دانشگاه علوم پزشکی و خدمات بهداشتی درمانی شهید بهشتی
مرکز تحقیقات علوم دارویی

Protein-Protein Interaction as a New Strategy to Inhibit HIV-1 Integrase

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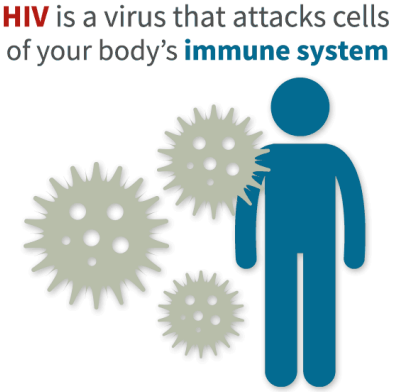
AIDS

- Acquired ImmunoDeficiency Syndrome (AIDS)

HIV is the Etiological agent of the AIDS

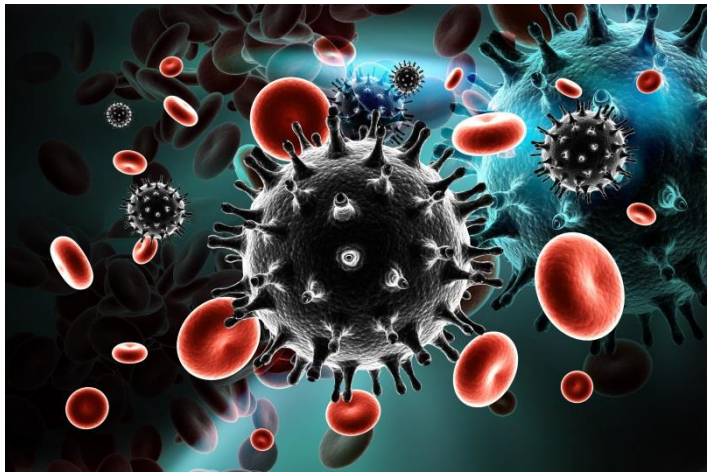
H= Human

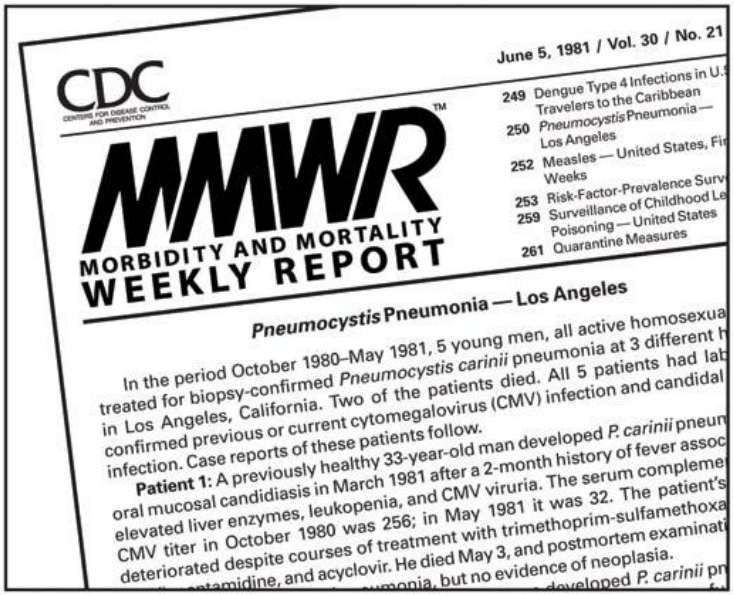
I= Immunodeficiency



V= Virus

HIV is a Virus





CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

June 5, 1981 / Vol. 30 / No. 21

MMWR™
MORBIDITY AND MORTALITY WEEKLY REPORT

249 Dengue Type 4 Infections in U.S. Travelers to the Caribbean
250 *Pneumocystis* Pneumonia — Los Angeles
252 Measles — United States, Fi... Weeks
253 Risk-Factor-Prevalence Surv...
259 Surveillance of Childhood Le... Poisoning — United States
261 Quarantine Measures

***Pneumocystis* Pneumonia — Los Angeles**

In the period October 1980–May 1981, 5 young men, all active homosexua... treated for biopsy-confirmed *Pneumocystis carinii* pneumonia at 3 different h... in Los Angeles, California. Two of the patients died. All 5 patients had lab... confirmed previous or current cytomegalovirus (CMV) infection and candidal... infection. Case reports of these patients follow.

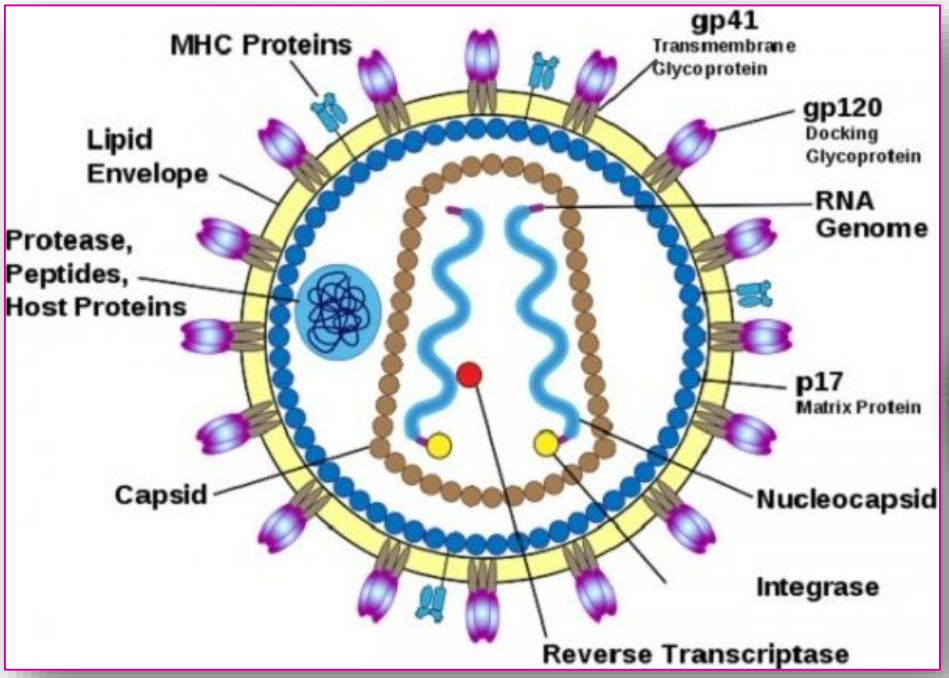
Patient 1: A previously healthy 33-year-old man developed *P. carinii* pneun... oral mucosal candidiasis in March 1981 after a 2-month history of fever assoc... elevated liver enzymes, leukopenia, and CMV viruria. The serum compleme... CMV titer in October 1980 was 256; in May 1981 it was 32. The patient's... deteriorated despite courses of treatment with trimethoprim-sulfamethoxa... ...tamidine, and acyclovir. He died May 3, and postmortem examinati... ...monia, but no evidence of neoplasia. ...developed *P. carinii* pn...

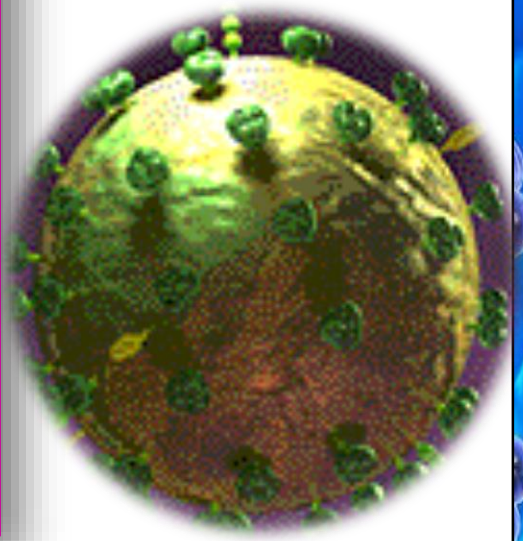
A new disease…

1981

A new virus

1983



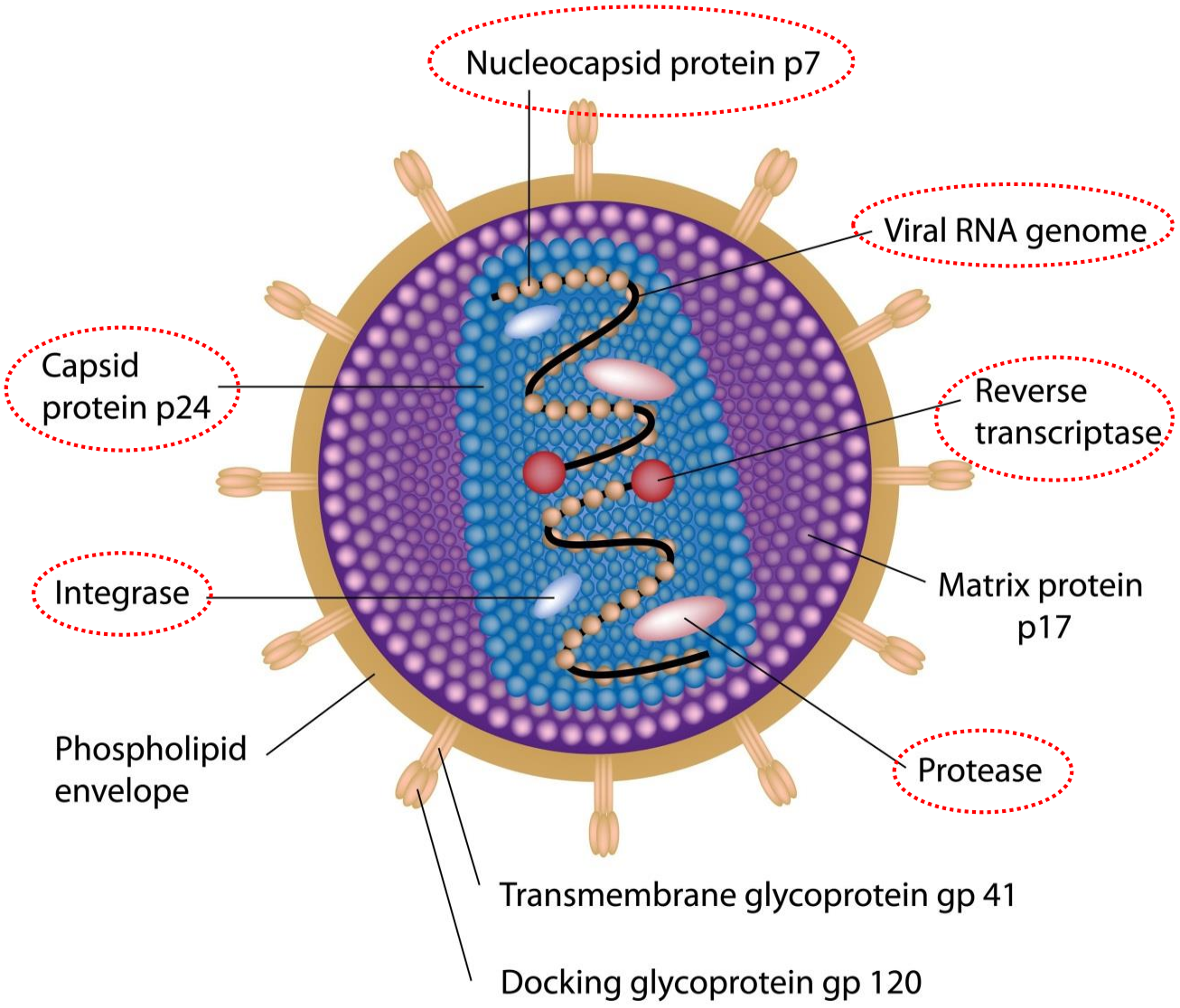


Summary of the global HIV epidemic (2018)

	People living with HIV in 2018	People newly infected with HIV in 2018	HIV-related deaths 2018
Total	37.9 million [32.7 million – 44.0 million]	1.7 million [1.4 million – 2.3 million]	770 000 [570 000 – 1.1 million]
Adults	36.2 million [31.3 million – 42.0 million]	1.6 million [1.2 million – 2.1 million]	670 000 [500 000 – 920 000]
Women	18.8 million [16.4 million – 21.7 million]	–	–
Men	17.4 million [14.8 million – 20.5 million]	–	–
Children (<15 years)	1.7 million [1.3 million – 2.2 million]	160 000 [110 000 – 260 000]	100 000 [64 000 – 160 000]

Source: UNAIDS/WHO estimates

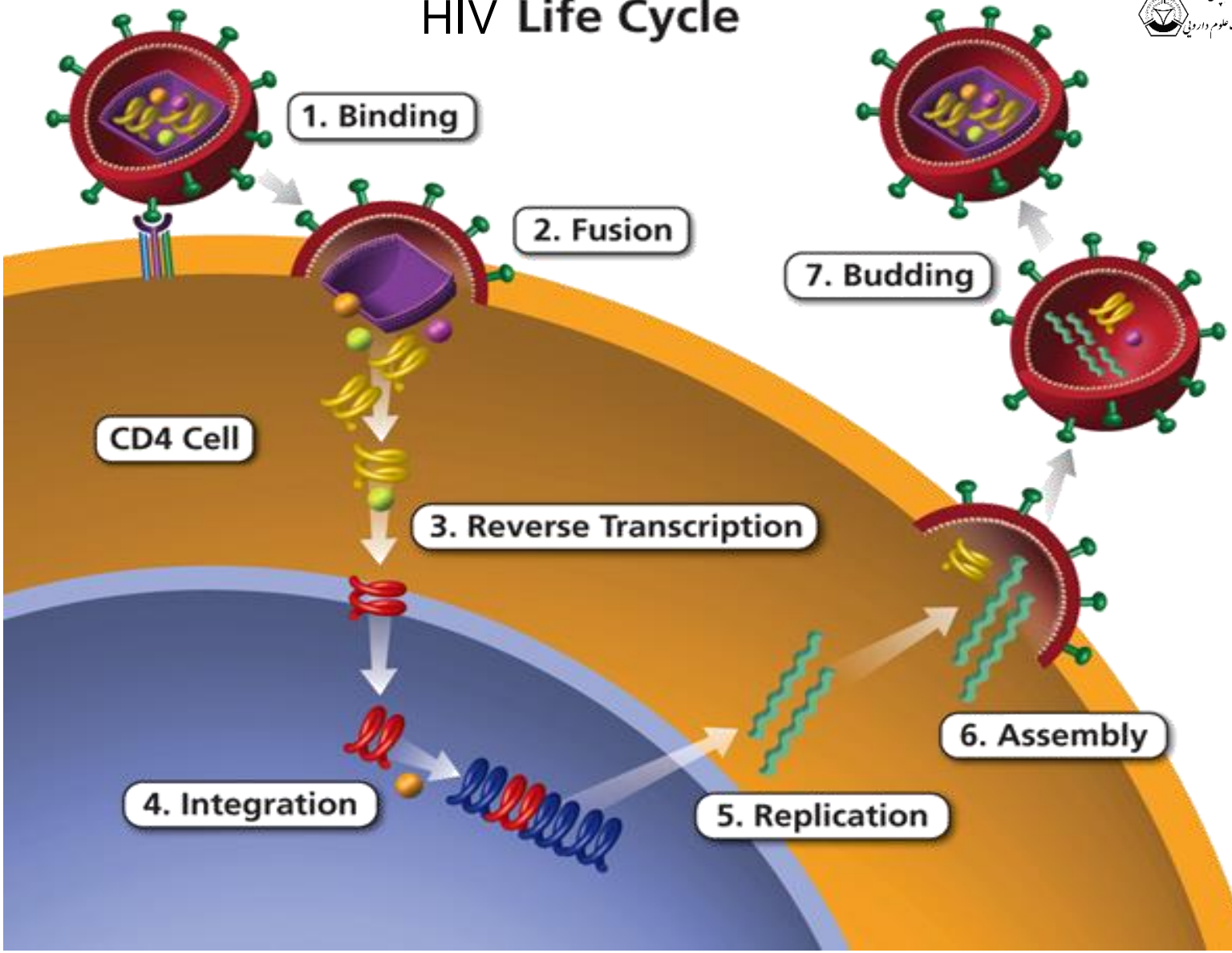
World Health Organization


Nucleocapsid protein p7
Viral RNA genome
Capsid
protein p24
Reverse
transcriptase
Integrase
Matrix protein
p17
Phospholipid
envelope
Protease
Transmembrane glycoprotein gp 41
Docking glycoprotein gp 120

HIV Virus

- HIV virus is a lentivirus of the family Retroviride
- Biological nanostructure (around 100-150 nm)
  - Host-derived Membrane
  - Nucleocapsid
    - Genetic material in the form of RNA
    - Protease
    - Reverse Transcriptase
    - Integrase
    - Capsid protein P24 , Nucleocapsid protein P7

HIV Life Cycle



Current Treatment of HIV/AIDS

- First Anti HIV Drug : AZT (1987)

- Highly Active Antiretroviral Therapy (HAART) (1996)
  - Make AIDS from Acute disease to Chronic disease

- Antiretroviral therapy is the best option for prolonged and maximal viral suppression

FDA Approval of HIV Medicines

1981: First AIDS cases are reported in the United States.

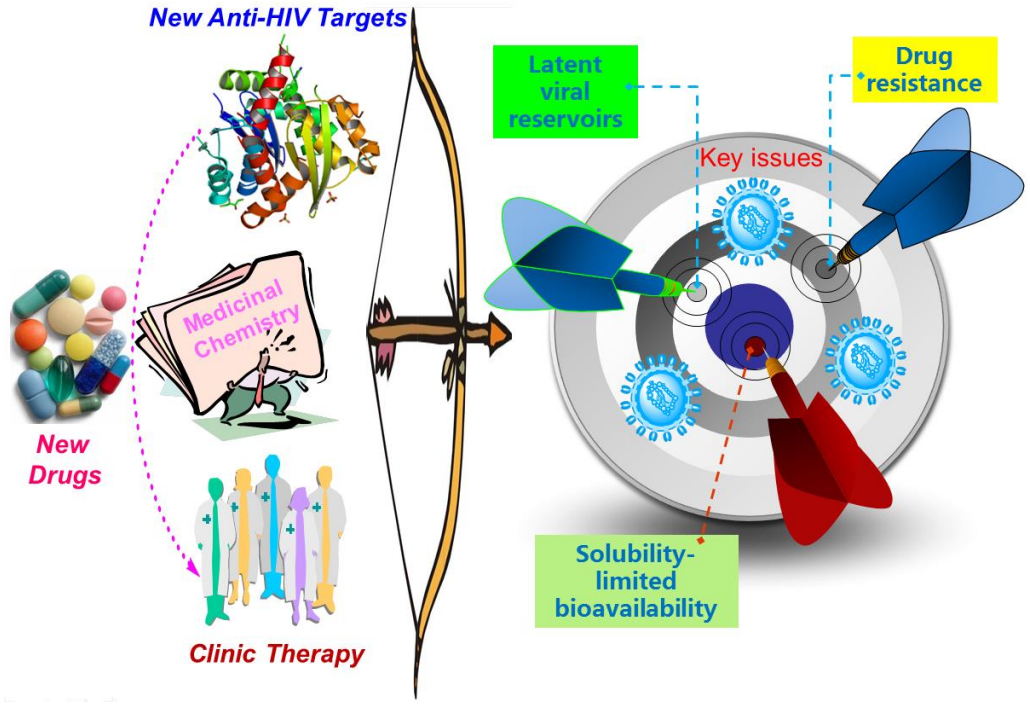
'85-'89	**1987** Zidovudine (NRTI)				
'90-'94	**1991** Didanosine (NRTI)	**1992** Zalcitabine (NRTI)	**1994** Stavudine (NRTI)		
'95-'99	**1995** Lamivudine (NRTI) Saquinavir (PI)	**1996** Indinavir (PI) Nevirapine (NNRTI) Ritonavir (PI)	**1997** Combivir (FDC) Delavirdine (NNRTI) Nelfinavir (PI)	**1998** Abacavir (NRTI) Efavirenz (NNRTI)	**1999** Amprenavir (PI)
'00-'04	**2000** Didanosine EC (NRTI) Kaletra (FDC) Trizivir (FDC)	**2001** Tenofovir DF (NRTI)	**2003** Atazanavir (PI) Emtricitabine (NRTI) Enfuvirtide (FI) Fosamprenavir (PI)	**2004** Epzicom (FDC) Truvada (FDC)	
'05-'09	**2005** Tipranavir (PI)	**2006** Atripla (FDC) Darunavir (PI)	**2007** Maraviroc (CA) Raltegravir (INSTI)	**2008** Etravirine (NNRTI)	
'10-'14	**2011** Complera (FDC) Nevirapine XR (NNRTI) Rilpivirine (NNRTI)	**2012** Stribild (FDC)	**2013** Dolutegravir (INSTI)	**2014** Cobicistat (PE) Elvitegravir (INSTI) Triumeq (FDC)	
'15-'19	**2015** Evotaz (FDC) Genvoya (FDC) Prezcobix (FDC)	**2016** Descovy (FDC) Odefsey (FDC)	**2017** Juluca (FDC)	**2018** Biktarvy (FDC) Cimduo (FDC) Delstrigo (FDC) Doravirine (NNRTI) Ibalizumab-uiyk (PAI) Symfi (FDC) Symfi Lo (FDC) Symtuza (FDC) Temixys (FDC)	**2019** Dovato (FDC)

Why New HIV Drugs ?

I. Still few treatment Option

II. The harsh side effects
   - Low compliance

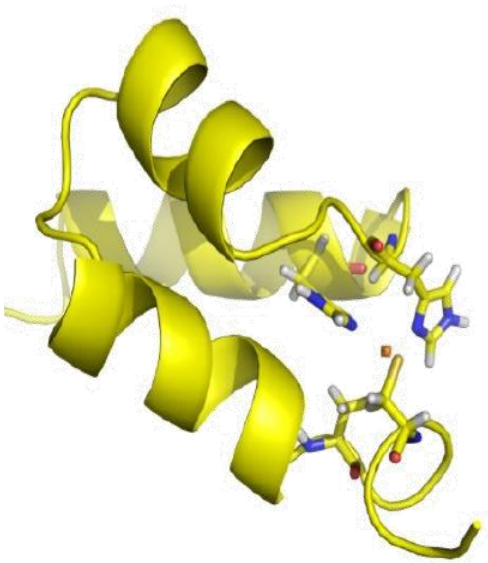
III. Multi drug resistance

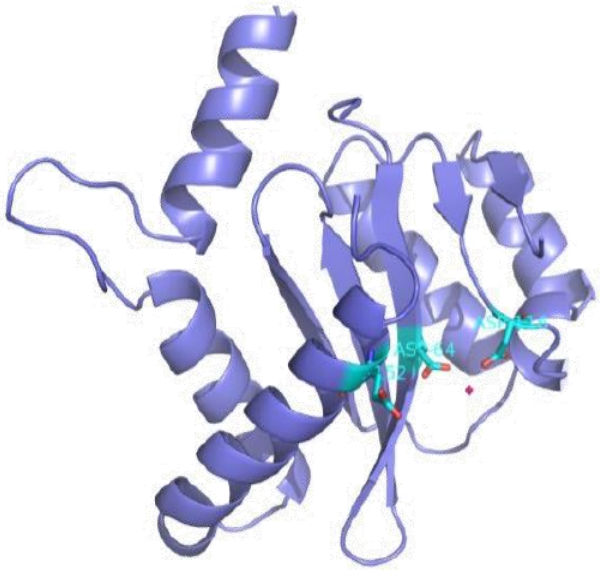


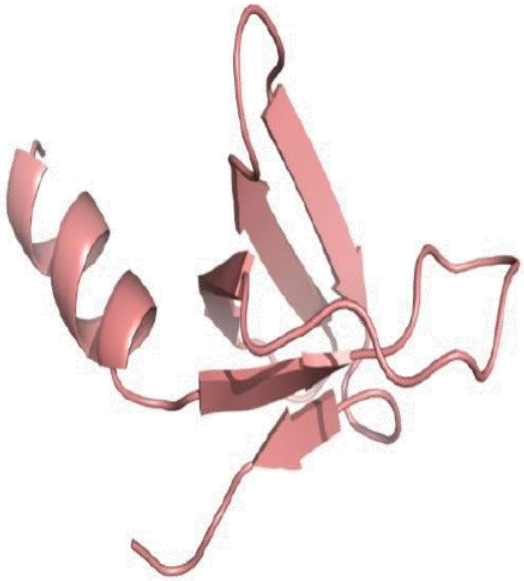
HIV-1 Integrase

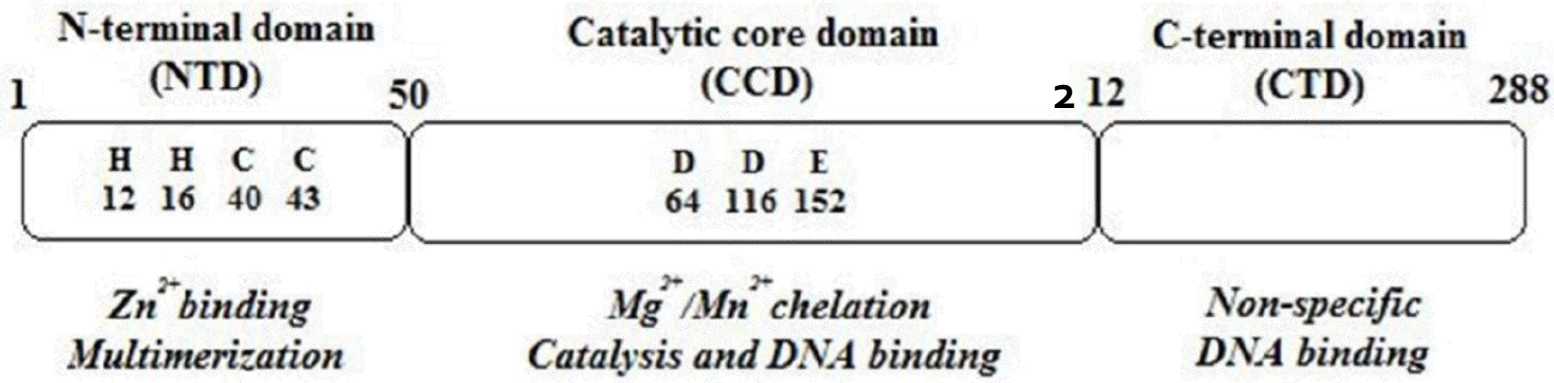
- Polynucleotidyl transferase/esterase
- 32 kDa , 288 amino acids
- First reported to be cloned and expressed in 1990 by Fyfe and Sherman at Wellcome Research Laboratories

Integrase








N-terminal domain
(NTD)
1
50
H H C C
12 16 40 43
Zn2+ binding
Multimerization
Catalytic core domain
(CCD)
2 12
D D E
64 116 152
Mg2+/Mn2+ chelation
Catalysis and DNA binding
C-terminal domain
(CTD)
288
Non-specific
DNA binding

HIV-1 integration

1) **Integrase-HIV DNA binding**
2) **3’ Processing of HIV DNA**
3) **Nuclear translocation**
4) **Host DNA binding & Strand transfer**
5) **Gap repair**

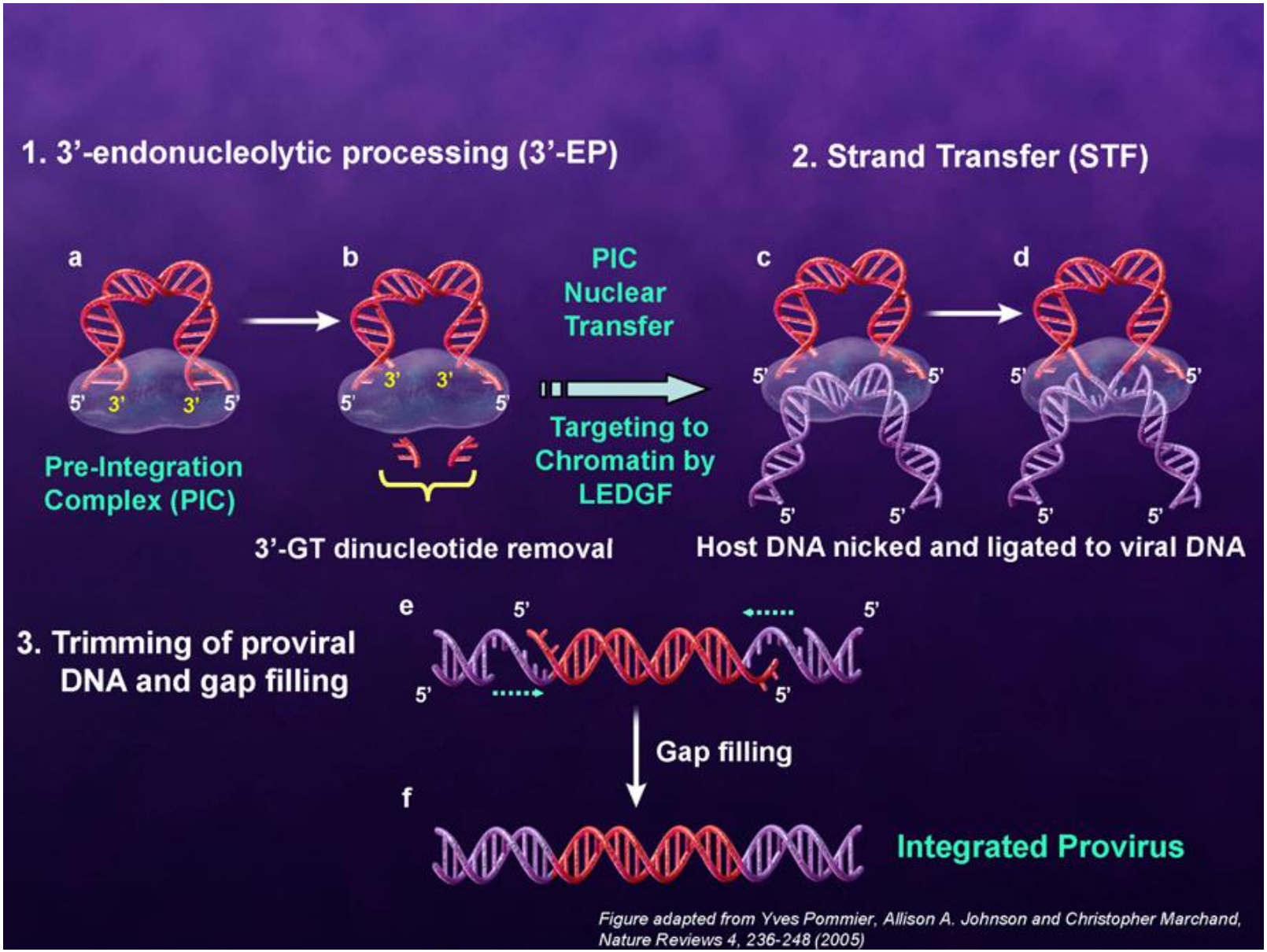
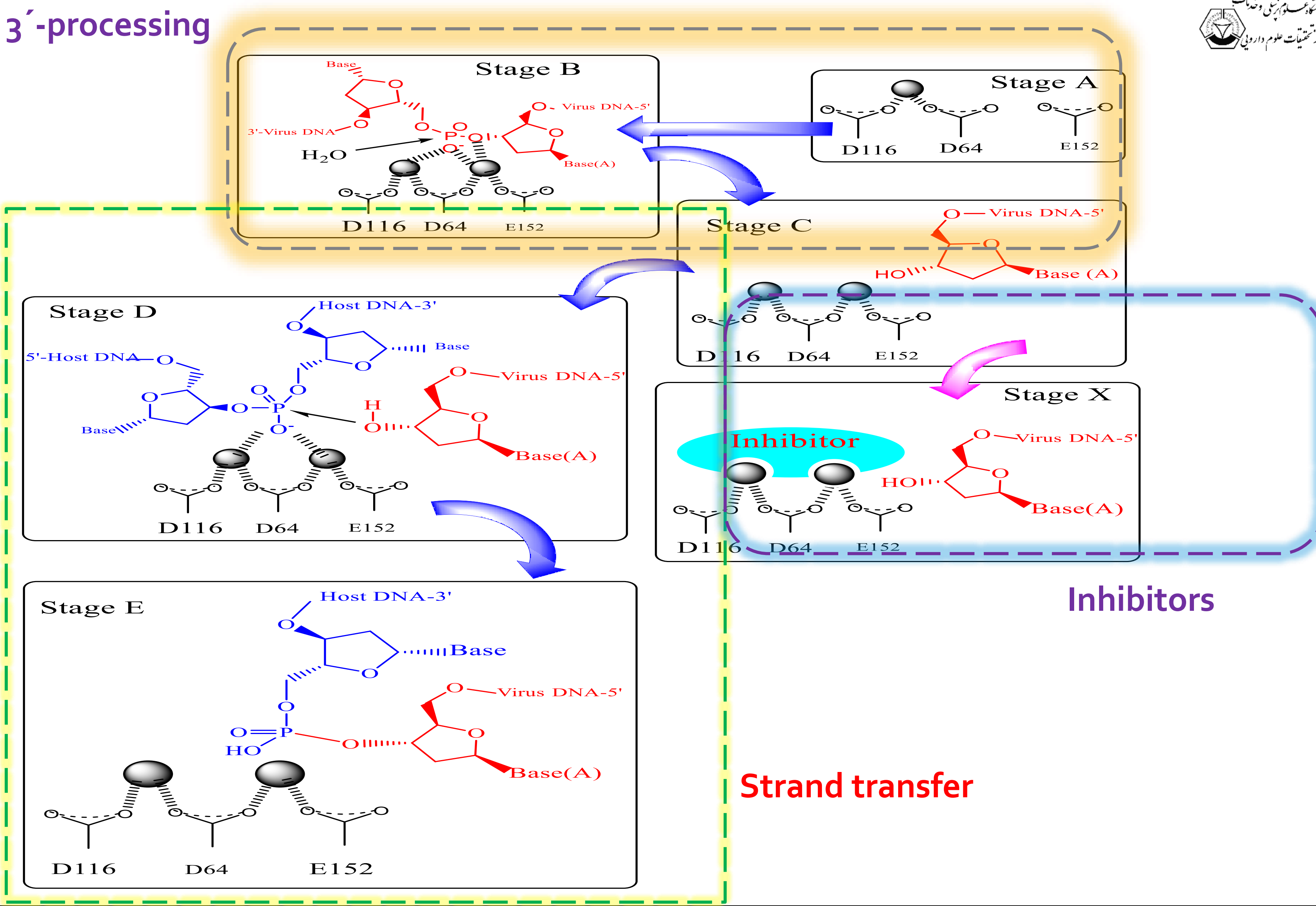
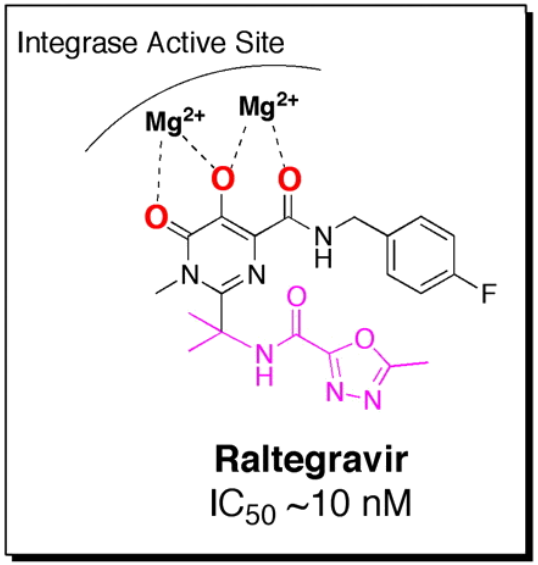


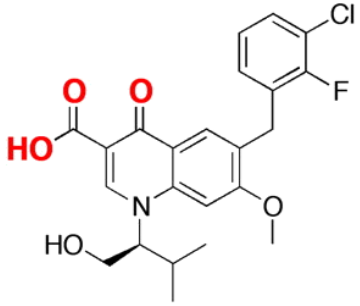
Figure adapted from Yves Pommier, Allison A. Johnson and Christopher Marchand, Nature Reviews 4, 236-248 (2005)


3´-processing
Stage A
D116
D64
E152
Stage B
Base
3'-Virus DNA
H_2O
Virus DNA-5'
Base(A)
Stage C
HO
Base (A)
Stage D
Host DNA-3'
5'-Host DNA
Base
Stage E
Stage X
Inhibitor
Inhibitors
Strand transfer

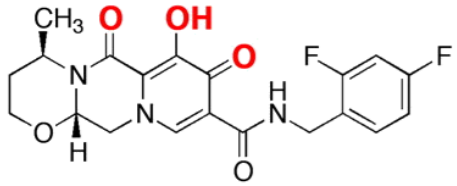
INSTIs



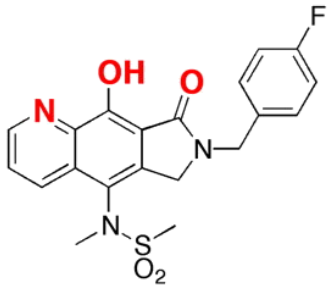
Raltegravir
IC_{50} ~10 nM



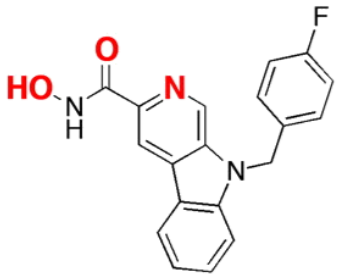
Elvitegravir



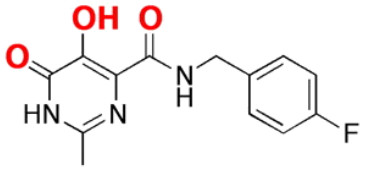
Dolutegravir



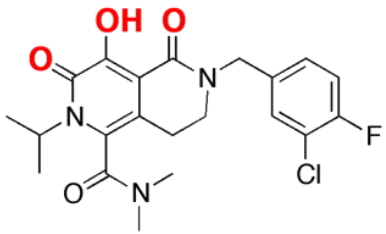
GS9160



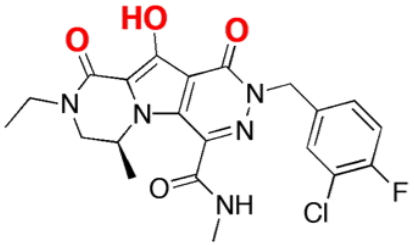
PICA



RCD-1
IC_{50} ~60 nM

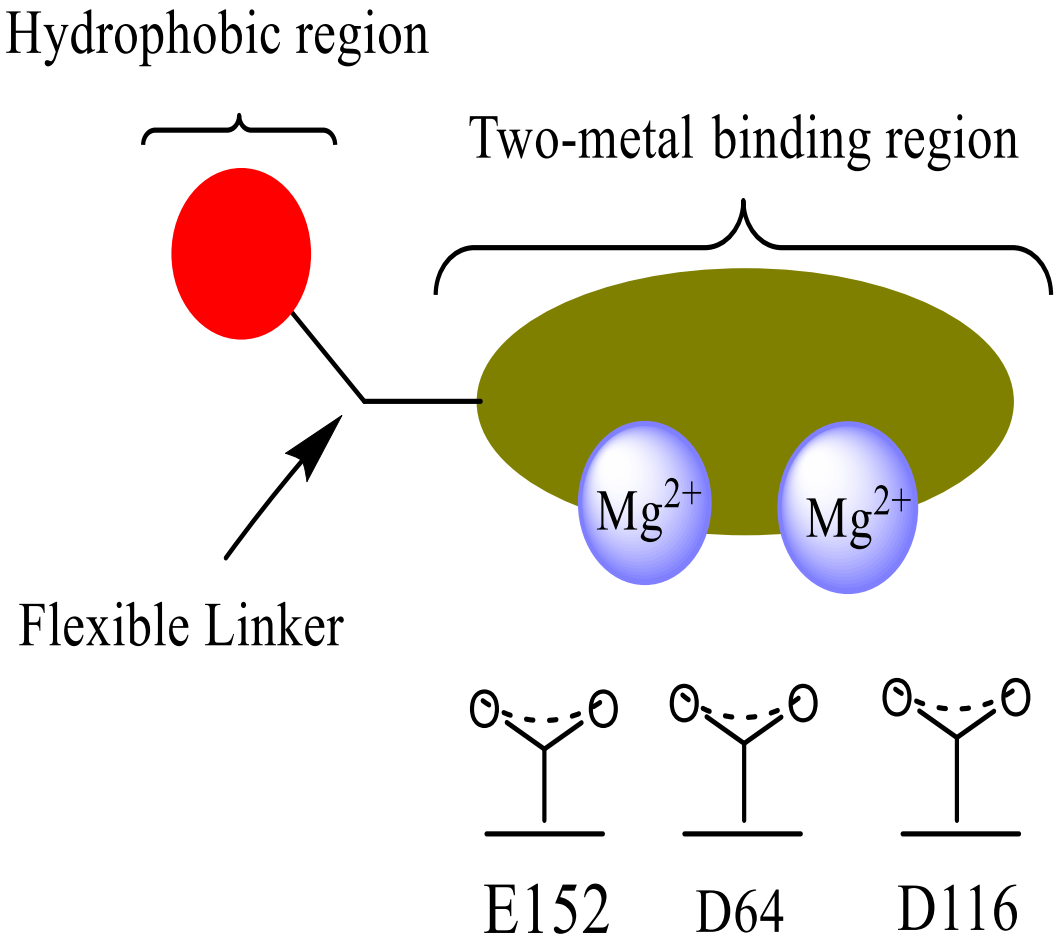


MK0536



MK2048

INSTIs Pharmacophores



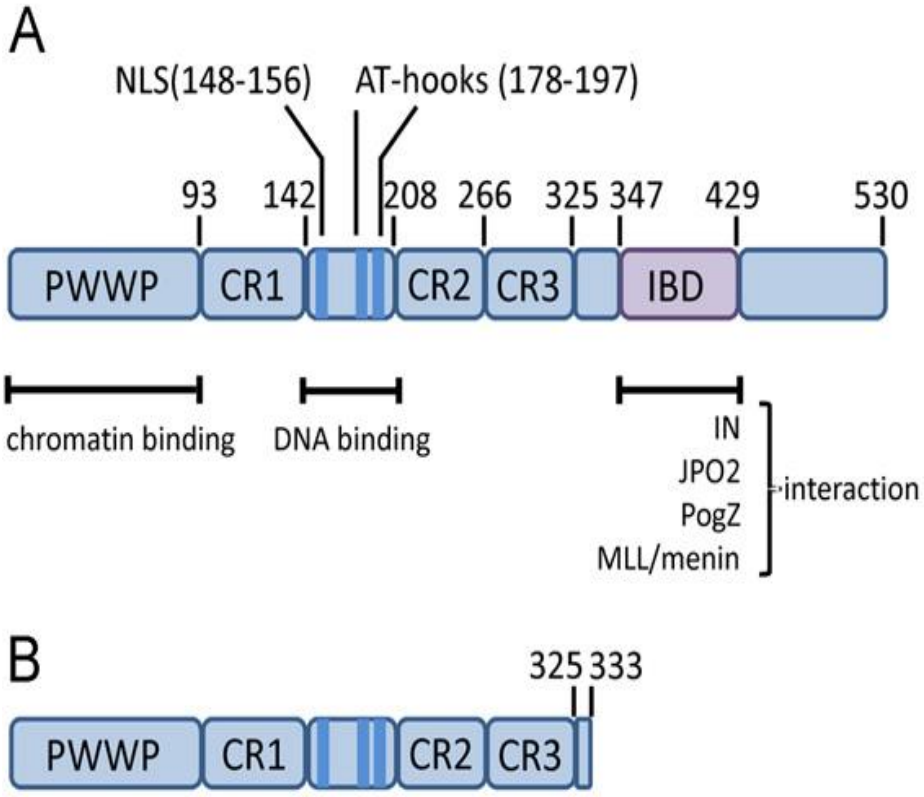
LEDGF/p75

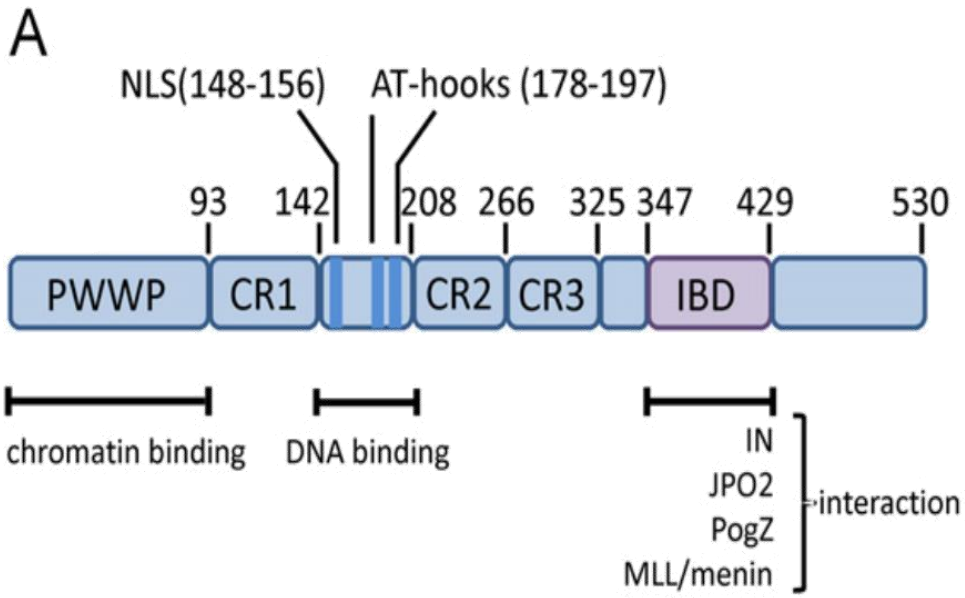
- LEDGF is the main chromatin-tethering factor for IN
- The lens epithelium-derived growth factor (LEDGF)
- Function:
  - Transcriptional co-activator
  - Common nuclear autoantigen
  - Growth/survival factor
- LEDGF/p52 and LEDGF/p75

LEDGF/p75

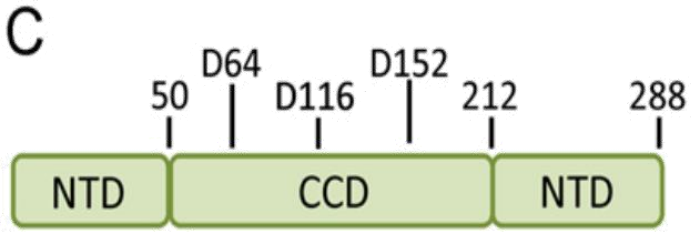
- N-terminal Pro-Trp-Trp-Pro (PWWP) chromatin binding domain

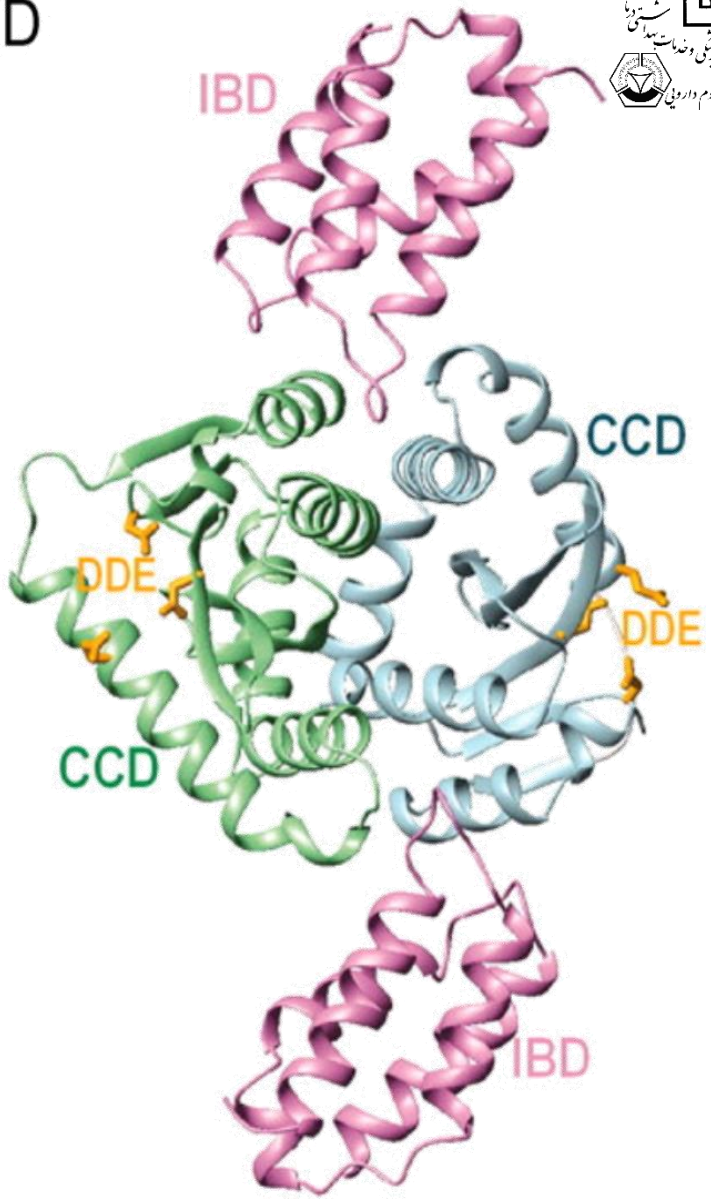
- integrase binding domain (IBD)

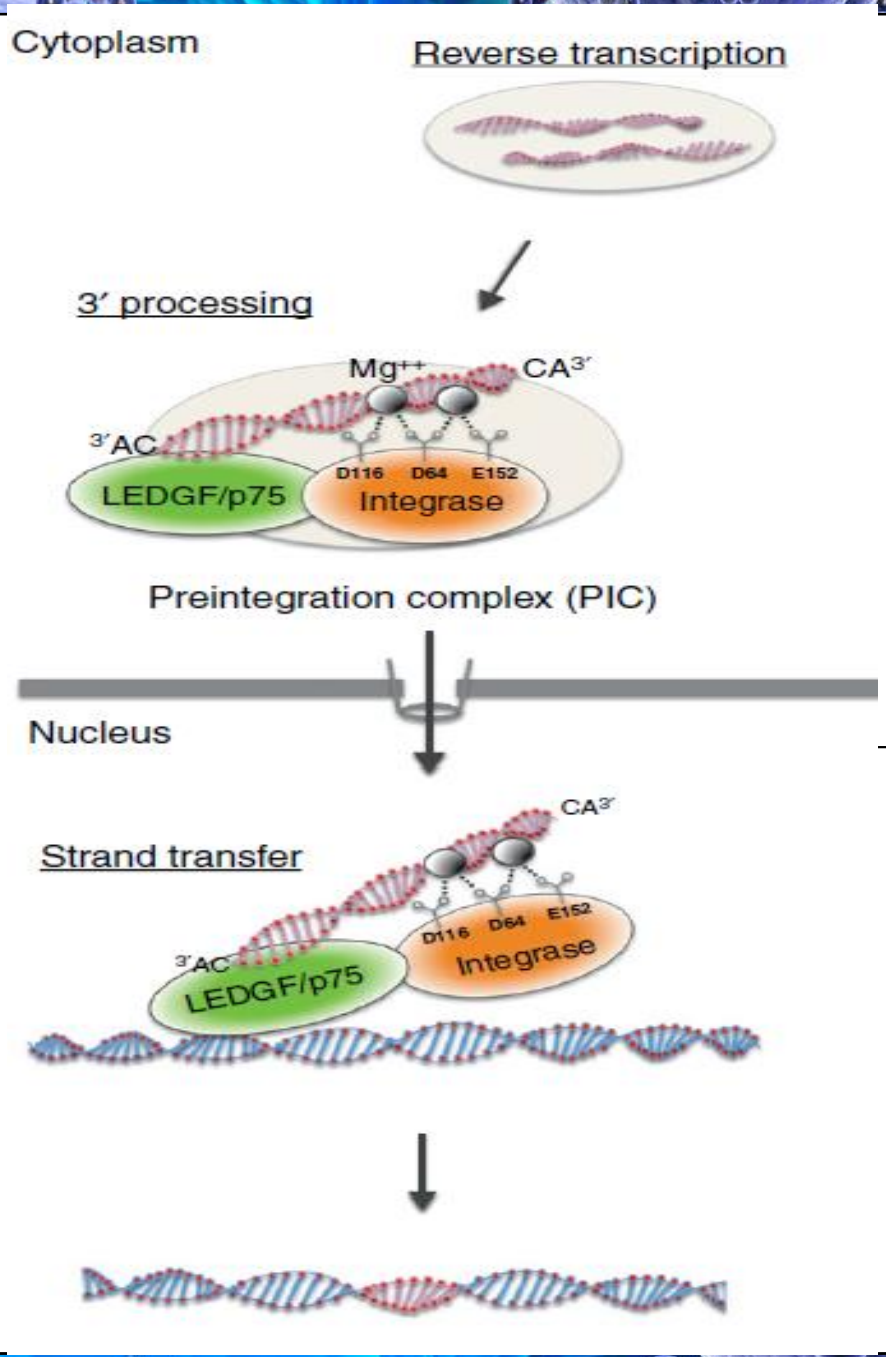


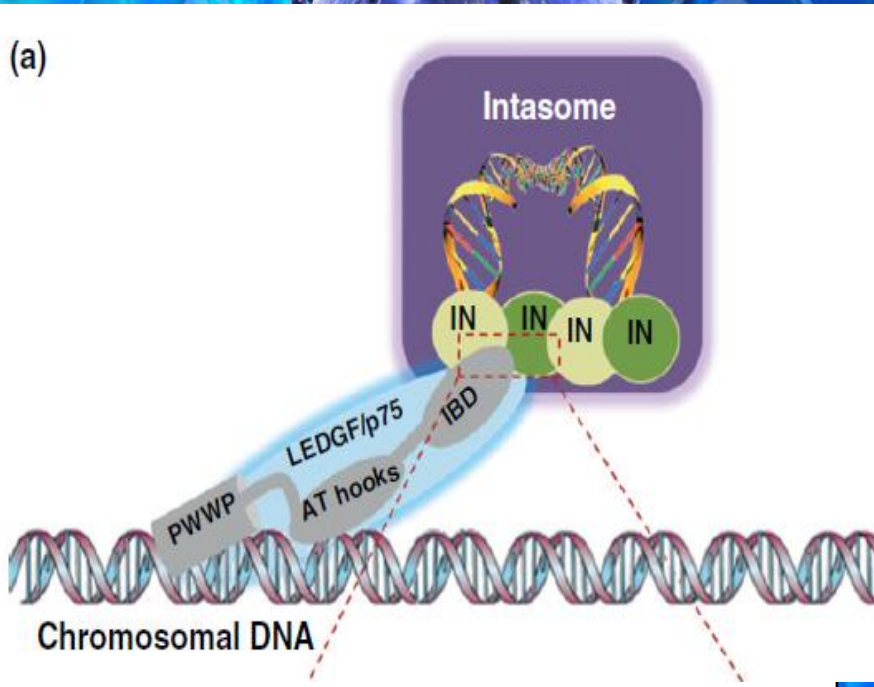

A
NLS(148-156)
AT-hooks (178-197)
93
142
208
266
325
347
429
530
PWWP
CR1
CR2
CR3
IBD
chromatin binding
DNA binding
IN
JPO2
PogZ
MLL/menin
interaction


B
325
333
PWWP
CR1
CR2
CR3


C
50
D64
D116
D152
212
288
NTD
CCD
NTD


D
IBD
CCD
DDE
DDE
CCD
IBD

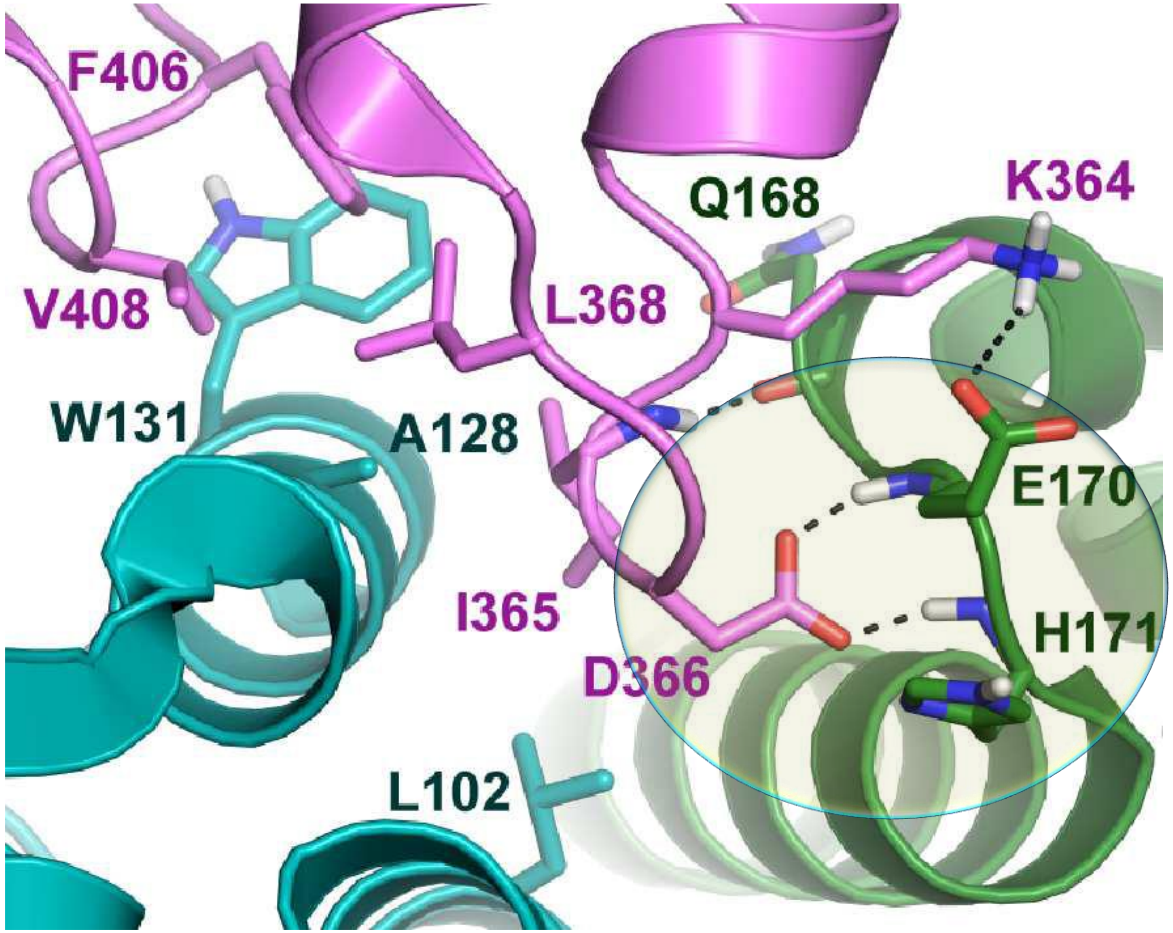

Cytoplasm
Reverse transcription
3′ processing
Mg++
CA3′
3′AC
D116
D64
E152
LEDGF/p75
Integrase
Preintegration complex (PIC)
Nucleus
Strand transfer
CA3′
3′AC
D116
D64
E152
LEDGF/p75
Integrase


(a)
Intasome
IN
IN
IN
IN
LEDGF/p75
IBD
PWWP
AT hooks
Chromosomal DNA

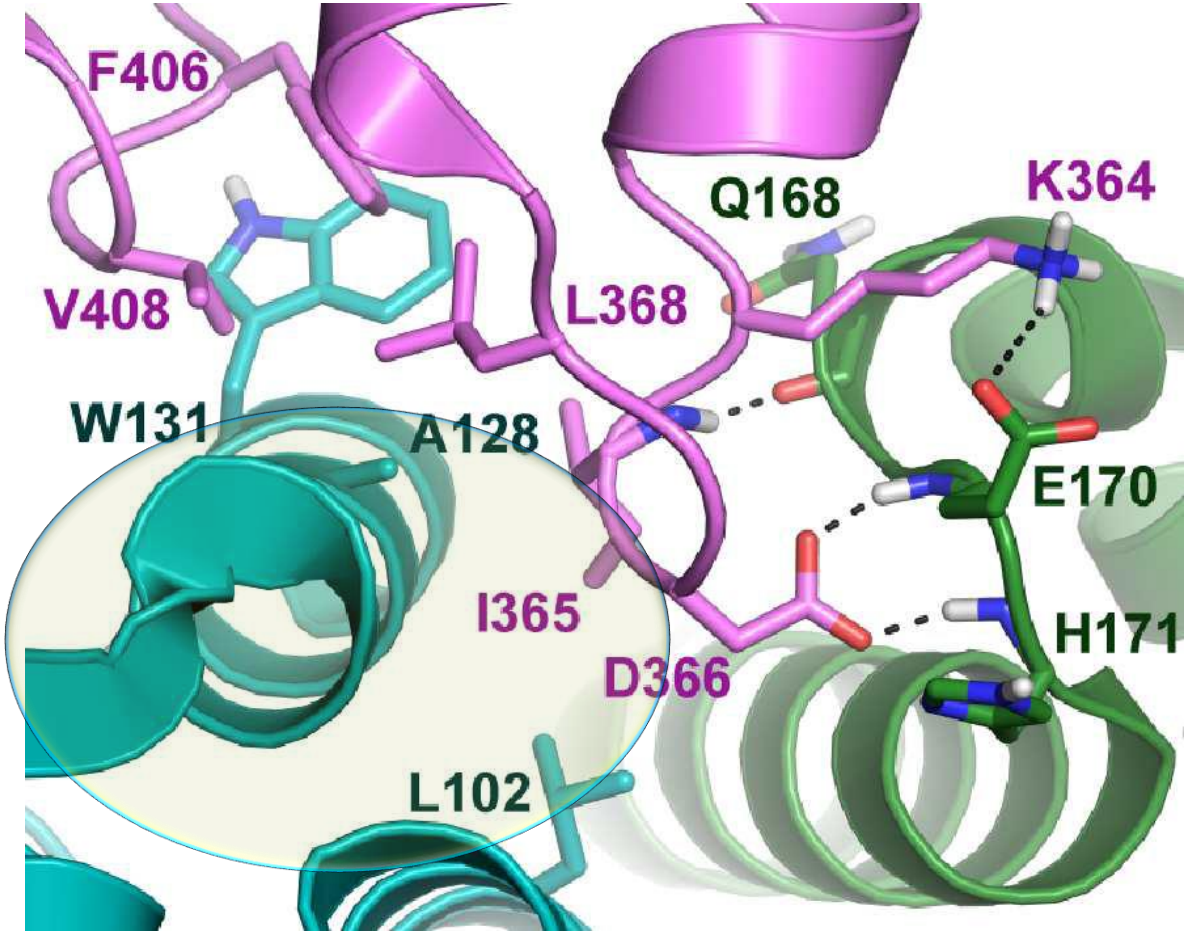
LEDGF/p75 IBD

- Compact right-handed bundle of five α-helices
- Binds to the catalytic core domain (CCD) and the N-terminal domain (NTD) of integrase

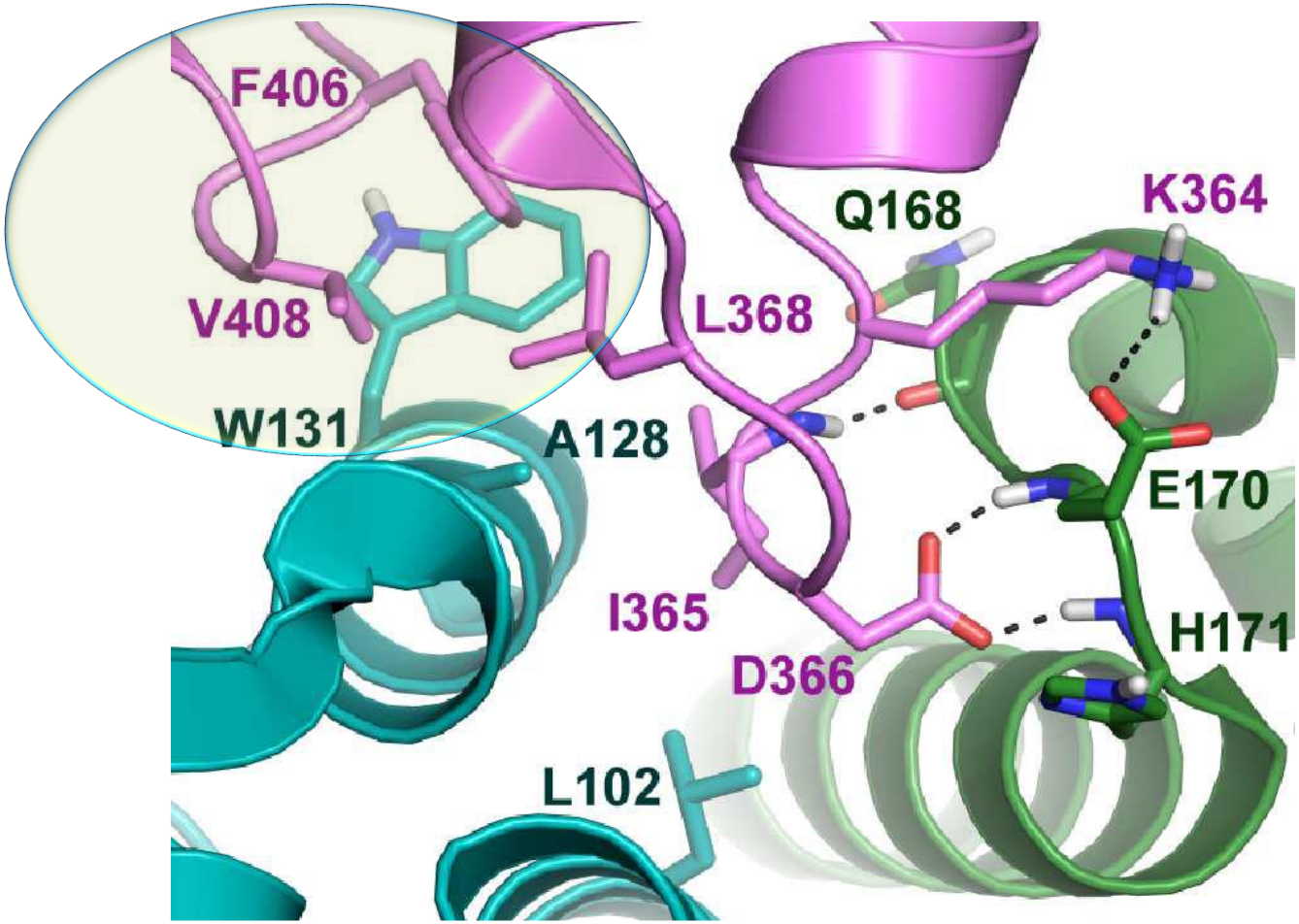
- **Asp-366** makes a bidentate hydrogen bond to the main-chain amides of IN residues **Glu-170 and His-171** in chain A



- The side chain of LEDGF/p75 residue **Ile365** projects into a hydrophobic pocket formed by the IN B-chain residues **Leu102, Ala128, Ala129, and Trp132** and the A-chain residues **Thr174 and Met178**.



- LEDGF/p75 residues **Phe-406 and Val-408** contact **Trp-131** in the IN B chain



- **LEDGF/p75 facilitates the IN activity in different ways including**

  - (i) Binding of LEDGF/p75 to integrase promotes **organization of the enzyme as a tetramer**
  - (ii) LEDGF/p75 **tethers the integration complex to the host chromatin** thereby facilitating the integration process and viral replication
  - (iii) LEDGF/p75 **stimulates the catalytic activity** of IN
  - (iv) LEDGF **protects IN from degradation** through the ubiquitin-proteasome pathway.

Protein-protein Interaction Inhibitors

- Protein-protein interaction surface of LEDGF/p75 and IN might be a feasible target for inhibition by small molecules due to its **limited extension** and **multiple hydrophobic and hydrogen bond interactions**.

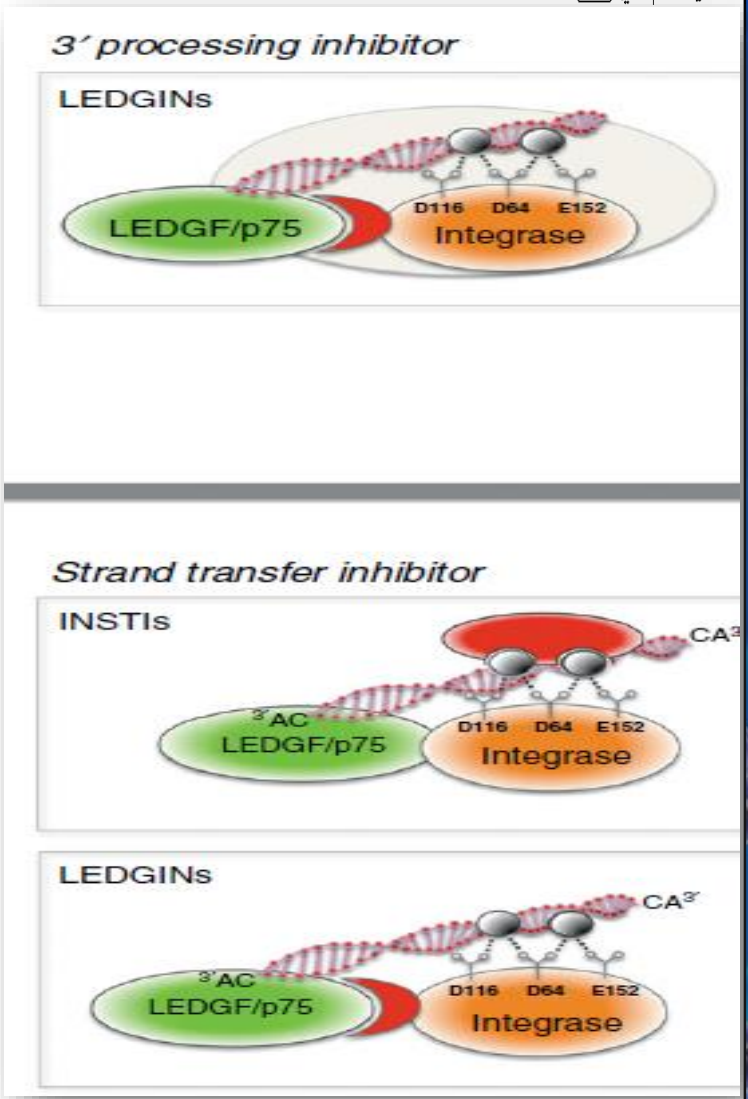
- LEDGIN (LEDGF/p75-integrase interaction site)
- ALLINIs (allosteric-integrase inhibitors)
- NCINIs (non-catalytic site integrase inhibitors)
- INLAIs (integrase-LEDGF allosteric inhibitors)

LEDGINs (allosteric inhibitors)

- Inhibiting the strand transfer and 3’ processing reactions

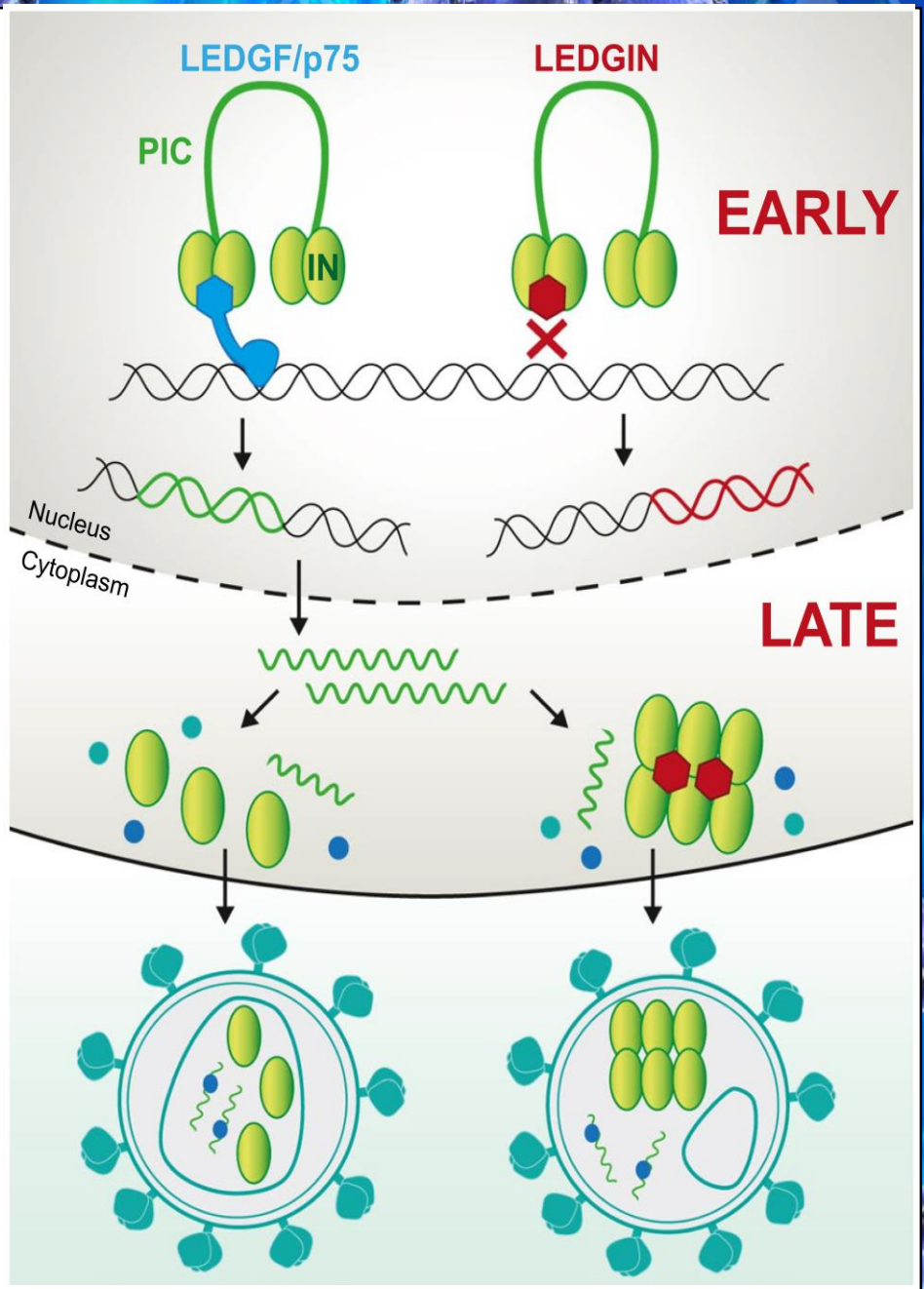
- No cross-resistant with INSTIs

- Acting in an additive or synergistic way with INSTIs



- LEDGINs bind to the LEDGF/p75 binding pocket on HIV-1 IN and blocks INLEDGF/p75 interaction disrupting chromatin tethering of the IN (**Early effect**)

- LEDGINs can interfere with proper maturation due to enhanced IN multimerization in the progeny virions, when present during virus production (**late effect**)

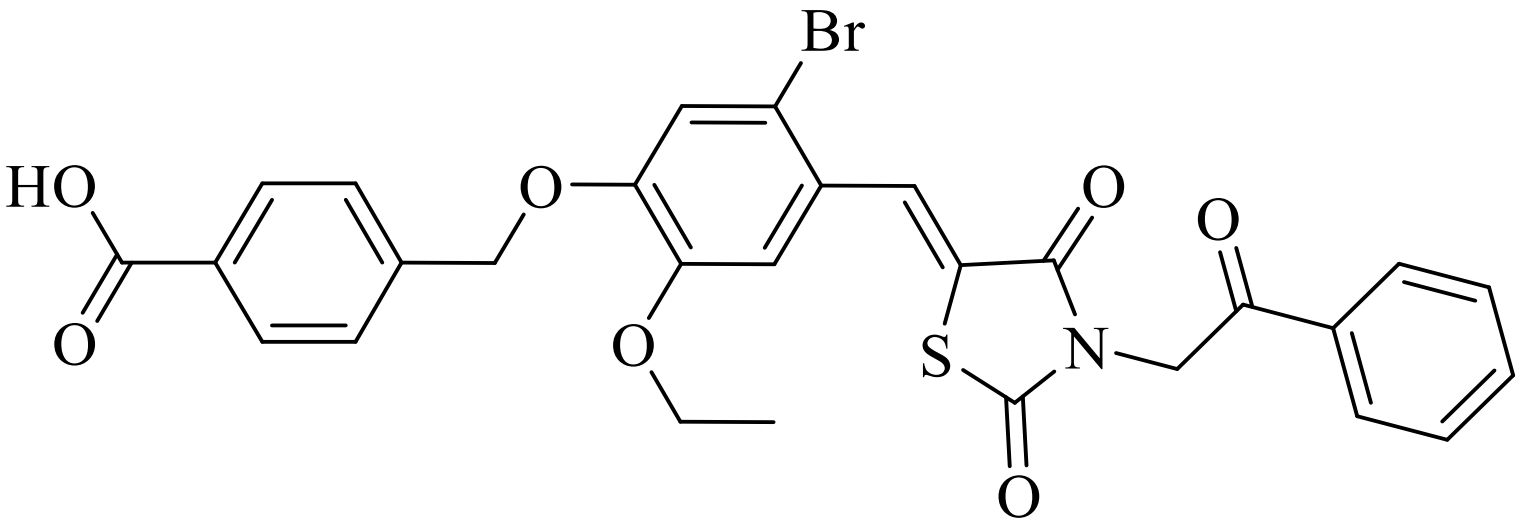


IN-LEDGF/p75 interaction Inhibitors

- **Peptides**
- **Small molecules (LEDGINs)**

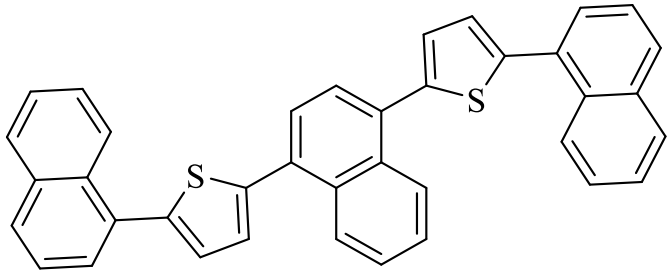
LEDGINs

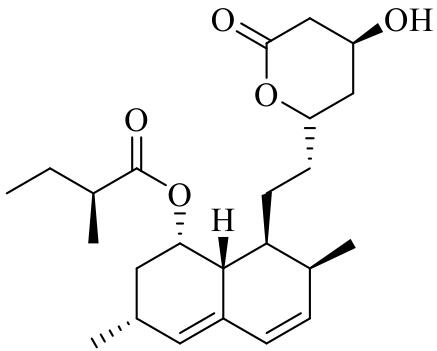
- Compound D77 was the first small-molecule that showed inhibition activity against IN-LEDGF/p75 interaction

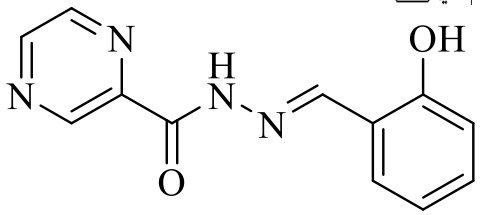


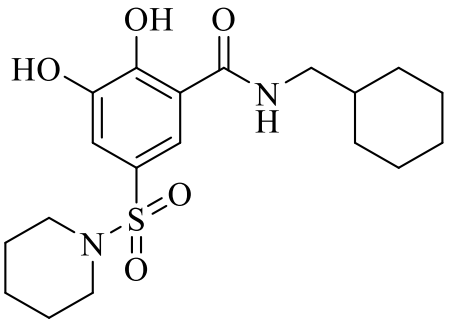
EC50 = 23.8 μg/mL in MT-4 cell
CC50 = 76.82 μg/mL in MT-4 cells

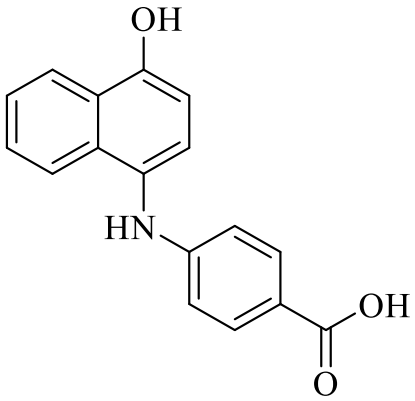
LEGINs

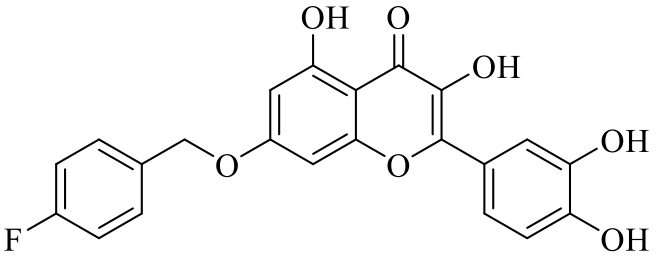


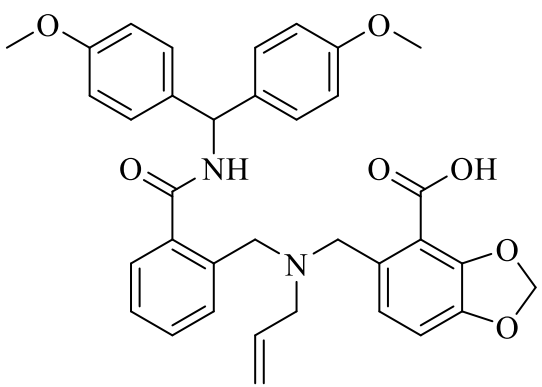


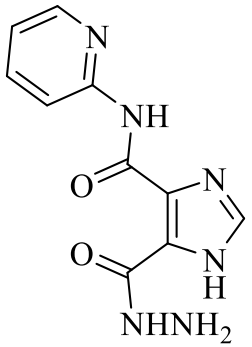


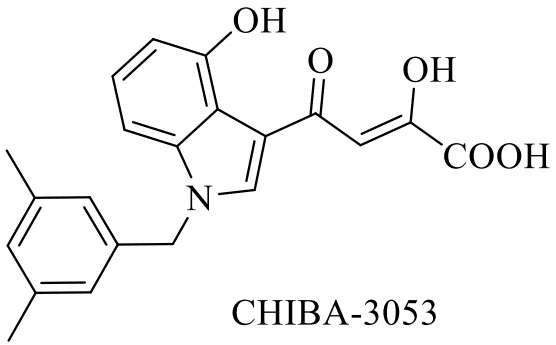












CHIBA-3053

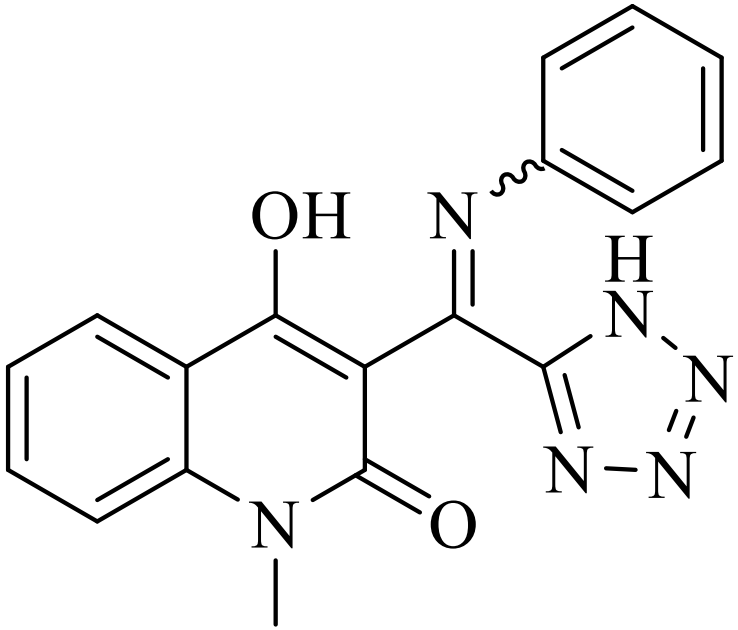
LEDGINs

- 2-(quinolin-3-yl)acetic acid derivatives

- The first class of small-molecule allosteric inhibitors to display antiretroviral activity tied to a specific disruption of the IN-LEDGF/p75 interaction

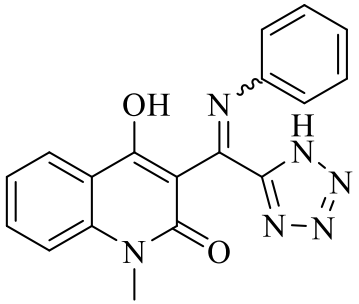
Discovery of 2-(quinolin-3-yl)acetic acid derivatives

- Screening of a set of 200,000 commercially available compounds
  - Pharmacophore based screening and docking studies
  - the 25 best-scoring compounds were selected for biological evaluation

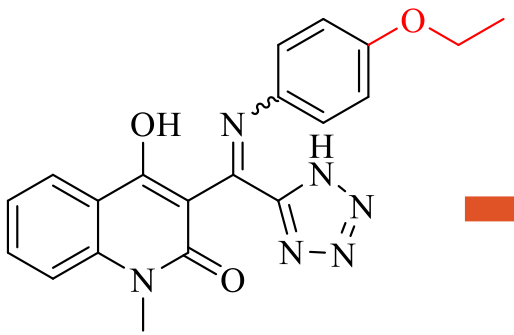


LEDGIN 1

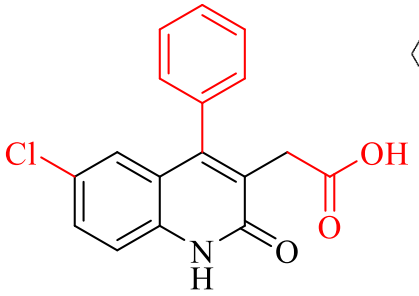
36% inhibition of the LEDGF/p75-IN interaction at 100 µM



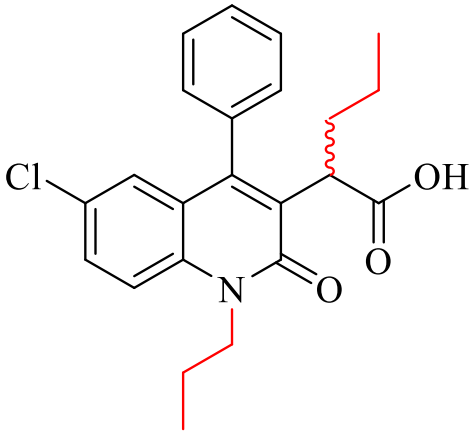
LEDGIN 1



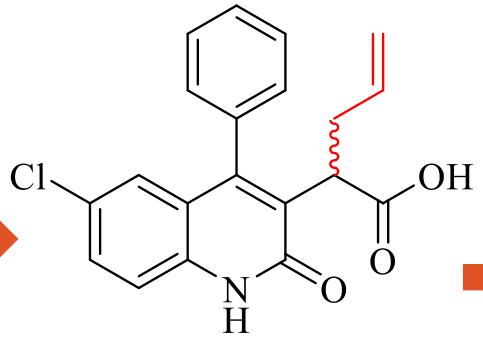
LEDGIN 2



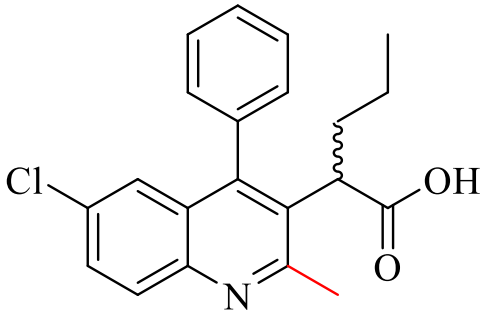
LEDGIN 3



LEDGIN 4

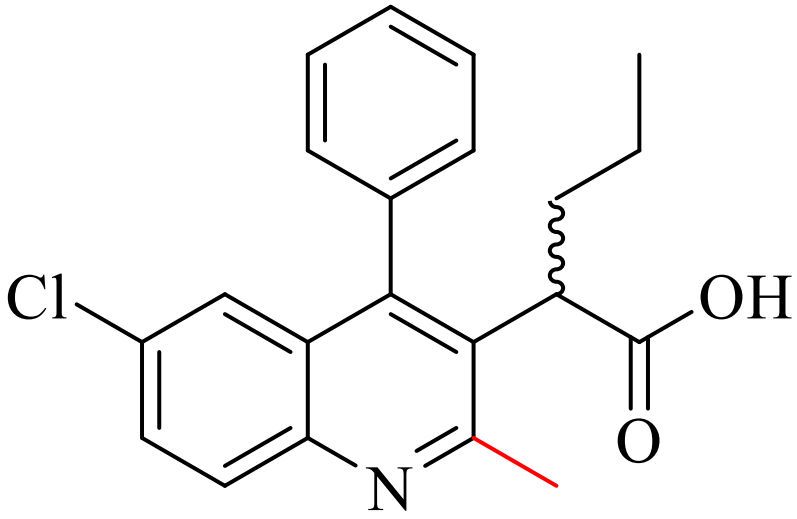


LEDGIN 5



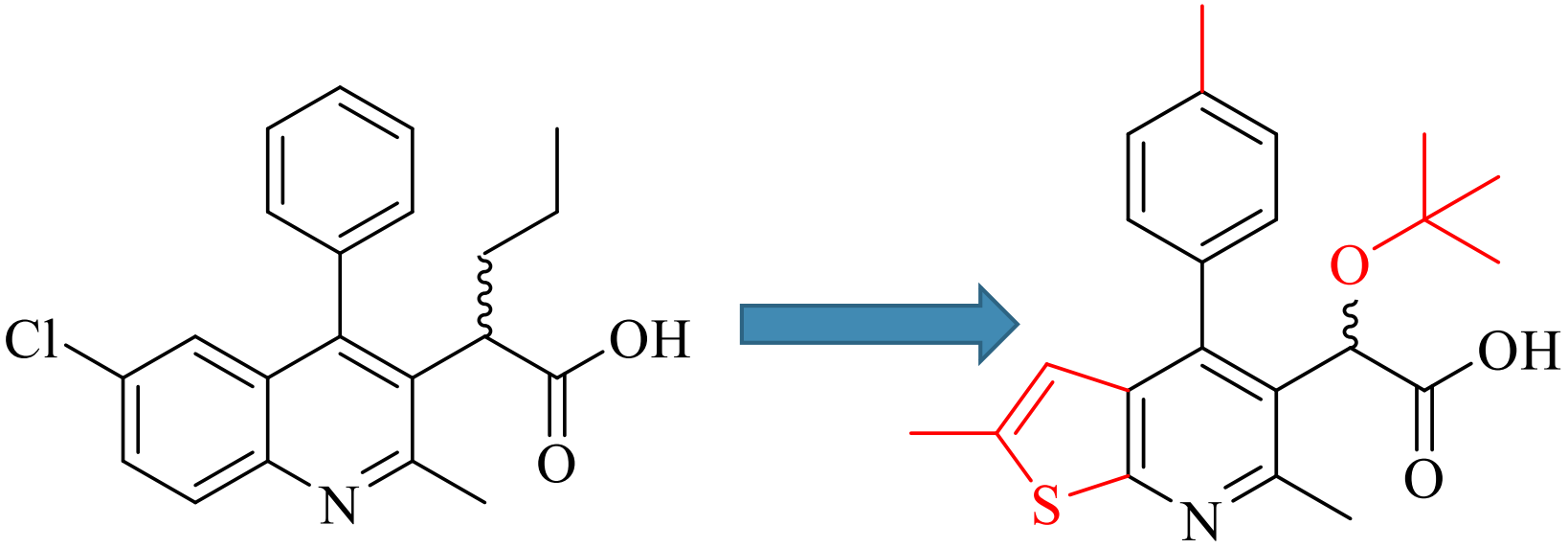
LEDGIN 6
CX05168

Christ F, et al. Nat Chem Biol 2010;6(6):442-8.



LEDGF/p75-IN interaction IC50 = 1.37 μM
EC50 = 2.35 μM in MT-4 cells
CC50 = 59.8 μM in MT-4 cells

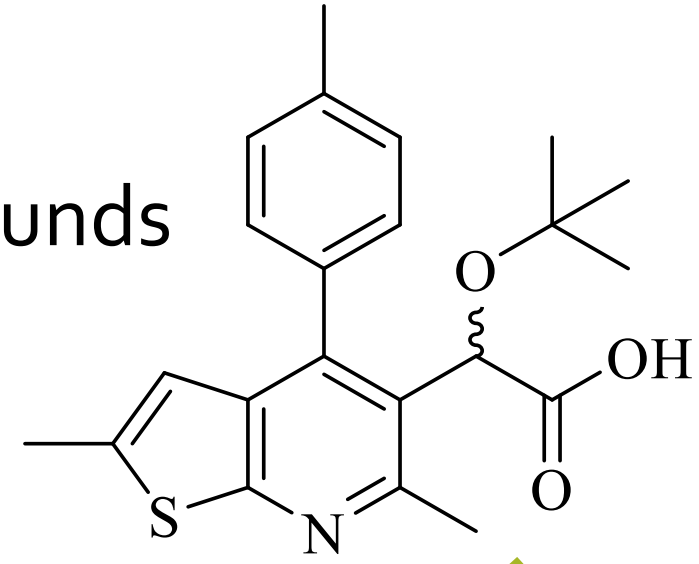
LEDGIN 6
CX05168

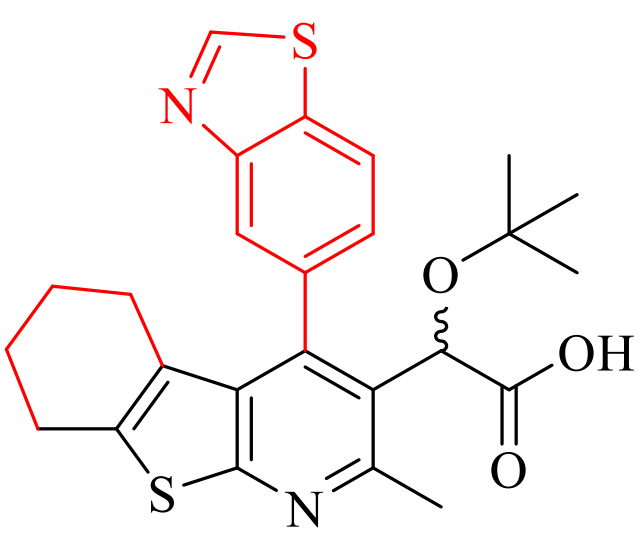


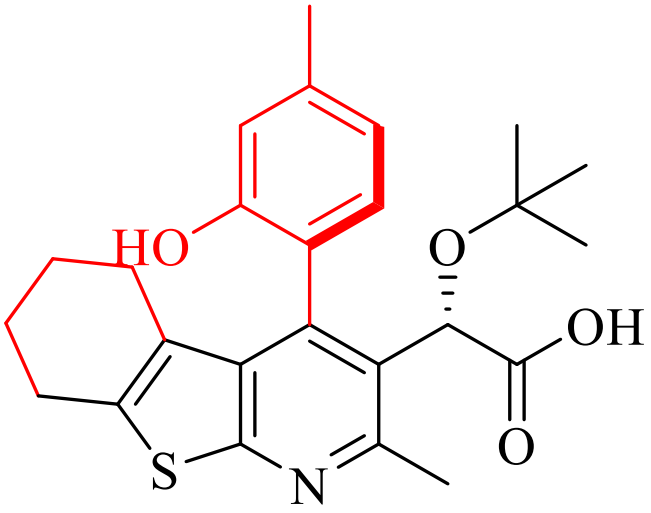
CX14442

LEDGF/p75-IN interaction IC50 = 0.046 μM
EC50 = 0.069 μM in MT-4 cells
CC50 = 96 μM in MT-4 cells

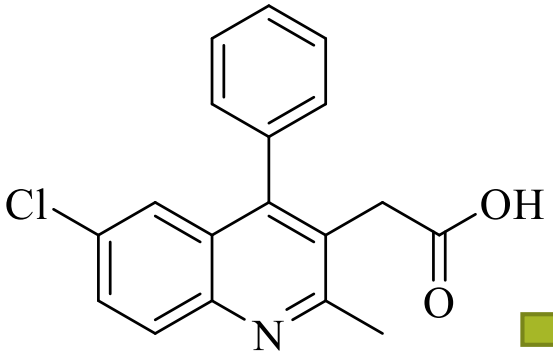
Patent Compounds



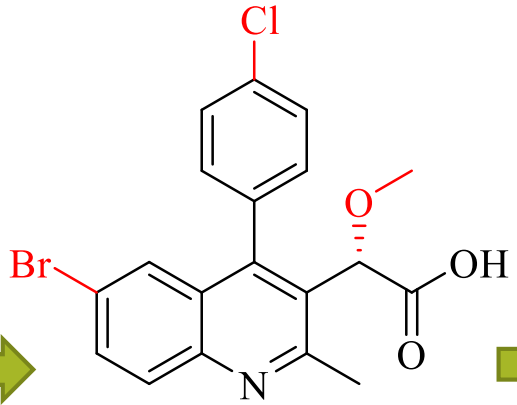




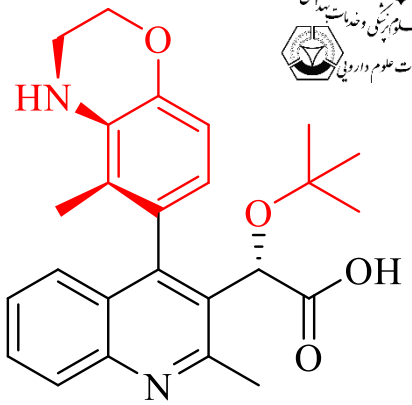
- In another independent study by researchers of Boehringer Ingelheim Ltd. (BI), Canada, a series of highly similar molecules were identified in a high throughput screening for discovery of IN 3P inhibitors.



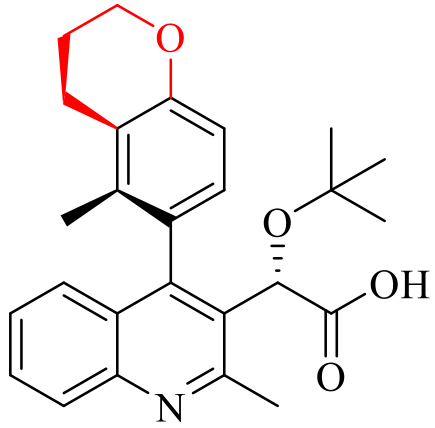
BI-A



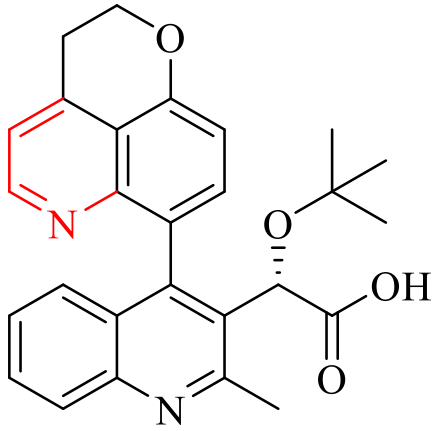
BI-B
BI-1001



BI-C

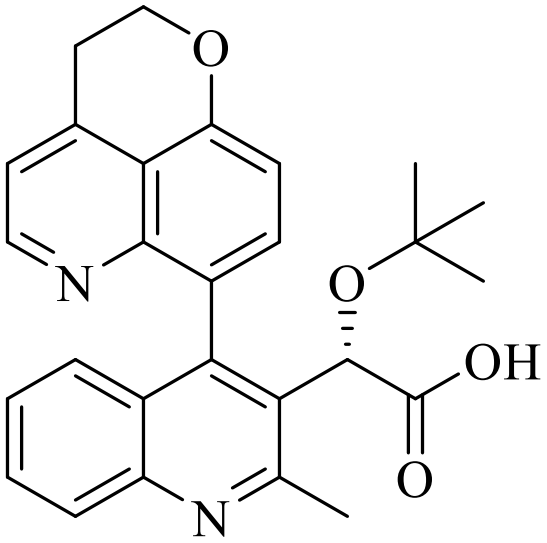


BI-D



BI 224436

- BI 224436 was progressed into Phase I clinical trial as the first IN-LEDGF interaction inhibitor

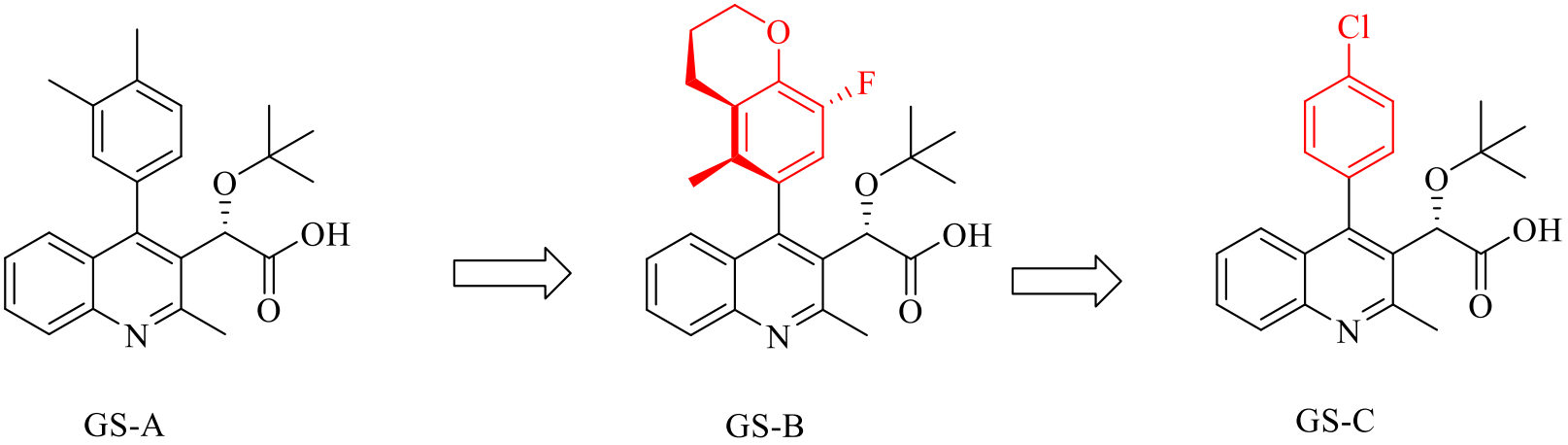


BI 224436

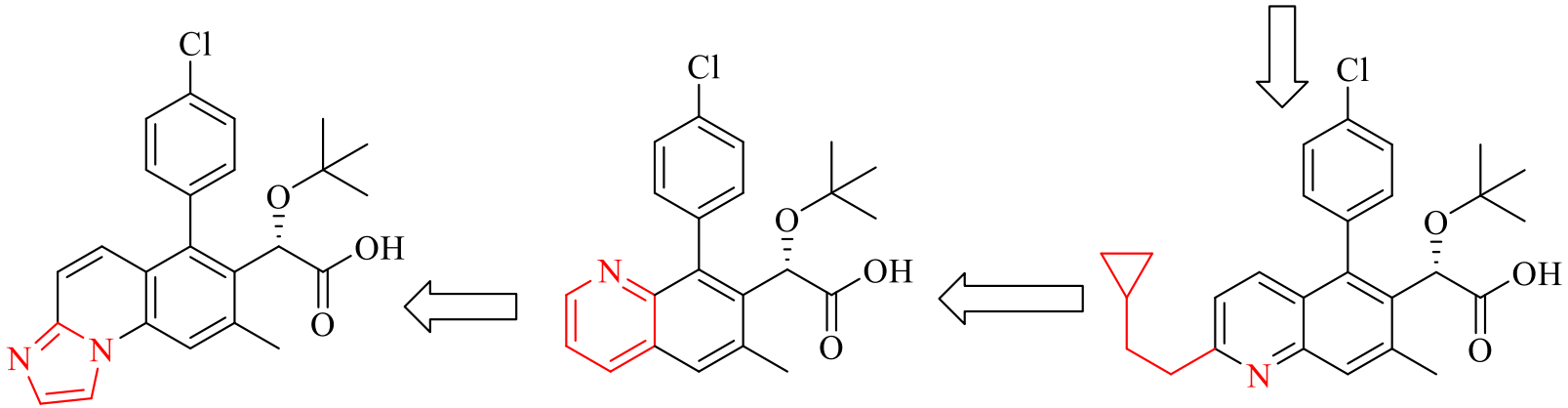
$PAEC_{95}$ = 22 nM

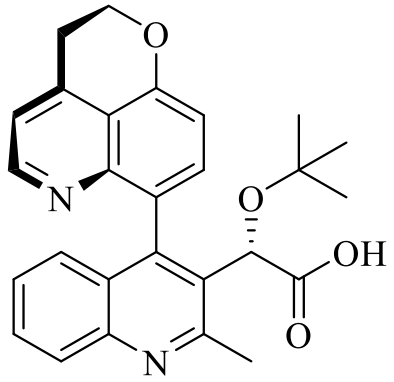
CC_{50} > 90 μM

- Clinical trial studies and development of BI 224436 was licensed to Gilead Sciences in 2011. Since then, Gilead reported the development of three structures from these series: GS-A, GS-B, and GS-C

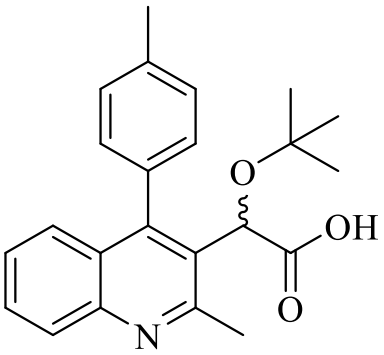


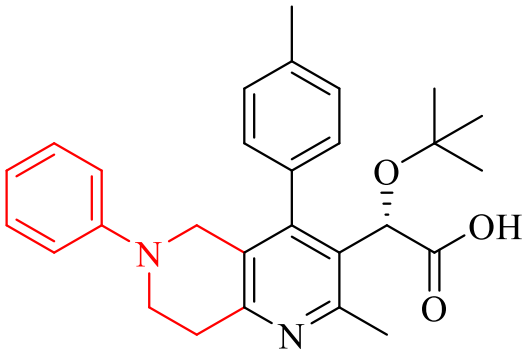
GS-A GS-B GS-C



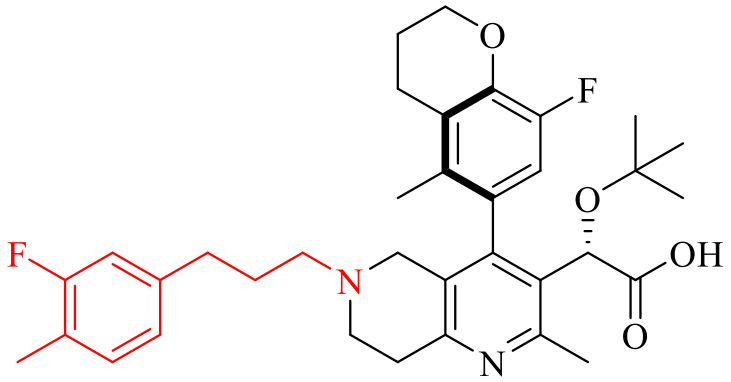


BI 224436





EC50 = 470 nM



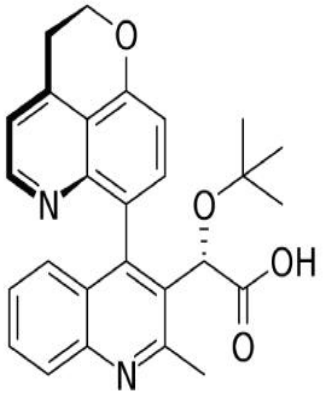
EC50 = 1nM

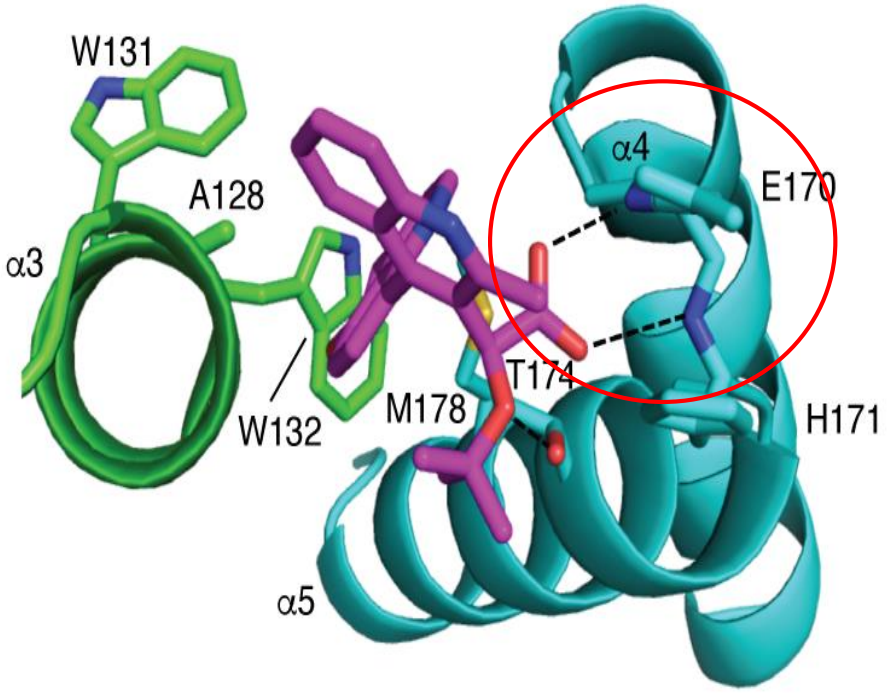
Peese et al. J. Med. Chem. 2019

Mechanism of action

- The compounds' **carboxylic acid group** mimics the carboxylate side chain of LEDGF/p75 residue **Asp366** by forming hydrogen bonds with the backbone amides of IN residues Glu170 and His171.

BI 224436

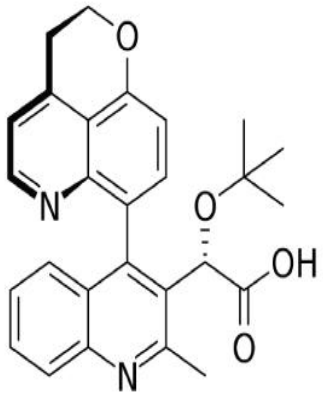


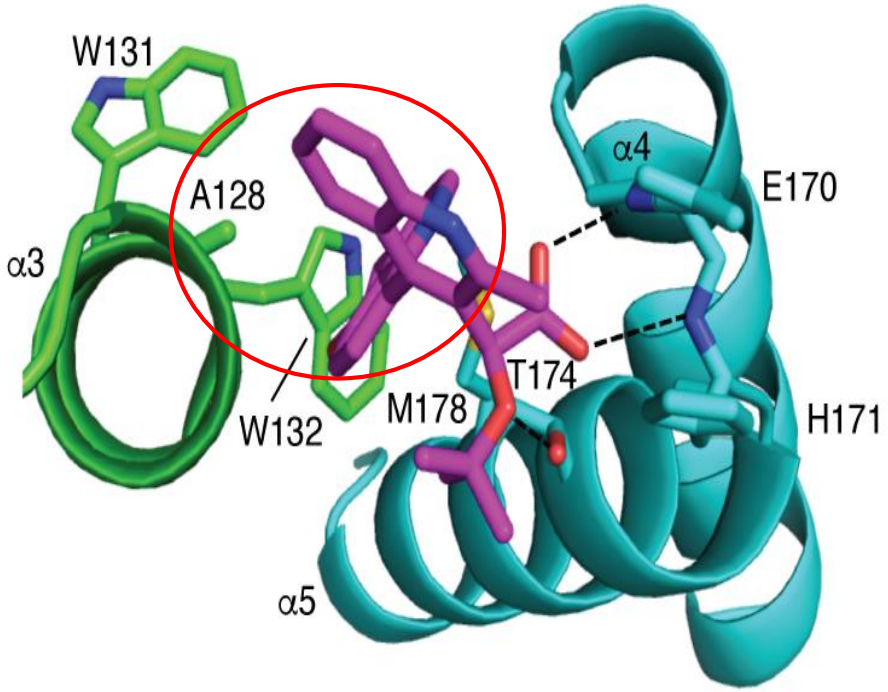


Mechanism of action

- **Central rings of compounds** mimic LEDGF/p75 residue **Ile365** by occupying a hydrophobic pocket formed by the IN B-chain residues Leu102, Ala128, Ala129, and Trp132 and the A-chain residues Thr174 and Met178.

BI 224436

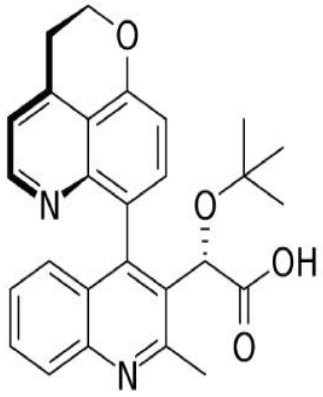


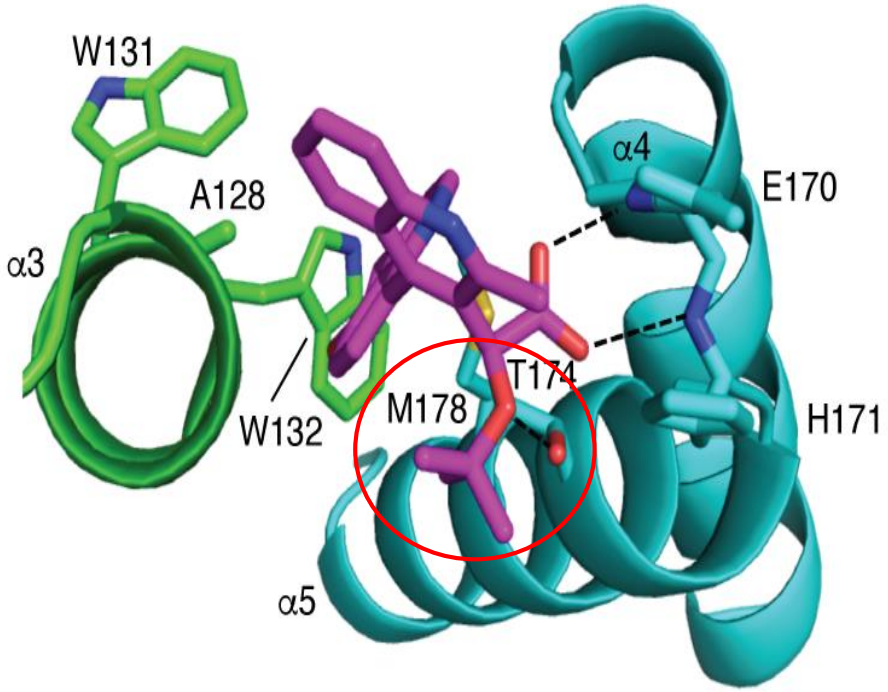


Mechanism of action

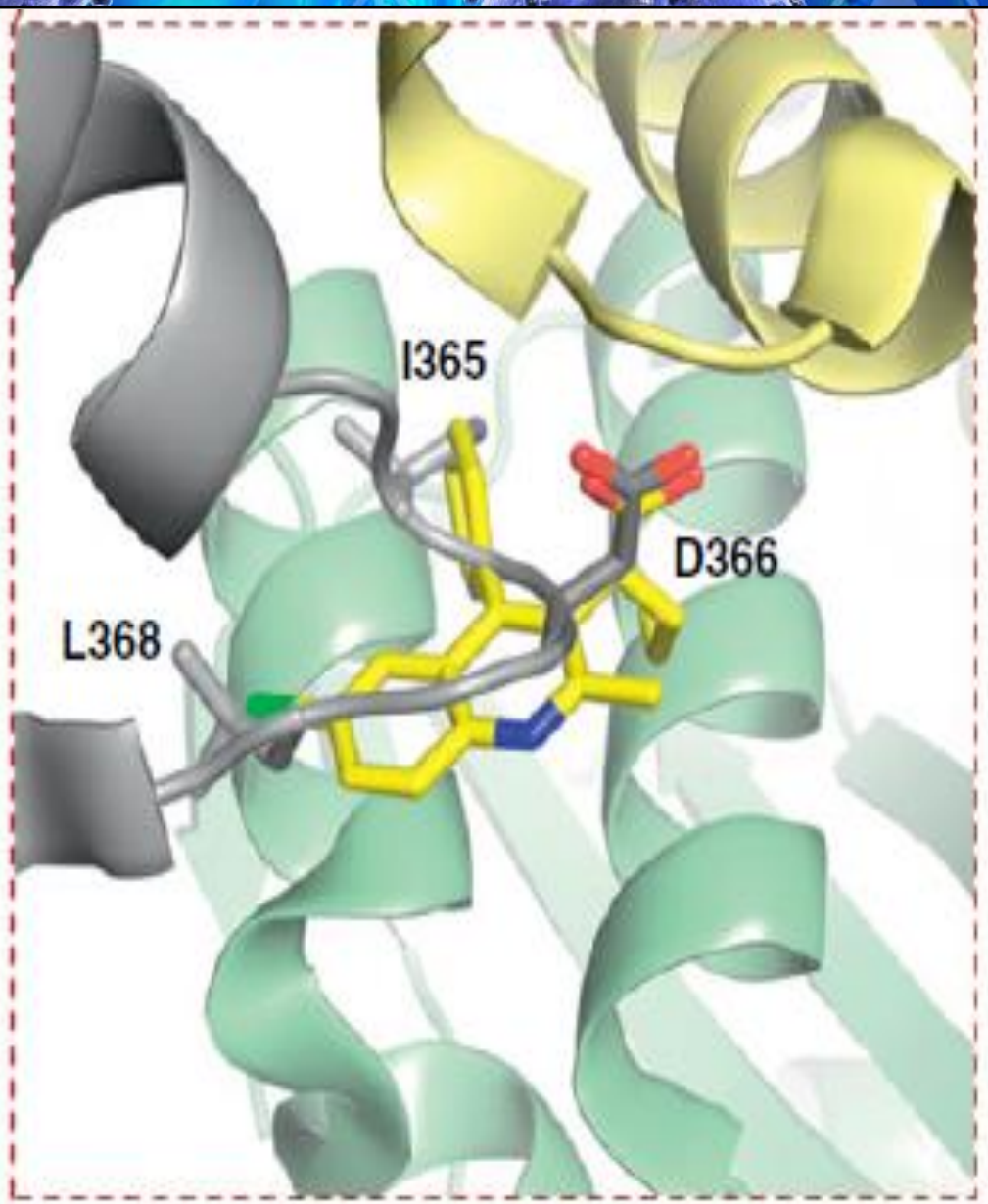
- The ***t*-butoxy group** of compounds interacts with IN A-chain residue **Thr174**.

BI 224436





Mechanism of action



Conclusion

- LEDGINs are still early in development
- Literature, however, reveals that almost all major pharmaceutical companies active in the treatment of HIV/AIDS have taken a significant interest in this class.
- Combined **early and late effects** present LEDGINs as unique within all classes of anti-HIV-drugs identified so far and might predestinate them for use in prevention and first in line therapy.
- They may well become the next class of antiretroviral agents to be added to HAART.

Thanks for your attention