

POSTER 49**A SMALL MOLECULE INHIBITOR OF HIV-1 INFECTION HAS A CYCLOPHILIN-DEPENDENT MECHANISM AND ACTS BY DESTABILISING THE VIRAL CAPSID**

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Following fusion of HIV-1 particles with target cells, the viral core, consisting of the ribonucleoprotein complex surrounded by the conical capsid, is released into the cytoplasm where it undergoes uncoating. Proper uncoating is critical for reverse transcription, as mutations that destabilise the capsid impair HIV-1 DNA synthesis. Using a full replication antiviral screen, we identified a new small molecule inhibitor, PF-3450074. Time-of-addition experiments indicated that PF-3450074 inhibits the virus early in its life cycle. When added at the time of inoculation, PF-3450074 inhibited wild type HIV-1 as well as HIV-1 particles pseudotyped by VSV-G, indicating that inhibition is not specific for HIV-1 Env-mediated fusion. The compound inhibited reverse transcription in target cells but not *in vitro*, suggesting that it targets an early postentry step in infection. Resistance selection experiments demonstrated that a combination of 5 mutations, clustered in the N-terminus of the CA protein, conferred significant resistance to PF-3450074 and related inhibitors. Calorimetric and crystallographic studies provided further evidence that the compounds bind specifically to the N-terminal domain of HIV-1 CA *in vitro*. Crystal structures of bound compound indicate a pocket predicted to lie at the interface of the N and C termini of capsid hexamers positioned between residues that mutate to confer resistance. PF-3450074 also bound specifically to HIV-1 particles, resulting in destabilisation of the viral capsid *in vitro*. Analysis of HIV-1 CA mutants revealed a link between sensitivity to PF-03450074 and the intrinsic stability of the viral capsid. Combination antiviral assays using PF-03450074 and cyclosporine A revealed strong antagonism, suggesting a role for cyclophilins in the antiviral activity of PF-3450074 in a manner reminiscent of HIV-1 restriction by TRIM5 α . However, the compound did not inhibit the binding of cyclophilin A to CA nor was the antiviral activity dependent upon target cell expression of the human TRIM5 α protein. Collectively, our results highlight the therapeutic potential of pharmacologic agents that target CA and perturb normal HIV-1 uncoating.