A new class of capsid-targeting inhibitors that specifically block HIV-1 nuclear import

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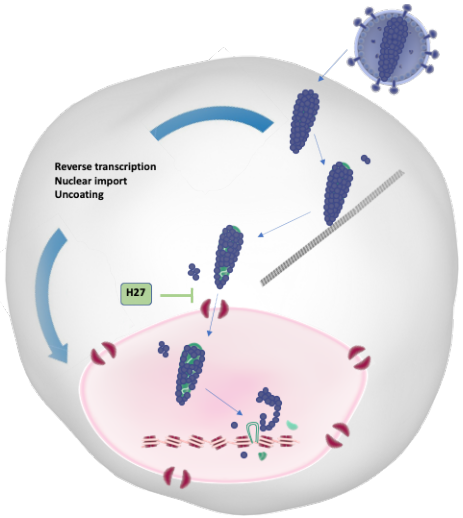
BACKGROUND

- HIV-1 nuclear import: the viral genome is carried across by its capsid
- To overcome the diffusional barrier of nuclear pore complexes, HIV-1 capsids bind to:
  - Nuclear transport receptors (NTRs, e.g. Transportin-1, -3)
  - Nuclear pore components (e.g. Nup153, Nup98, Nup358)
- Despite ART, HIV-1 persists in the nucleus of infected cells in the form of integrated and unintegrated provirus

We hypothesised that **compounds that prevent HIV-1 capsids from engaging with the nuclear import machinery of their target cells could have antiviral properties**, by disrupting uncoating, nuclear import, or both.

METHODS

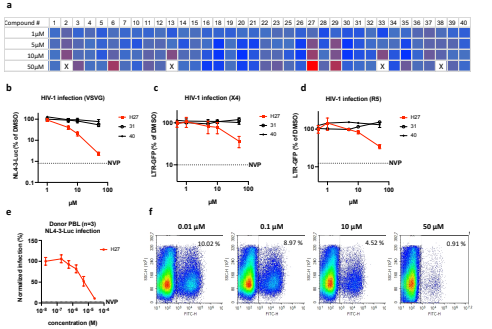
- 40 compounds tested following *in silico* screening targeting TRN-1 :: CA (Ref. 1).
- Antiviral activity: primary lymphocytes, clinical isolates from treatment-naive patients, ART-resistant mutants.
- Nuclear import: quantitative PCR, AlphaCentauri (Ref. 2), confocal imaging.
- Binding experiments: surface plasmon resonance, methyl-Trosy NMR, co-IP, capsid pulldown, proximity ligation assay



New CA inhibitors that block nuclear import without affecting CA assembly / disassembly

RESULTS

- Virtual screening of the HIV-1 CA hexamer - TRN-1 interface identifies H27 as an antiviral molecule
- H27 inhibits HIV-1 nuclear import without disrupting reverse transcription
- H27 does not inhibit the late assembly or maturation steps of HIV-1
- Differences in the PR loop may account differential sensitivity to H27 across divergent primate lentiviruses
- PF74- and LEN-resistant mutants are sensitive to H27
- Unlike LEN, H27 does not affect CA assembly or disassembly kinetics
- H27 reduces HIV-1 CA binding to TRN-1
- Primary isolates from treatment-naïve patients and multi-resistant HIV-1 are inhibited by H27



IC50 values	H27	Analogues of H27				
		43	46	49	55	84
Infectivity	1.0 - 2.9 µM	0.6 - 1.3 µM	1.4 - 5.3 µM	1.0 - 8.7 µM	1.5 - 1.6 µM	0.4 - 0.8 µM
Nuclear import	1.8 - 3.4 µM	3.7 - 5.6 µM	0.6 - 3.2 µM	1.8 - 6.0 µM	1.6 -6 µM	0.2 - 1.0 µM

Fig. 1: H27 and analogues inhibit HIV-1 infection at low µM concentrations

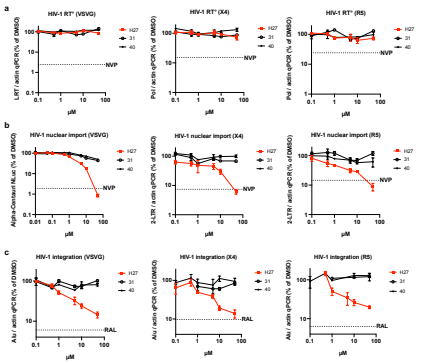


Fig. 2: H27 blocks HIV-1 nuclear import without disrupting reverse transcription

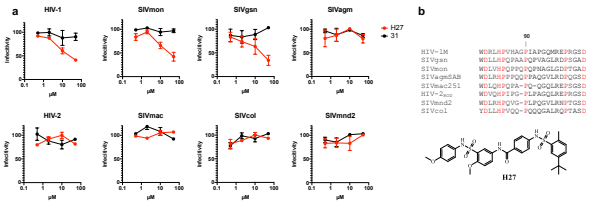


Fig. 3: Determinants for sensitivity to H27 across divergent lentiviral capsids

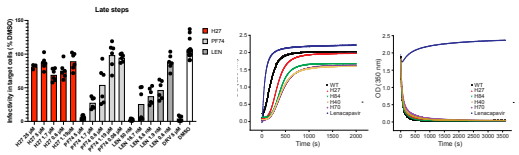


Fig. 4: Effect of H27, PF74 and LEN on CA assembly and disassembly

CONCLUSIONS

- We identified a new class of HIV-1 inhibitors that specifically target HIV-1 nuclear entry and display an antiviral activity.
- H27 was potent against all tested HIV-1, irrespective of their tropism, including HIV-1 isolates from the plasma of treatment-naïve patients and mutant viruses commonly associated with resistance to the four main classes of ART.
- Potency was demonstrated in primary human lymphocytes, with minimal toxicity, and an acceptable metabolic stability *in vivo*.
- Mechanism is distinct from previously described CA inhibitors (e.g. PF-74, LEN): H27 inhibits nuclear import specifically, no effect on CA assembly/disassembly.

LIMITATIONS

Although we found that H27 reduces the binding of HIV-1 capsid to TRN-1, we cannot exclude that it also blocks other key interactions with other NTRs, Nups, or cellular co-factors important for nuclear import. Alternatively, H27 binding to capsids may alter their flexibility, and hence their ability to pass through NPCs.

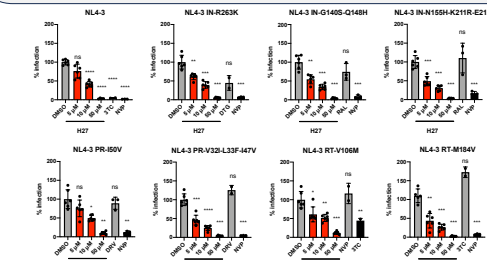


Fig. 5: Potency of H27 on resistant mutants to NRTIs, NNRTIs, PIs and INSTIs

REFERENCES

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