

Discovery of a novel class of orally bioavailable HIV-1 fusion inhibitors



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Background

During virus entry, the HIV-1 envelope glycoprotein (Env) undergoes a complex series of conformational changes induced by interactions with CD4 and coreceptor on the target cell surface (Figure 1). These conformational changes culminate in the formation of a six-helix bundle (6HB) structure in the gp41 transmembrane subunit. The formation of this structure drives fusion of the viral and target cell membranes. These conformational changes present attractive targets for drug discovery.

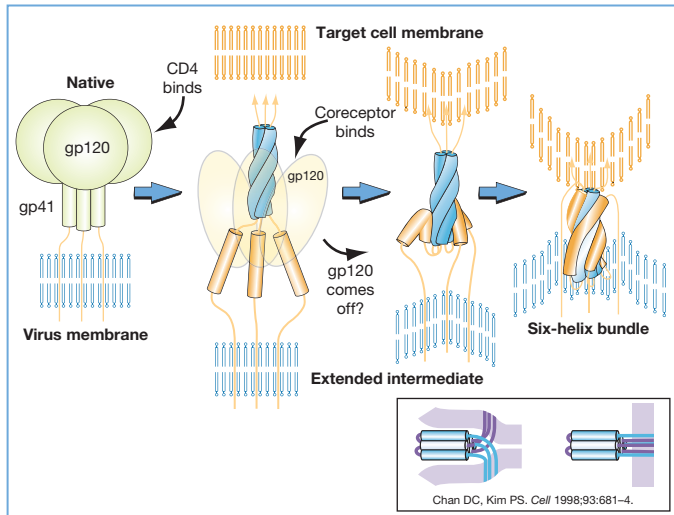


Figure 1. HIV gp41 six-helix bundle (6HB) formation

High-throughput assay design

We developed a proprietary high-throughput screening (HTS) assay to identify inhibitors of Env conformational changes that are necessary for membrane fusion and virus entry (Figure 2D). This assay uses conformation-specific antibodies to detect 6HB formation as a surrogate for membrane fusion. Compounds that inhibit the conformational changes in either gp120 or gp41 that are a prerequisite for 6HB formation and fusion are identified by a reduction in antibody binding to Env-expressing cells following triggering.

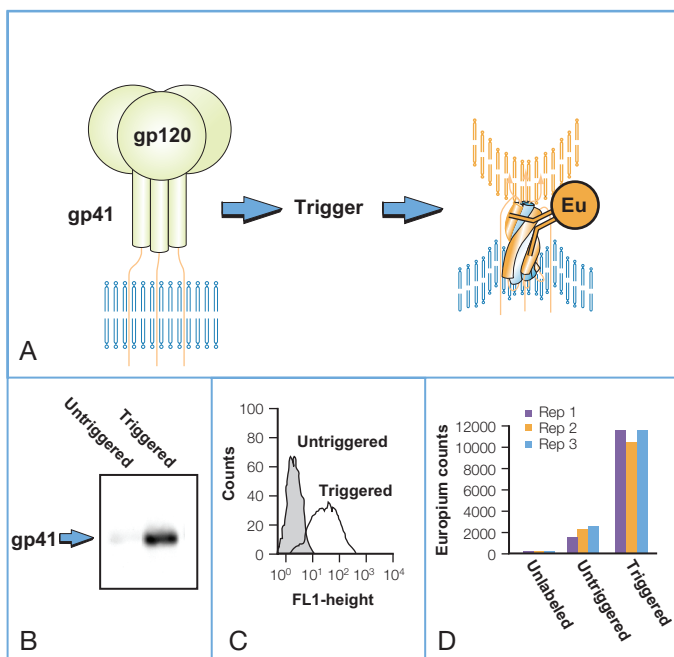


Figure 2. Detection of HIV gp41 6HB formation. a) Antibodies specific for the 6HB were used to detect Env conformational changes. 6HB-specific antibodies recognize receptor-triggered HIV Env by b) cell-surface immunoprecipitation, c) flow cytometry and d) time-resolved fluorometry (HTS assay)

Identification of orally bioavailable HIV-1 fusion inhibitors

A diverse library of over 400,000 drug-like compounds was screened in the 6HB inhibition HTS assay. Eight distinct tractable hit series were identified (~0.002% success rate; ~1/50,000 compounds). Three series were found to share a unique mechanism of action (Table 1 & Figure 3). Hit-to-lead medicinal chemistry increased the potency of the original Series A micromolar hit by ~3.5 logs. Median molecular weight and cLogP for the compounds in Table 1 were ~470 and ~4.3, respectively.

Table 1. Characterization of analogs from three chemically distinct hit series that share a unique mechanism of action.

Each series has undergone partial hit-to-lead optimization. 6HB assay: HIV-1_{RF} (CXCR4-tropic; clade B). Infection assay: HIV-1_{NL4-3} (CXCR4-tropic; clade B) infection of TZM-bl cells; detected using the GalScreen kit (Applied Biosystems). HIV-2: Infection assay using HIV-2_{ROD} (CXCR4-tropic). Cytotoxicity: XTT staining of TZM-bl cells. CD4 binding: binding of sCD4 to HIV-1_{RF}-expressing cells

Series	Potency; IC ₅₀ , μM		Specificity	Cytotoxicity	CD4 binding	PK
	6HB assay	Infection assay				
A	0.0005	0.004	>100	>100	>20	19% (mouse)
B	0.3	1	>100	>100	>20	30% (rat)
C	0.05	0.04	>100	>100	>20	ND

Table 2. Cross-clade inhibition of HIV-1 clinical isolates. Series A was tested against a single clinical isolate from each clade in the infection assay (average of two experiments). All isolates were CCR5-tropic, except for HIV-2_{ROD}.

Clade	HIV-1								HIV-2 _{ROD}	SIV _{mac239}	
	Group M							Group O			
	A	B	C	D	E	F	G	H			
IC ₅₀ , μM	0.03	0.13	2.70	0.02	>20	1.59	2.00	14.09	16.85	>20	>20

These partially optimized compounds were broadly active against diverse HIV-1 clades (Table 2). By comparison, the small molecule attachment inhibitor, BMS-378806, was reported to have median EC₅₀ values of 1.13 μM for clade A, and >2 μM for clades D, F, and G.¹

Novel mechanism of action

These compounds represent a new class of fusion inhibitors with a novel mechanism of action (Figure 3). Unlike enfuvirtide, they affect gp120 conformation by binding to native Env prior to receptor engagement (Figure 4) and enhancing exposure of conserved neutralization epitopes on gp120 (Figure 5). The compounds also bind CD4-bound Env (Figure 4) and stabilize the association of gp120 with gp41, thus inhibiting sCD4-induced gp120 shedding (Figure 5). Unlike coreceptor antagonists, the compounds are active against both CXCR4- and CCR5-using viruses (Tables 1 & 2). Unlike attachment inhibitors, they have no effect on CD4 binding (Table 1). The compounds act at a late stage in HIV fusion (Figure 6A) and inhibit exposure of the N-helical coiled-coil, to which enfuvirtide binds (Figure 6B). Consequently, these compounds have a resistance profile distinct from that of enfuvirtide (Figure 7 & Table 3). Thus, these compounds appear to block HIV fusion by interacting with a unique target site in Env.

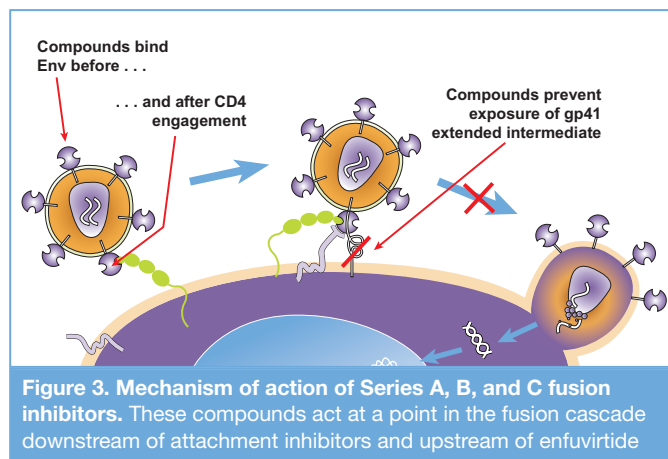


Figure 3. Mechanism of action of Series A, B, and C fusion inhibitors. These compounds act at a point in the fusion cascade downstream of attachment inhibitors and upstream of enfuvirtide

Compounds interact with Env both pre- and post-CD4 binding

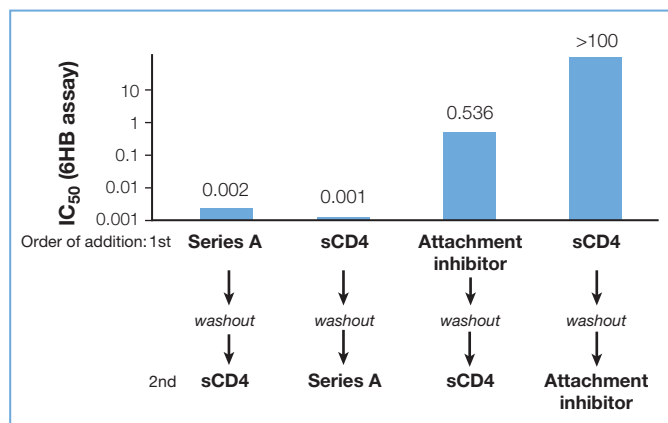


Figure 4. Compound addition pre- or post-sCD4 incubation. Env-expressing cells were pre-incubated with Series A, an attachment inhibitor, or sCD4. Following washout of the initial reagent, inhibitor, or sCD4 was added as indicated. 6HB formation was quantitated by flow cytometry using 6HB-specific antibodies

Compounds enhance exposure of conserved gp120 neutralization epitopes and stabilize gp120-gp41 association

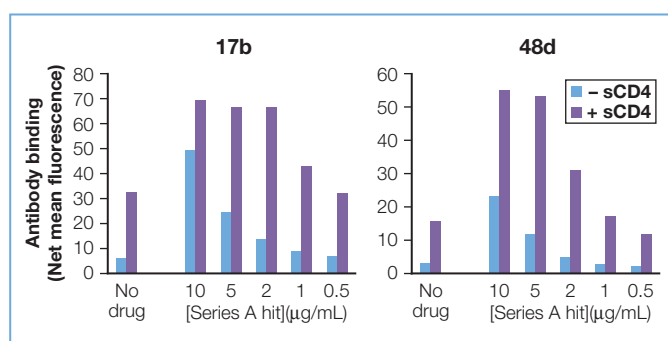


Figure 5. Induction of 17b and 48d epitope exposure and inhibition of sCD4-induced gp120 shedding. The effects of the original Series A hit compound on gp120 conformation and sCD4-induced shedding of gp120 were determined by flow cytometry using the human mAbs 17b and 48d, which recognize CD4-induced neutralization epitopes on gp120

Compounds block exposure of the gp41 N-helical coiled-coil

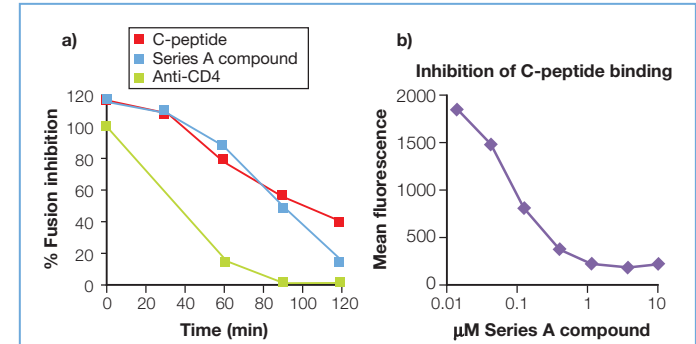


Figure 6. Time-of-addition and inhibition of C-peptide binding to the gp41 N-helical coiled-coil. a) Compounds act at a late step in virus-cell fusion (detected using HeLa-CD4-LTR-β-gal cells). b) Compounds block C-peptide binding to triggered Env as detected by T26 Mab binding (flow cytometry). C-peptide: a 34-amino acid peptide similar to enfuvirtide

Distinct resistance profile

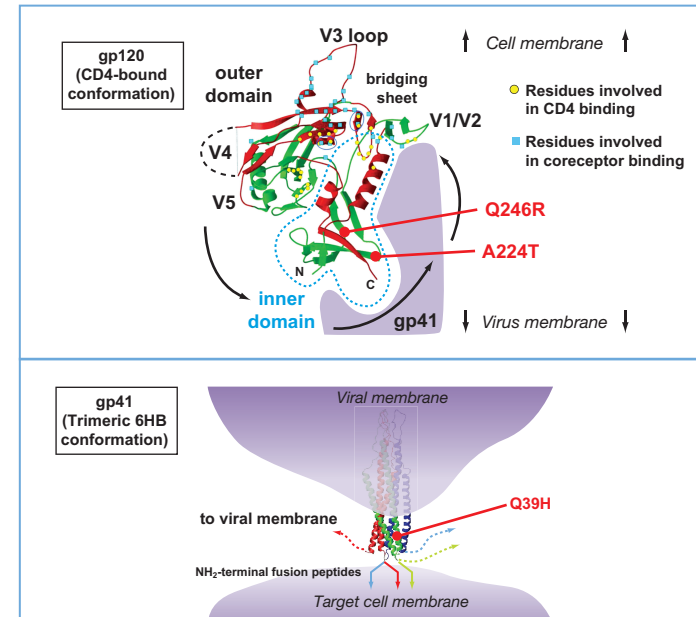


Figure 7. In vitro resistance selection. Repeated passage in the presence of suboptimal concentrations of drug identified 3 separate mutations that each conferred ~10–30-fold resistance: gp120-A224T, gp120-Q246R, and gp41-Q39H

Table 3. Compounds have a resistance profile distinct from that of enfuvirtide. Infection assay in HeLa-CD4-LTR-β-gal cells. Mutant viruses were normalized for MOI. C-peptide inhibitor: a 34-amino acid peptide similar to enfuvirtide

Enfuvirtide resistance mutant	Fold resistance	
	Series A	C-peptide
L33S	11.7	3.0
V38E	0.5	60.5
I37K	4.8	6.3
N42T/N43K	1.2	9.3
V38A/N42T	0.9	7.3
V38E/N42S	0.5	23.3
V38A	2.0	9.5

Red=>2-fold resistance

Summary

- We have used a novel screening approach to identify orally bioavailable HIV-1 fusion inhibitors with a novel mechanism of action
- We attribute the success of this approach to the use of native, oligomeric, membrane-anchored Env as the screening target. No assumptions were made about the target site or mechanism of inhibition
- These compounds act upstream of exposure of the target site for enfuvirtide and are active against most enfuvirtide-resistant viruses
- The most potent of these compounds specifically inhibit HIV-1 infection *in vitro* with an IC₅₀ of 4 nM. No cytotoxicity was observed at up to 100 μM, yielding a therapeutic index >10,000
- Unlike the peptide fusion inhibitor, enfuvirtide, these compounds have the potential for delivery in an oral form.
- Further optimization is ongoing to identify a lead compound to take forward into formal pre-clinical studies

Acknowledgments

We thank James Robinson (Tulane University) for 17b and 48d mAbs. This work was supported in part by NIH-AI-56915.

Reference

1. Lin PF, et al. *Proc Natl Acad Sci USA* 2003;100:11013–8