A Novel Class of Multimerization Selective Inhibitors of HIV-1 Integrase

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Introduction

HIV-1 integrase (IN) is necessary for viral replication and therefore can be exploited as a drug target. A tetramer of IN catalyzes the insertion of viral DNA (vDNA) into host chromosomal DNA through two steps, 3'-processing and strand transfer. Its cellular cofactor lens epithelium derived growth factor (LEDGF)/p75 tethers the stable synaptic IN-vDNA complex to chromatin and stimulates integration.

Quinoline-based compounds have been shown to be promising leads for the development of clinical HIV-1 IN inhibitors. These compounds allosterically modulate IN function and are termed allosteric IN inhibitors (ALLINIs). ALLINIs bind at the catalytic core domain (CCD) dimer interface in the LEDGF/p75 binding pocket. Consequently, ALLINIs inhibit replication in two ways: (1) impair IN-LEDGF/p75 binding and (2) promote inactive higher-order oligomers of IN.

The multifunctional nature of ALLINIs presents significant limitations for exploiting these compounds as investigational probes to study HIV-1 molecular biology. Additionally, ALLINIs have a low genetic barrier needing only the A128T IN mutation to confer resistance.

Here we have exploited the x-ray crystal structure of quinoline-based ALLINIs bound to the IN CCD dimers to rationally modify existing compounds to design a new class of inhibitors that selectively promote aberrant IN multimerization.

We present multimerization IN inhibitors (MINIs), which potently promote aberrant IN multimerization without significantly inhibiting IN-LEDGF/p75 binding. MINIs also overcome the resistance of HIV-1 to ALLINIs caused by the IN mutation A128T.

Experimental approaches

We have rationally designed and synthesized pyridine-based inhibitors KF115 and KF116.

Homogeneous time-resolved fluorescence (HTRF)-based assays have been developed to examine the effects of MINIs on aberrant IN multimerization and IN-LEDGF/p75 binding.

Antiviral activities were determined by treatment with MINIs during the early or late stage of HIV-1 replication.

The effects of KF116 for promoting aberrant IN multimerization during the late stage of HIV-1 replication were examined by sucrose gradient fractionation, BS^3 crosslinking and TEM of virions.

X-ray crystallography was employed to elucidate interactions of MINIs with the HIV-1 IN CCD dimer.

KF116 resistance mutations were selected in cell culture.

Rational design of MINIs

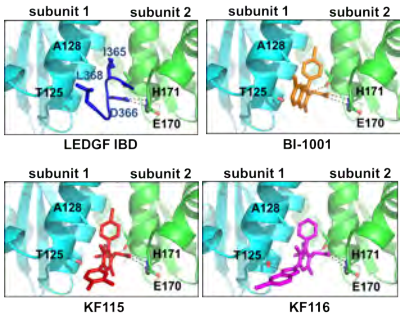


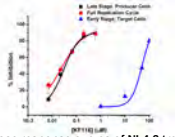
Fig 1. HIV-1 IN CCD dimer crystal structures with (A) LEDGF IBD, (B) ALLINI BI-1001, (C) KF115 or (D) KF116 bound.

MINIs are multimerization selective

	BI-1001	KF115	KF116
IN Multimerization IC50, μM	4.9 ± 0.3	0.274 ± 0.025	0.086 ± 0.006
IN – LEDGF/p75 binding IC50, μM	1.0 ± 0.1	13.0 ± 1.5	5.03 ± 0.36
Selectivity Ratio	0.2	47.4	58.5
Antiviral Activity (EC_{50}), μM	5.8 ± 0.1	0.121 ± 0.004	0.024 ± 0.003
Cytotoxicity (CC_{50}), μM	>100	>100	>100
Selectivity Index	>17	>826	>4167

Table 1. Inhibitory activities of ALLINI BI-1001 and MINIs KF115 and KF116.

KF116 is potent during late stage



	EC_{50} (μM)
Full Replication Cycle	0.024 ± 0.003
Late Stage	0.030 ± 0.002
Early Stage	50.9 ± 4.3

Fig 2. Dose response curves of NL4-3 treated during the late stage, early stage or full replication cycle

KF116 promotes aberrant IN multimerization

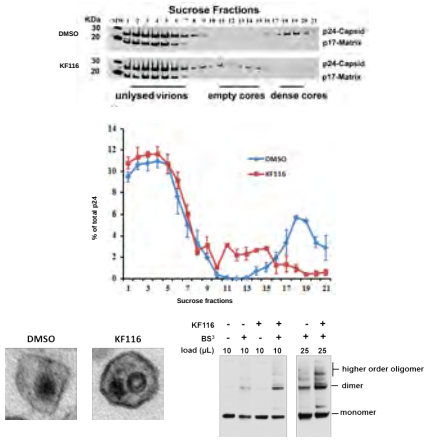


Fig 3. KF116 promotes IN multimerization in virus particles and impairs the formation of electron dense viral cores

KF116 overcomes A128T resistance

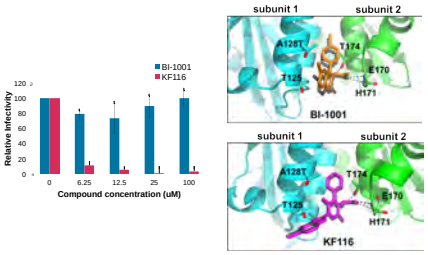


Fig 4. Inhibition of NL4-3(IN A128T) by BI-1001 or KF116, and crystal structures of BI-1001 and KF116 bound to HIV-1 IN A128T CCD dimer.

Evolution of resistance mutations

HIV-1 IN Mutations	Passage 5, (%)	Passage 10, (%)
T124N/V165I/T174I	0	70.7
T124D	0	20.7
T124N	100	3.7
T124N/T174I	0	3.7
T124N/V165M/T174I	0	1.2

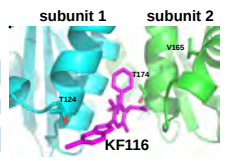


Fig 5. Mutations in HIV-1 NL4-3 IN gene of resistant viruses selected with KF116, and the crystal structure of KF116 bound to the HIV-1 IN CCD dimer highlighting Thr-124, Val-165 and Thr-174 residues.

Conclusions

Here we have rationally modified ALLINIs by altering the rigid quinoline moiety and designed pyridine-based molecules with a rotatable single bond to allow these compounds to bridge between interacting IN subunits optimally. These modified inhibitors potently promote the aberrant IN multimerization and are not effective inhibitors of IN-LEDGF/p75 binding. Therefore, we have termed pyridine-based compounds multimerization IN inhibitors or MINIs.

The most potent pyridine-based inhibitor, KF116, potently (EC_{50} of 24 nM) blocked HIV-1 replication by inducing aberrant IN multimerization in virus particles, whereas it was not effective when added to target cells.

KF116 is able to overcome the resistance to ALLINIs conferred by the A128T IN mutation in infected cells. The crystal structure of the IN CCD dimer bound to ALLINI BI-1001 shows a change in the conformation of BI-1001 when compared to CCD dimer with the A128T substitution, whereas there is no change in the orientation of KF116 binding to either wild type or A128T IN CCD dimer.

While a single A128T mutation was sufficient to confer marked resistance to a number of ALLINIs, selection of HIV-1 variants under genetic pressure of KF116 revealed a triple T124N/V165I/T174I mutant as the predominant variant suggesting an increased genetic barrier imposed by KF116 in comparison to ALLINIs.

Future work

Our current studies are focused on understanding the mechanism of resistance to MINIs. We have shown that under KF116 selective pressure HIV-1 evolve the IN mutation T124N at passage five, whereas after 10 passages the triple IN mutant, T124N/V165I/T174I emerges. We are working to delineate how each substitution works together to confer resistance to KF116.