD (yellow) is mixed with His₆-tagged IN CCD (green) at the desired concentrations. After incubation at room temperature, a double-tagged PPI complex with N-terminal GST-tag and C-terminal His₆-tag is established between two protein partners. The PPI complex is captured by Ni²⁺-coated magnetic beads (red) through His₆-tag, followed by the addition of alkaline phosphatase conjugated anti-GST antibody (dark red). Therefore, the product could be measured by the alkaline phosphatase conjugated-coupled enzyme reaction (Fig. 1A).

Detection of the protein-protein interaction between LEDGF/p75 IBD and IN CCD. A His₆-tag pull down assay was performed to validate the PPI between LEDGF/p75 IBD and IN CCD *in vitro*. The proteins in the PPI complex are identified in a SDS-PAGE gel stained with Coomassie Brilliant Blue R-250 (Fig. 1B). When 1 μg LEDGF/p75 IBD was added to 1 μg IN CCD, two bands can be identified (lane 1–3). In contrast, the negative controls indicated that without IN CCD, no band was identified. These results suggested that the recombinant LEDGF/p75 IBD and IN CCD can interact with each other *in vitro*. Previously, Busschots *et al.*²⁸ has reported that compared with the C-terminal His₆-tagged IN, N-terminal His₆-tagged IN pulled down less LEDGF/p75 and N-terminal domain of IN enhanced the affinity of IN to LEDGF/p75. In this study, C-terminal His₆-tagged IN CCD can pull down LEDGF/p75 IBD *in vitro*, which is concordant with previous studies by Busschots *et al.*²⁸.

Assay development and validation. This study aimed to establish a high-throughput assay for screening inhibitors targeting the PPI between IN CCD and LEDGF/p75 IBD. The optimum concentration ratio of the IN CCD and LEDGF/p75 IBD binding partners was initially determined by titrating, with increased concentrations of LEDGF/p75 IBD against a fixed concentration of 20 nM IN CCD (Fig. 2). A ratio of 1:1 IN CCD: LEDGF/p75 IBD was chosen for the subsequent assays as it was well below the binding capacity of the beads while still provided a robust signal that was within the linear range of the assay and not within the hooking zone.

To test the effectiveness and robustness of the assay, we measured the signal of all reactions under optimal reaction conditions. Furthermore, well-to-well variations in 96-well plates were evaluated by calculation of standard statistical parameters S/N, S/B and z' factor. As shown in Fig. 3, the negative controls in the absence of LEDGF/p75 IBD or IN CCD and in the presence of IN CCD_{W131E}-LEDGF/p75 IBD or IN CCD-LEDGF/p75 IBD_{D366N} showed background readings lower than 0.01 measured the absorbance at 405 nm (A₄₀₅). In contrast, the positive control using the GST-His₆ double tagged protein showed a signal of 0.76 and the IN CCD-LEDGF/p75 IBD PPI gave a signal of 0.66 at 405 nm respectively. Accordingly, the S/N, S/B and z' factor values of the assay

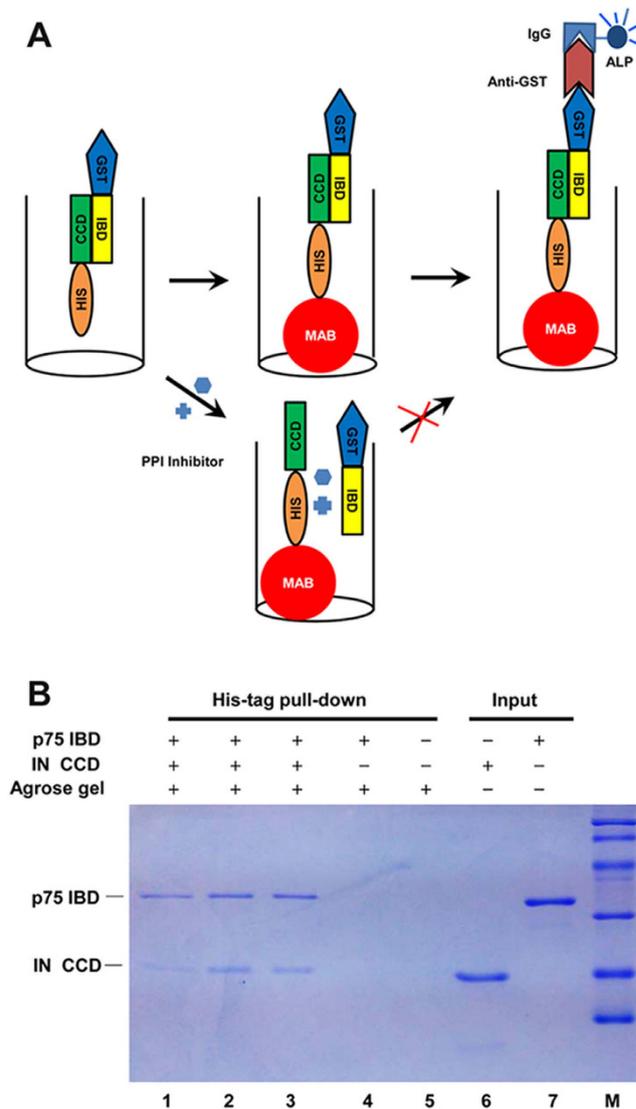


Figure 1. The schemes of the established assay for IN CCD-LEDGF/p75 IBD interaction and SDS-PAGE gel of His₆-tag pull-down assay for IN CCD-LEDGF/p75 IBD interaction. (A) GST-IBD interacts with His₆-CCD in the microplate, forming the PPI complex which is captured by Ni²⁺-coated magnetic beads (MAB). And the amounts of PPI complex are quantified by using ALP-conjugated anti-GST antibody and ALP substrate. In the presence of the PPI inhibitors, GST-IBD cannot interact with His₆-CCD, resulting in no ALP activity. (B) Recombinant LEDGF/p75 IBD was incubated with HIV-1 IN CCD containing a His₆-tag at the C terminus (lane 1–3). The complexes were bound on a Ni²⁺ chelating resin. Lanes 6 and 7 reflect the protein input in the reactions. IN CCD was omitted in lane 4 and only agarose gel was added in lane 5. The gel was stained using Coomassie Blue R-250. The results showed that IN CCD could interact with LEDGF/p75 IBD (lane 1–3), no band was detected in controls (lane 4–5).

were 326, 146 and 0.98 respectively, which exceeded the minimum criteria ($z' > 0.5$, $S/N > 10$, and $S/B > 3$). These values of statistical parameters reflected that the assay developed showed high sensitivity, specificity, and robustness and was suitable to be applied for high-throughput screening (HTS).

To test whether the HTS assay was effective for screening inhibitors of the PPI through detection of reduced signal, we tested optimized IN CCD-LEDGF/p75 IBD binding assay in the presence of increasing concentrations of the peptide IL-23, which was previously reported as an inhibitor of IN-LEDGF/p75 PPI²⁹. As shown in Fig. 4, the assay signal decreased with the increase in IL-23 concentration, implying that the PPI was inhibited by IL-23. The IC₅₀ value was calculated based on the assay results using a non-linear regression curve fit. In this assay, IL-23 inhibited the signal production with an IC₅₀ of 22.0 μM (95% confidence interval [CI], 19.7 μM–24.6 μM) which was comparable to previous experiment result of 25.0 μM, indicating that the assay can robustly detect the decreases in signal and was therefore effective and suitable for a HTS application²⁹.

Compared with other three screening systems currently used in HTS for IN-LEDGF/p75 interaction inhibitors^{18–20}, the present ELISA-based assay has several improvements: (1) economic (expensive equipment and reagents are not required); (2) convenience (the value is read on an ELISA reader and could be widely used in a

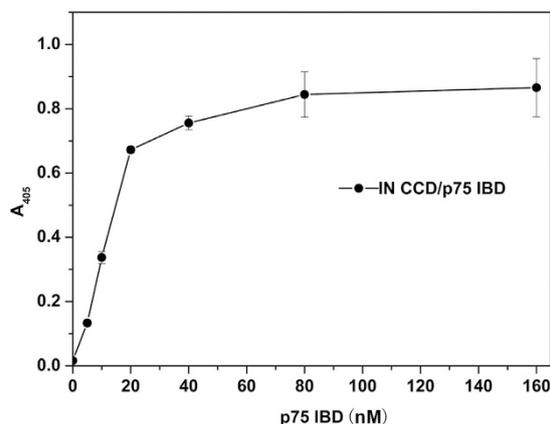


Figure 2. Determination of the optimal IN CCD and LEDGF/p75 IBD ratio and protein concentrations for the assay. The assay signal was measured using a fixed concentration of IN CCD (20 nM) against a titration of LEDGF/p75 IBD. Signal was obtained as A_{405} . Error bars represent SD from 3 replicate values. A ratio of 1:1 IN CCD : LEDGF/p75 IBD was chosen for the subsequent assays.

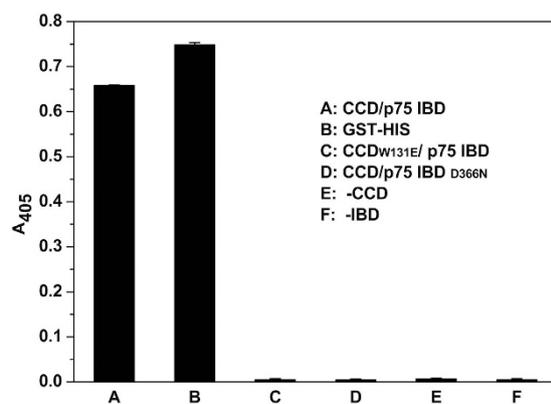


Figure 3. Assay performance under optimal reaction conditions. Signal was obtained as A_{405} . Error bars represent SD from 3 replicate values. The S/N, S/B and z' factor values of the assay were 326, 146 and 0.98 respectively, which exceeded the minimum criteria ($S/N > 10$, $S/B > 3$ and $z' > 0.5$). These values of statistical parameters reflected that the assay developed showed high sensitivity, specificity, and robustness and was suitable to be developed for high-throughput screening (HTS).

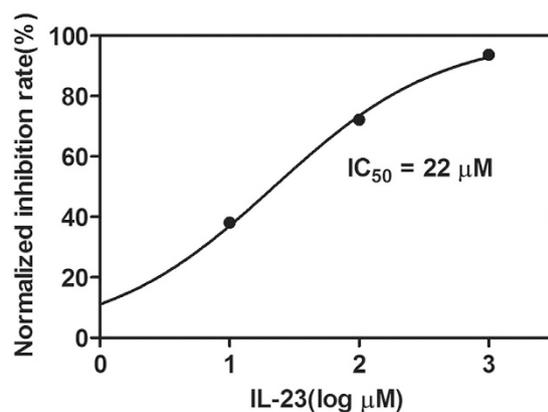


Figure 4. Inhibition of interaction between IN CCD and LEDGF/p75 IBD by peptide IL23. Compounds were diluted in DMSO to a final concentration of 10% (v/v) DMSO in the reaction volume. PPI binding mutant controls (single-point mutation) were set as negative controls, and the positive control contained 10% DMSO only. Error bars represent SD from 3 replicate values.

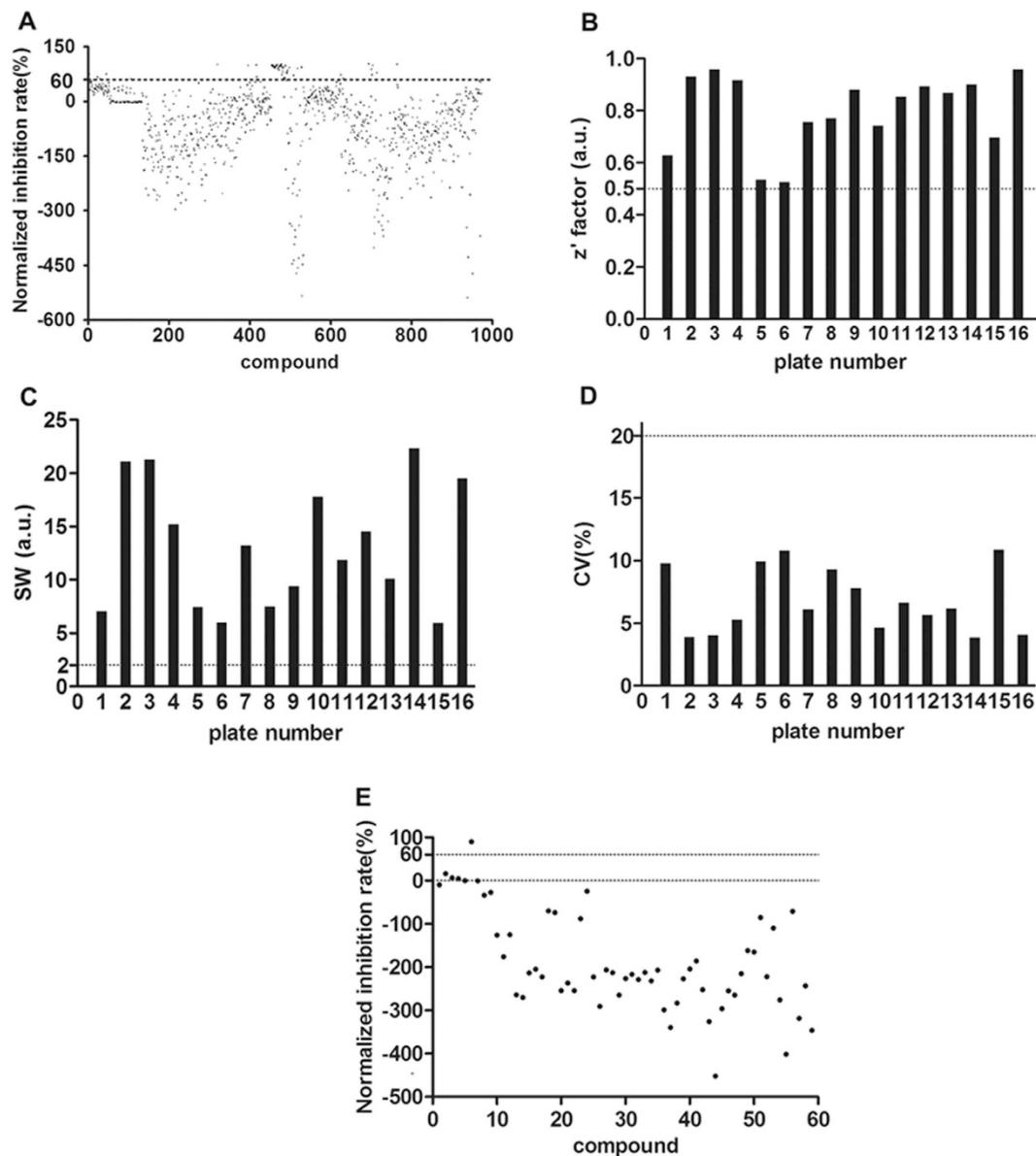


Figure 5. The results of HTS screening and assay quality control. (A) Scatterplots of the primary screening using the developed assay. Dashed line shows the “hits” selection criteria (more than 60% disruption of a PPI complex); Z' factor (B), SW (C) and CV (D) of each plate in the primary screening. Dashed lines show the minimum pass criteria ($z' > 0.5$, $S/N > 10$, $S/B > 3$, $SW > 2$, $CV < 20\%$). (E) Scatterplots of the confirmatory screening using the developed assay. Dashed line shows the “hits” selection criteria (more than 60% disruption of a PPI complex). Z' factor, SW and CV of the confirmatory screening were 0.99, 7.32 and 14% respectively.

normal laboratory); (3) lower background (using the no-binding surface microplate), whereas the molecular-level screening methods need a fluorescence microplate reader and agents that is a bit expensive for some laboratories and a cell-based screening is not convenient for HTS.

High-throughput screening for inhibitors of IN CCD-LEDGF/p75 IBD Interaction. The optimized IN CCD-LEDGF/p75 IBD PPI assay was used to screen a partial library of pharmacologically active compounds for inhibitors of PPI. Compounds were screened as described in the materials and methods section, with appropriate comparison controls included in each plate. Hits were scored as compounds that showed an inhibition ratio of the signal higher than 60% in the assays. The z' , signal window (SW), and coefficient of variation (CV) of each plate were calculated and then compared with the minimum pass criteria ($z' > 0.5$, $SW > 2$, $CV < 20\%$) to evaluate the quality of the HTS data. As shown in Fig. 5A, at the concentration of $30 \mu\text{M}$, 59 of the 1000 initial compounds were identified as hits from the primary screen, with more than 60% inhibition of the signal representing a hit rate of 5.9%. All HTS campaigns in the primary screening exceeded the minimum criteria ($z' > 0.5$, $SW > 2$, and $CV < 20\%$) (Fig. 5B–D), indicating that the results of the screening were reliable and could be used for further investigation. These 59 primary hits were further confirmed as described in the primary screening. Among them,

dexlansoprazole showed the most potent inhibitory effect with an inhibitory rate of 90.3% (Fig. 5E). All HTS campaigns in the confirmatory screening exceeded the minimum criteria ($z' > 0.5$, $SW > 2$, and $CV < 20\%$), indicating that the results of the screening were reliable. Therefore, we focused our follow-up studies on dexlansoprazole.

In the primary screening, each compound was tested in one replicate (Fig. 5A). While in the confirmatory screening, each compound was used in triplicate. Therefore, results in the primary screening were less credible than that in the confirmatory screening. The repeatability of the primary 59 hits was poor as shown in Fig. 5E. Also, in the assay we developed, two kinds of beads were used, and many compounds may interact with the reaction system, leading to the nonspecific interference shown in Fig. 5A. It was reported that compounds which modulate the protein-protein interaction include two types: inhibitor and stabilizer³⁰. In our manuscript, the target was LEDGF/p75-IN interaction, therefore, several compounds may stabilize the target and indicated as enhancers.

Identification of pan-assay interference compounds. ‘Hits’ from the drug screening may become tools for studying the disease, as well as starting points in the hunt for treatments. But many hits are artefacts, as their activity does not depend on a specific, drug-like interaction between molecule and protein³¹. These molecules are previously termed as pan-assay interference compounds (PAINS) or ‘promiscuous inhibitors’ or ‘frequent hitters’^{32–34}. The PAINS have defined structures, and mainly cover 10 classes of compounds, such as alkylidene rhodanines, aralkyl pyrroles, cyclopentene-fused tetrahyd roquinolines, alk-ylidene pyrazolidinediones, alkylidene barbiturates and thiobarbiturates, phenolic Mannich bases, hydroxyphenylhydrazones, quinones, catechols and cyanine dye³². The structure of dexlansoprazole (Fig. 6C) belonged to neither of current published PAINS, indicating that dexlansoprazole may be a specific inhibitor of PPI.

Non-specificity counterscreen validation. The false positive compound identified in this HTS may affect the assay by forming hydrogen bonds interaction with His₆-tag of IN CCD or forming chelating complexes with Ni²⁺-ion covering magnetic beads surface. To verify that dexlansoprazole specifically inhibited the signal of the assay by interfere with PPI itself rather than with the assay system, we used a His₆-GST protein control that bound to the Ni²⁺ coupling magnetic beads and could be detected by GST antibody, generating an extremely strong signal. Any compound that interfered with bead or with the His₆-tag of double-tagged protein would result in a decreased signal in the counterscreen assay. As shown in Fig. 6A, dose-response performed in non-specificity counterscreen indicated dexlansoprazole almost had no effect on the assay system, whereas displayed a dose-dependent effect on IN CCD-LEDGF/p75 IBD PPI with an IC₅₀ value of 4.8 μM (Fig. 6B).

Dexlansoprazole directly binds to LEDGF/p75. It is generally believed that molecules targeting the LEDGF/p75 partner of the protein-protein interaction may have the higher genetic barrier to resistance, in contrast to previously reported compounds which block the IN partner^{35,36}. We therefore utilized biolayer interferometry (BLI) assay to biophysical characterization of dexlansoprazole binding to LEDGF/p75. BLI results demonstrated direct binding of dexlansoprazole to LEDGF/p75 with a calculated dissociation constant K_d value of 330 nM ± 2.6 nM (Fig. 7A). Finally, the binding mode analysis of dexlansoprazole at the PPI interface was performed using PyMOL. The result illustrated in Fig. 7B showed that the binding site amino acid residues which are involved in H-bond interactions with the potent compounds are Thr399 and Lys402 in IBD. All these results indicated that dexlansoprazole was potent small molecular inhibitor for IN CCD-LEDGF/p75 IBD interaction.

Drug repositioning, as a cost effective reduced-risk strategy for developing new drug products, has become a mainstream drug development strategy for major pharmaceutical companies²⁵. In this study, we identified dexlansoprazole, a FDA-approved proton pump inhibitor, as IN CCD-LEDGF/p75 interaction inhibitor through a newly developed assay. In a previous drug repositioning screening with cancer xenograft models, dexlansoprazole, as a primary screen hit, was not under further investigation due to its poor anti-cancer activity³⁷. Here, we provide the first report, to our knowledge, that dexlansoprazole has a moderate inhibitory activity for IN-LEDGF/p75 interaction. As a matter of fact, in our study other five FDA-approved proton pump inhibitors, such as esomeprazole, pantoprazole, lansoprazole, rabeprazole, omeprazole and tenatoprazole, also displayed weak inhibitory activities against IN CCD-LEDGF/p75 IBD interaction (Table 1) and thus were not be used for further investigation.

In conclusion, we have developed a highly sensitive and effective screening method based on Ni²⁺-NTA coupling magnetic agarose beads to identify inhibitors of IN-LEDGF/p75 interaction, and have successfully identified a FDA-approved drug dexlansoprazole that may prove useful in this regard. Furthermore, dexlansoprazole was proved to be a novel small molecule inhibitor of IN-LEDGF/p75 interaction and has the potential to be a lead compound for further optimization.

Materials and Methods

Materials. All general biochemical reagents were obtained from AMRESCO (Solon, USA). Ni-NTA resin and GST resin were purchased from Smart-Lifesciences (Changzhou, China). Ni-NTA Magnetic Agarose Beads were purchased from BEAVER Nano-technologies (Suzhou, China). 96-well nonbinding surface (NBS) microplates were purchased from Corning (New York, USA). Rabbit anti-mouse IgG alkaline phosphatase conjugated antibody and mouse anti-GST antibody were purchased from Sigma (St. Louis, USA). INH5 was synthesized by GL Biochem (Shanghai, China).

Construction of protein expression plasmids. The full-length human LEDGF/p75 cDNA was chemically synthesized in Genewiz, Inc. (Suzhou, China) and cloned into the *Bam*H I-*Sal* I sites of bacterial protein expression vector pGEX-4T-1 resulting in the recombinant plasmid pGEX-4T-LEDGF/p75. The LEDGF/p75 IBD domain encoding sequence was amplified by PCR and subcloned into the *Bam*H I-*Sal* I sites of the vector pGEX-4T-1, creating pGEX-4T-IBD construct for expression of recombinant N-terminal GST-tagged IBD

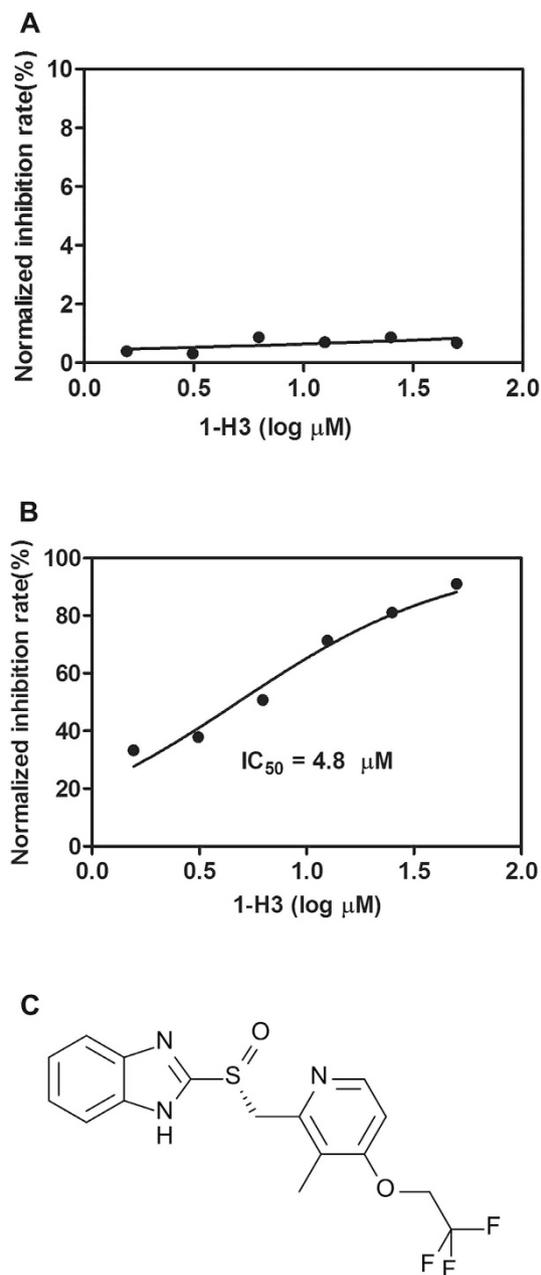


Figure 6. Counterscreen and dose response assay for dexlansoprazole. (A) Counterscreen using His₆-GST protein was performed to analyze the interference between dexlansoprazole and the assay system. (B) Dexlansoprazole identified in precious screening exhibited a sigmoidal dose-dependent reduction of assay signal. (C) The structure of dexlansoprazole.

protein. The recombinant plasmid pGEX-4T-IBD_{D366N} for expression of N-terminal GST-tagged mutation IBD/D366N protein was constructed based on pGEX-4T-IBD using site directed mutagenesis with *Dpn* I. The coding region of the IN CCD (residues 50–212), including the F185K-solubilizing mutation, was PCR amplified from pET28a-IN (conserved in our lab) and subcloned into *Nco* I-*Xho* I sites of the vector pET28a to create the pET28a-CCD for expression of recombinant C-terminal His₆-tagged CCD protein. The recombinant plasmid pET28a-CCD_{W131E} for expression of C-terminal His₆-tagged mutation CCD/W131E protein was constructed based on pET28a-CCD using site directed mutagenesis with *Dpn* I. The pGEX-4T-His₆ plasmid for expression of recombinant C-terminal His₆-tagged GST (GST-His₆) used for counterscreen was constructed by inserting the His₆-tag encoding sequences (CACCACCACCACCACCTGA) to the *Bam*H I-*Sal* I sites of the vector pGEX-4T-1.

Recombinant protein expression and purification. LEDGF/p75 IBD, D366N LEDGF/p75 IBD, IN CCD, and W131E IN CCD were expressed and purified as previously described²². The double-tagged GST-His₆ protein was produced using standard conditions as previously described¹⁷. The concentrations of all proteins

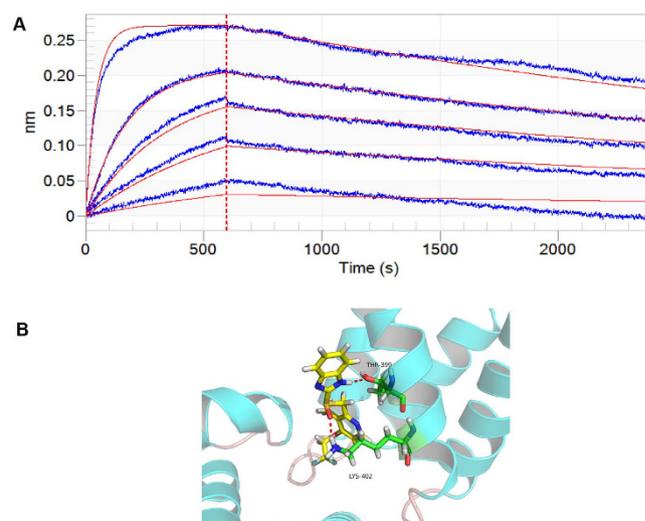


Figure 7. Dextralansoprazole binds directly to LEDGF/p75. (A) The BLI results confirmed that dextralansoprazole bound to LEDGF/p75 with a calculated dissociate constant K_d value of $330 \text{ nM} \pm 2.6 \text{ nM}$. The experiments were performed and analyzed using the ForteBIO Octet system. (B) Binding mode of dextralansoprazole at the interface of LEDGF/p75-IN interaction (ligand colored in yellow). Red dash lines represent hydrogen bonds interactions between the ligand and receptor.

were determined by the Bradford assay with bovine serum albumin (BSA) as a standard. 12% SDS-PAGE was performed to determine the purity of proteins.

His₆-tag pull-down assay. A His₆-tag pull down assay was performed as described previously²⁸. In brief, His₆-tagged IN CCD was pre-incubated with LEDGF/p75 IBD in pull down (PD) buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20 mM imidazole, 0.1% NP-40, 1 mM MgCl₂) for 30 min at room temperature (RT). Then, the mixtures were centrifuged for 2 min at 13,000 rpm at 4 °C and supernatants were incubated with 40 μl Ni-NTA-agarose (Novagen, San Diego, CA) and stirred for an additional 30 min at RT. The agarose beads were recovered by centrifugation for 2 min at 500 × g at 4 °C and washed three times with 500 μl of PD buffer. The beads binding protein-protein complexes were subjected to SDS-PAGE for separation and visualized by Coomassie Blue stain.

The IN CCD-LEDGF/p75 IBD PPI assay. The IN CCD-LEDGF/p75 IBD interaction assay was developed based on Ni-NTA Magnetic Agarose Beads (BEAVER Nano-technologies Co., Ltd. Suzhou, China). The assay was optimized for use in 96-well nonbinding surface microplates (Corning, New York, USA) in a final volume of 100 μl per well. Briefly, the assay works as follows. Compounds and proteins were diluted to 10 × and 5 × working solutions in the 1 × assay buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 0.05% BSA [v/w]) respectively. Firstly, 10 μl compound dilution, 50 μl 1 × buffer, 20 μl each of protein dilutions were pipetted into the wells. The plate was sealed and left to incubate at 200 rpm for 3 h at RT. Secondly, 10 μl of Ni-NTA Magnetic Agarose Beads were added followed by incubating at 900 rpm for 30 min at RT. Thirdly, the wells holding the mixture were placed on a magnetic concentrator, the supernatant was discarded, and the wells were washed 3 times with 200 μl wash buffer (50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 0.005% tween-20 [v/v], 20 mM imidazole). Fourthly, the wells were incubated with 100 μl of a 1:4000 solution of mouse anti-GST antibody (Sigma, USA) in antibody dilution buffer (50 mM Sodium Phosphate, pH 7.4, 150 mM NaCl, 0.005% Tween-20 [v/v], 0.5% [w/v] BSA) at 900 rpm for 30 min at 37 °C, followed by washing three times. Fifthly, the wells were incubated with 100 μl of a 1:5000 solution of rabbit anti-mouse IgG alkaline phosphatase conjugated antibody (Sigma, USA) in antibody dilution buffer at 900 rpm for 30 min at 37 °C, followed by washing 3 times. Lastly, the color reaction was developed with 100 μl substrate buffer (10 mM diethanolamine, pH 9.8, 0.5 mM MgCl₂, 8 mM p-NPP) for 30 min at 37 °C. The absorbance (450 nm) of each well was measured in an Enspire multimode reader (PerkinElmer, USA). The reproducibility, signal stability, and robustness of this PPI assay were determined to ensure its compatible for HTS. Furthermore, IL23, an IN CCD-LEDGF/p75 IBD PPI inhibitor, was used to validate the assay efficiency²⁹.

Library screening for inhibitors targeting the IN CCD-LEDGF/p75 IBD PPI. In total, 1000 compounds selected at random from ‘The Spectrum Collection’ were purchased from National compound resource center. The library, which is composed of 2,320 compounds in 10 mM DMSO solution, lists primarily FDA-approved human therapeutic drugs, drug-like compounds, and natural products. Each compound was diluted in DMSO to reach a concentration of 300 μM, which was used as the 10 × compound stock³⁸. In all screening campaigns, the negative control was based on the use of PPI binding mutant controls (single-point mutation) that would prevent the PPI from forming, and the positive control contained 10% (v/v) DMSO only. The negative and positive control with 8 replicates wells each located randomly among the 96 wells of each plate.

A counterscreen was carried out as described above, with the replacement of purified HIV-1 IN CCD and LEDGF/p75 IBD with 10 nM His₆-tagged GST protein.

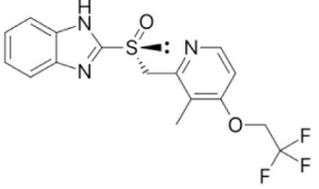
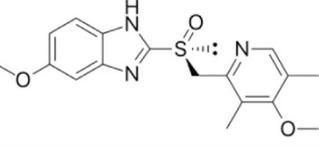
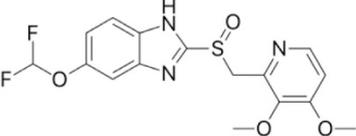
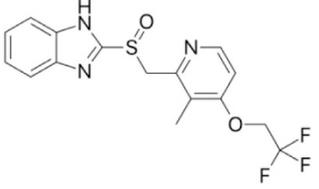
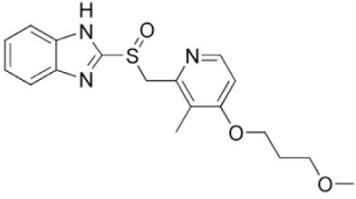
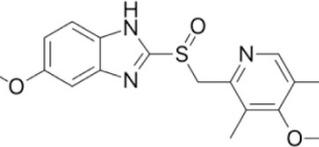
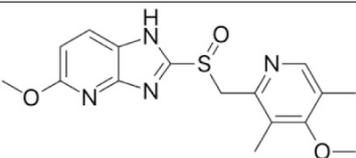
Drug name	Drug structure	Inhibition (%) ^a	IC ₅₀ (μM)
Dexlansoprazole		75.2	4.8
Esomeprazole		22.0	>30
Pantoprazole		-23.9	ND
Lansoprazole		-86.2	ND
Rabeprazole		14.5	>30
Omeprazole		26.2	>30
Tenatoprazole		33.6	>30

Table 1. Chemical structures of seven proton-pump inhibitors and their inhibitory data against PPI between LEDGF/p75 IBD and IN CCD. ^aData were collected at 30 μM for each proton-pump inhibitor.

Biolayer interferometry. Biolayer interferometry (BLI) was used to study the kinetics of dexlansoprazole binding to LEDGF/p75 on a ForteBio Octet, according to the manufacturer's instructions and published methods. Streptavidin biosensors tips were pre-wetted with buffer and immobilized with biotinylated protein. The biosensors were then transferred into wells containing dexlansoprazole in serial dilutions from 32 μM to 1 μM concentrations. Protein–ligand binding was measured by monitoring the changes in the interferometric profile of the wavelength of light passing through the sensor. Binding curves were analyzed using ForteBIO software, which performs a global fit according to the 1:1 Langmuir model to obtain the kinetic rate constants for each set of interaction conditions.

Data Analysis. The z' factor, signal-to-noise ratio (S/N), signal-to-background ratio (S/B), signal window (SW), and coefficient of variation (CV) were calculated and then compared with the minimum pass criteria ($z' > 0.5$, $S/N > 10$, $S/B > 3$, $SW > 2$, $CV < 20\%$)^{39–42}. The “hits” for PPI assays were classified as compounds that led to more than 60% disruption of a PPI complex. All HTS data were processed using Excel (Microsoft Corp.) and visualized using Prism 5.0 (GraphPad Software).

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Author Contributions

Z.D.W., M.Z.X. and G.S.X. conceived and designed the experiments. Z.D.W. performed the experiments. Z.D.W. and L.M.M. analyzed the data. Z.D.W. and H.H.Q. wrote the manuscript. All authors reviewed the manuscript.

Additional Information

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