

NEW LESSONS FROM HIV RNase H INHIBITORS

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High throughput screening of NCI libraries of synthetic and natural compounds identified the vinylogous ureas 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide (NSC727447) and N-[3-(aminocarbonyl)-4,5-dimethyl-2-thienyl]-2-furancarboxamide (NSC727448) as inhibitors of the ribonuclease H (RNase H) activity of HIV-1 and HIV-2 reverse transcriptase (RT). A Yonetani-Theorell analysis demonstrated that NSC727447 and the active site hydroxytropolone RNase H inhibitor β -thujaplicinol were mutually exclusive in their interaction with the RNase H domain. Mass spectrometric protein footprinting of the NSC727447 binding site indicated that residues Cys280 and Lys281 in helix I of the thumb subdomain of p51 were affected by ligand binding. Although DNA polymerase and pyrophosphorolysis activities of HIV-1 RT were less sensitive to inhibition, protein footprinting indicated that NSC727447 occupied the equivalent region of the p66 thumb. Site-directed mutagenesis using reconstituted p66/p51 heterodimers substituted with natural or non-natural amino acids demonstrates that altering Tyr501 of the p66 RNase H primer grip significantly decreases inhibitor sensitivity, while the enzyme with a T473C mutation is highly sensitive. Unlike hydroxytropolone RNase H inhibitors, vinylogous ureas cannot be displaced by nucleic acid binding and represent a novel class of RNase H antagonists with a mechanism of action differing from active site, divalent metal-chelating inhibitors that have been reported*.

Although the nucleic acid binding cleft separating the N- and C-terminal domains of RT can accommodate structurally-diverse duplexes, it is currently unknown whether bound nucleic acids can simultaneously contact both the polymerase and the RNase H active sites. Data will be presented showing that ligands which stabilize the RT-DNA/RNA complex in pre- and post-translocational states, respectively, affect specificity of RNase H cleavage without altering the efficiency of the reaction. This data provides strong evidence for a model in which the nucleic acid substrate can engage both active sites at the same time. Most importantly, the bound and intact DNA/RNA hybrid restricts access of the RNase H active site inhibitor β -thujaplicinol. Inhibition of RNase H activity is limited to secondary cleavage events. These findings raise the question whether efficient RNase H inhibition *in vivo* requires blockage of the primary cleavage step.

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