The design and investigation of the mechanism of action of the novel, potent HIV-1 entry inhibitor SC12

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Summary

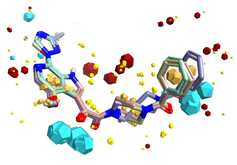
Background: The process of HIV-1 entry is key to the replication of the virus and viral Env serves as a hot target as a first protein complex encountering the target cell. We designed a novel potent HIV-1 entry inhibitor, **SC12** that binds viral Env and prevents the infection. In addition, **SC12** has better predicted ADME/PK properties (absorption, distribution, metabolism and excretion/pharmacokinetic; as assessed using StarDrop [Optibrium, UK]) compared to the lead chemotype in HIV-1 entry field, BMS compounds. Given the enormous potential of the small molecule entry inhibitor, **SC12**, we sought to investigate the mechanism of its action against HIV-1 viruses.

Methods: We utilized the single-round infection assay against HIV-1 viruses pseudotyped with HIV-1 Env and AMLV Env to identify the potency and specificity of **SC12**. Surface Plasmon Resonance (SPR) was employed to determine the binding site of **SC12** on viral Env and to investigate its potential to block sCD4-gp120 binding. We designed a novel attachment assay based on the temperature dependence of entry to determine the effect of **SC12** on viral attachment and viral fusion.

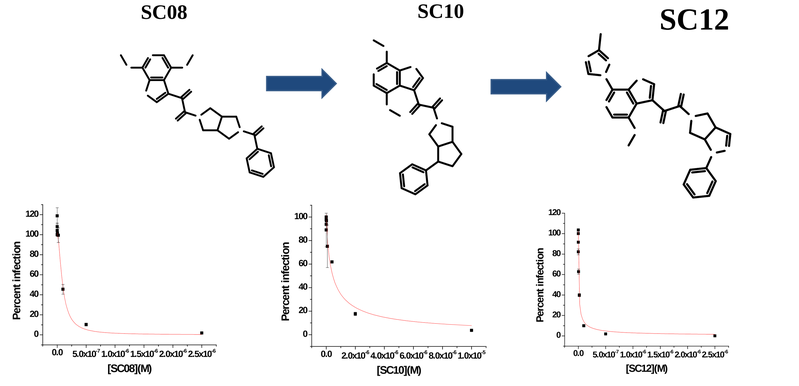
Results: **SC12** has low nM to low µM potency range against numerous isolates of HIV-1 clades A through D. In addition, **SC12** was determined to directly bind gp120 and the gradual increase of **SC12** concentration did not affect sCD4 binding to gp120. Moreover, **SC12** did not block viral attachment to the cells regardless the time of the addition of the compound: before, after or at the time of the viral infection.

Conclusions: We have designed a novel small molecule inhibitor that is potent against various HIV-1 isolates proposing that the binding site of **SC12** is conserved throughout the clades. We did not observe a concentration-dependent competition of **SC12** and sCD4 for the binding site on gp120, proposing that **SC12** is not an attachment inhibitor. Moreover, at the concentration of 80 nM **SC12** did not affect viral attachment to the cells , however, was able to inhibit over 50% of viral infectivity. Taken together, we propose that **SC12** is not an attachment inhibitor and blocks viral infection by another mechanism. The further characterization of its mechanism of action and optimization of **SC12** will yield important basic information about the process of HIV-1 entry and potentially lead to the novel therapies.

Design and testing of the pyrrolo-3,4-pyrazole SC12



We have previously designed and tested **SC08** that utilizes the dipyrrolidine scaffold. Templating this compound and BMS-488043 and BMS-626529 (using FieldTemplater, Cresset, UK; see figure on left) and taking note of the conformation of the C-terminal phenyl group, two new compounds were designed and synthesized — **SC10** and **SC12** (see below).

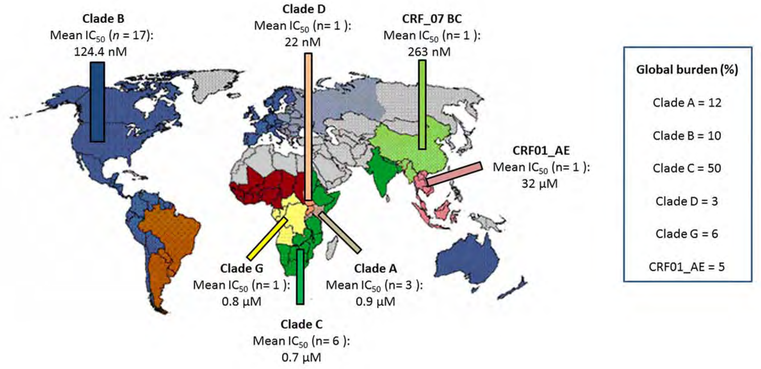


HIV-$1_{JR\text{-}CSF}$ IC_{50} = 0.09 ± 0.01µM

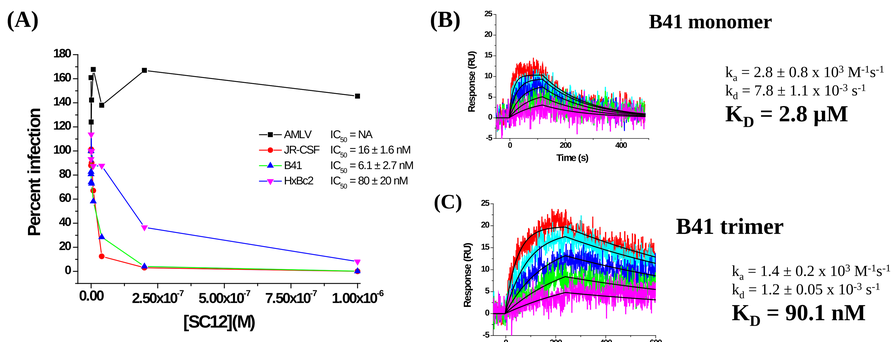
HIV-$1_{JR\text{-}CSF}$ IC_{50} = 0.42 ± 0.11µM

HIV-$1_{JR\text{-}CSF}$ IC_{50} = 8.0 ± 2.0 nM

Potency and therapeutic spectrum of SC12

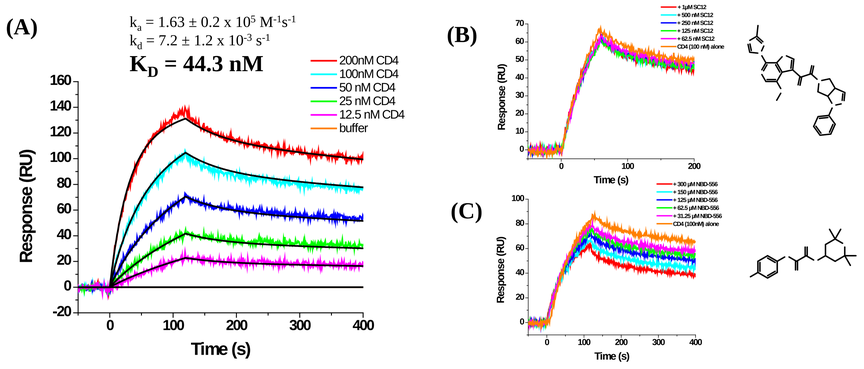


SC12 binds greater to SOSIP.664 Env than to monomeric gp120



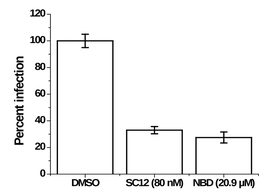
(**A**) Potency of **SC12** against AMLV, JR-CSF, B41 and HxBc2 Env pseudotyped HIV-1 (**B**) Direct binding of **SC12** to B41 monomeric gp120 (**C**) Binding of **SC12** to B41 SOSIP.664 trimer.

SC12 does not inhibit CD4 binding to SOSIP.664 Env

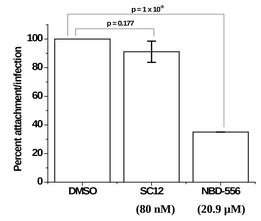


(**A**) Direct binding of 2-domain sCD4 to B41 SOSIP.664 (**B**) sCD4 competition using **SC12** and B41 SOSIP.664. Highest **SC12** concentration is 10-fold the K_D (**C**) sCD4 competition using NBD-556 to B41 SOSIP.664 trimer. Highest NBD-556 concentration is 10-fold the K_D.

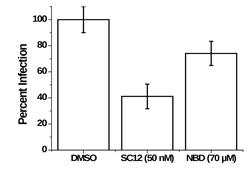
SC12 does not appear to block cellular attachment of the virus



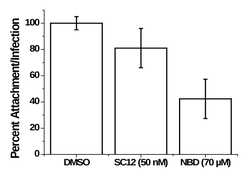
Above: Effect of 80 nM of SC12 and 20.9 µM of NBD-556 on the infection of U87.CD4.CXCR4 cells with HxBc2 Env pseudotyped single-round infective HIV-1.



Above: Effect of 80 nM of SC12 and 20.9 µM of NBD-556 on the attachment/infection of HxBc2 Env pseudotyped single-round infective HIV-1 to/with U87.CD4.CXCR4 cells



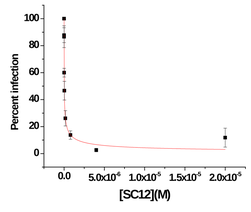
Above: Effect of 50 nM of SC12 and 70 µM of NBD-556 on the infection of Cf2.CD4.CCR5 cells with J1Hx Env pseudotyped single-round infective HIV-1.



Above: Effect of 50 nM of SC12 and 70 µM of NBD-556 on the attachment/infection of J1Hx Env pseudotyped single-round infective HIV-1 to/with Cf2.CD4.CCR5 cells.

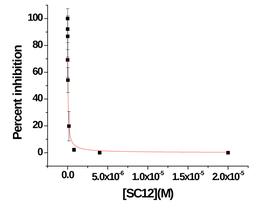
SC12 inhibits infection of CD4 independent virus

CD4-dependent J1Hx Env (Cf2.CD4.CCR5 cells)



HIV-1_{J1Hx} IC_{50} = 18.8 ± 4.3 nM

CD4-independent J1Hx(197) Env (Cf2.CCR5 cells)



HIV-1_{J1Hx} IC_{50} = 32.5 ± 13.6 nM

Conclusions

- SC12 represents a new core scaffold for the entry inhibitor class
- SC12 is broadly active against multiple isolates, except CRF02_AE
- SC12 interacts with trimeric Env greater than monomeric gp120
- SC12 does not inhibit CD4 binding to Env, attachment of virus to cells and inhibits the infection of the CD4-independent virus J1Hx(197) – indicating its primary mode of action is not blockade of the attachment of virus to the cellular receptor CD4.

Acknowledgments

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