

BIOCHEMICAL AND STRUCTURAL STUDIES OF NOVEL HIV-1 RIBONUCLEASE H INHIBITORS

Suhman Chung¹, Michaela Wendeler¹, Jason W. Rausch¹, Jennifer T. Miller¹, Alun Bermingham², Barry O' Keefe², John A. Beutler², In-Ja Byeon³, Angela Gronenborn³, and Stuart F.J. Le Grice¹

¹RT Biochemistry Section, HIV Drug Resistance Program, ²Molecular Targets Development Program, National Cancer Institute-Frederick, Frederick, MD 21702, USA; ³Department of Structural Biology, University of Pittsburgh, Pittsburgh, PA, USA

High throughput screening of NCI libraries of natural compounds and chemical entities (n ~ 250,000) recently identified the vinylogous urea 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[b]thiophene-3-carboxamide (NSC727447) as a moderately potent inhibitor of the ribonuclease H activity of HIV-1 RT. In the present study, we have undertaken a structure/function analysis by examining the effects of substituting the cycloalkane, 2-amino and 3-carboxamide components of the NSC727447 thiophene ring. With respect to cycloalkane substitution, a cycloheptane ring appears to be the optimal size. However, when the cycloheptane substituent is retained, modifying either the 2-amino or 3-carboxamide groups reduces potency. NSC727448, or N-[3-(aminocarbonyl)-4,5-dimethyl-2-thienyl]-2-furancarboxamide, represents a second class of vinylogous urea whose thiophene moiety contains a 4,5-dimethyl replacement of the cycloheptane ring. Interestingly, 2-amino substitutions of this basic scaffold resulted in increased potency relative to both NSC727447 and NSC727448.

Previous mass spectrometric protein footprinting data suggested NSC727447 did not bind to the RNaseH active site, but rather to the p51 thumb subdomain. Attempts were therefore made to dock active compounds into the p51 thumb *in silico*. In parallel, we exploited a bioorthogonal tRNA/aminoacyl tRNA synthetase pair to site-specifically introduce the unnatural, fluorinated amino acid 4-(trifluoromethoxy)-L-Phe into the p51 thumb subdomain and p66 RNase H domain via translational suppression, in order to examine inhibitor binding by ¹⁹F-NMR. Modified enzymes retained both RNase H activity and inhibitor sensitivity, indicating that the unnatural amino acid insertion was not deleterious to enzyme function. The current status of our ¹⁹F-NMR studies will be reviewed, together with a proposed model for binding of vinylogous ureas to the p51 thumb subdomain.