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 interaction with CD4 and co-receptor 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.

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 2). This assay uses conformation-specific inhibitors of Env conformational changes that are necessary for membrane fusion. Compounds that inhibit the conformational changes in either gp120 or gp41 fusion and virus entry (Figure 2D). This assay uses conformation-specific inhibitors of Env conformational changes that are necessary for membrane fusion.

Table 1. Characterization of analogs from three distinct hit series that share a unique mechanism of action. Each series has undergone partial hit-to-lead optimization, including: gB assay, HIV1-R5 (CXCR4-tropic), Bcl-2 Infection assay; HIV1-R5 (CXCR4-tropic), Bcl-2 Infection assay; HIV1-R5 (CXCR4-tropic), Bcl-2 Infection assay using 6HB Screen (Applied Biosystems). HIV-2: Infection using HIV1-IIIa (R5): CD4 + cells. Cytotoxicity (IC50): 6HB Screen (Applied Biosystems). 80 cells.

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 HIV2-MU clade.

Table 3. Compounds have a resistance profile distinct from that of enfuvirtide. Infection assay in H9-CD4-LTR-β-gal cells. Mutant viruses were normalized for MOI. C-peptide inhibitor: a 34-amino acid peptide similar to enfuvirtide.

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 the 6HB Screen HTS assay). b) Compounds block C-peptide binding to triggered Env as detected by TMB Max 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 resistant isolates of each clinical isolate. Each resistant isolate was tested against a single clinical isolate from each clade. All isolates were CCR5-tropic, except for HIV-2ROD.

Comprehensive characterization of the compounds showed that they have a novel mechanism of action.

The compounds interact with Env both pre- and post-CD4 binding.

These compounds represent a new class of orally bioavailable HIV-1 fusion inhibitors with a novel mechanism of action (Figure 3). Unlike enfuvirtide, they affect gp120 conformational change by targeting viral Env prior to receptor engagement (Figure 4) and enhancing exposure of conserved neutralization epitopes on gp120 (Figure 5). These compounds act at a late stage in HIV fusion (Figure 6) 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.

Compounds are orally bioavailable and have been shown to block HIV fusion in vivo.

Figure 8. In vivo resistance selection. Resistance in vivo was selected in mouse xenografts using HIV-1-expressing cells. Each resistant isolate was tested against a single clinical isolate from each clinical isolate. All isolates were CCR5-tropic, except for HIV-2ROD.

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 a novel, pharmacologically characterized Env as the screening target. No assumptions were made about the target cell or mechanisms of inhibition.

These compounds act upstream of selection of the target site for enfuvirtide and are active against most enfuvirtide-resistant strains.

The most potent of these compounds specifically inhibit HIV-1 infection in vitro with 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.

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