with a novel conformational structure formed upon coculture of virus and cells. The pyrimidinediones inhibit the replication and transmission of resistant viruses bearing RT or Env mutations alone (albeit with 100-fold loss of sensitivity to NNRTI-resistant viruses) and remain highly active against multi-drug resistant (MDR) viruses with mutations in RT and/or protease. With serial passage in increasing compound concentrations, a virus which is completely resistant to the selecting pyrimidinedione can be selected. The selection follows a defined progression consisting of the initial appearance of mutations in the RT, resulting in approximately 100-fold loss in sensitivity, followed by the accumulation of mutations in gp120, gp41 and gag proteins which allow the virus to escape entry or maturation inhibition, yielding viruses with 1000–10,000-fold resistance. Finally, multiple additional changes occur in RT, resulting in complete resistance. The mutational profile suggests that the compounds act as typical NNRTIs but target a unique conformational structure to inhibit entry requiring interaction of envelope and gag proteins. Each of the resistant viruses have been evaluated for their sensitivity to other nonnucleoside, nucleoside and nucleotide RT inhibitors, entry and fusion inhibitors, and protease inhibitors. Cross-resistance is only detected with other NNRTIs. In light of the ability of the pyrimidinediones to inhibit entry and cell–cell fusion, it is notable that the viruses do not have cross-resistance to Fuzeon. The pyrimidinediones thus represent excellent therapeutic and microbicide development candidates based on the inability of viruses resistant to one of the mechanisms of action to abrogate the activity of the second mechanism. The pyrimidinediones have the potential to replace Sustiva and Fuzeon in current therapy regimens, but with one small molecule with a higher genetic barrier to resistance.

doi:10.1016/j.antiviral.2008.01.064

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Azaindole-based HIV-1 Integrase Specific Inhibitors Display Potent Anti-Retroviral Activity

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We have synthesized and studied a series of compounds that share an azaindole core and additional groups such that the expected conformation would form a two-metal binding motif. We the characterization of the representative compound, PF-00558475, demonstrating that it is a potent and selective inhibitor of the strand transfer activity of HIV-1 Integrase and displays corresponding viral activity in cell culture HIV infections. We present biochemical, antiviral, and mechanistic studies indicating specific inhibitory activity against the HIV-1 Integrase enzyme. In addition, we have carried out resistance selection studies confirming that mutations to the Integrase gene are necessary and sufficient to reduce HIV-1 susceptibility to PF-00558475. Finally, we present results from a panel of clinical isolates indicating that this compound is a broad spectrum inhibitor of HIV-1.

doi:10.1016/j.sviral.2008.01.065

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Establishment of a Cell-based HTS System for Discovery of Anti-Flavivirus Drugs

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Flavivirus infections have become a global public health concern due to the severe nature of disease caused by these viruses and their prevalence in the human population. There is an urgent need for specific antiviral therapies to treat these infections. The viral NS3 serine protease is an attractive target for anti-flavivirus therapy because it is highly conserved and essential for viral replication. We have designed a cell-based high throughput screening system for the identification of small molecule inhibitors of the flavivirus protease. In this system, two expression constructs were generated that constitutively express a bi-cistronic mRNA encoding the viral protease; or a proteolytically inactive mutant protease, and a marker gene (GFP^{CSI}) containing a specific cleavage-site for the viral protease inserted within the GFP. Cleavage at this site by the viral protease results in a loss of GFP fluorescence. Coexpression of NS3 protease of Dengue Virus type 2 with the engineered GFP^{CSI} in 293T cells results in site-specific cleavage of GFPCSI, destabilization of GFPCSI conformation, and reduced fluorescence. The relative fluorescent signal for GFPCSI in cells expressing wild-type NS3 (2.3 MFI) was reduced to $72 \pm 5\%$ of that in cells expressing the active site mutant $NS3^{S135A}$ (3.2 MFI) as measured by flow cytometry. The system has been validated by quantifying the level of inhibition of proteolytic activity in the presence of known protease inhibitors and experimental compounds. In the presence of Aprotinin, a known serine protease inhibitor, the proteolytic inhibition was 67%. Likewise, an inhibitor compound (ST905) identified by high throughput screening that reduced NS3-specific protease activity by 51% at 5 μM showed an 18% inhibition of GFP cleavage in the cell-based assay. Taken together, the cell-based HTS system will facilitate discovery of anti-flavivirus therapeutics.

doi:10.1016/j.antiviral.2008.01.066

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Mevastatin Markedly Potentiates the Anti-HCV Activity of Selective Inhibitors of HCV Replication and Delays or Prevents the Emergence of Antiviral Resistance

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Statinsare 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors that are widely used for the treatment of hypercholesterolemia. Recently, it was reported that certain