Small Molecule HIV-1 Nef Inhibitors Disrupt Nef Homodimer Formation in vitro

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The HIV-1 Nef protein promotes viral replication and immune evasion of HIV-infected cells through diverse interactions with host cell proteins involved in signal transduction and endolysosomal trafficking. Previous crystallography studies revealed that Nef forms homodimers when complexed to SH3 and SH2 domains of Src-family kinases. Mutations at the crystallographic dimer interface of Nef impair most Nef functions, including infectivity enhancement, immune and viral receptor downregulation, and kinase activation. The X-ray crystal structure of the dimerization-defective Nef-L112D mutant bound to the SH3 domain of the Src-family kinase Hck revealed a monomeric 1:1 complex, in contrast to the 2:2 dimeric complex previously observed with wild-type Nef. The overall fold of the Nef core in the complex was nearly identical to that of wild-type Nef observed in previous structures, including the interaction interface with the SH3 domain, supporting the conclusion that the diverse phenotypic effects associated with these mutations are due to the dimerization defect. Recently, we developed a reversible split fluorescent reporter assay (SplitFAST) to assess the effects of hydroxypyrazole Nef inhibitors on dimer formation. Several inhibitors showed a significant, concentration-dependent reduction in fluorescence intensity, indicating that these inhibitors influence Nef homodimer formation. These findings mirror the antiretroviral activities of these compounds and suggest a mechanism of action involving perturbation of the active Nef dimer like that observed with the dimerization interface mutants. Collectively, these findings provide insight into the mechanism of Nef homodimer formation and support the continued development of targeted Nef inhibitors as potential antiretroviral therapeutics.