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Potent HCV NS5B Polymerase Inhibitors Derived From 5-Hydroxy-3(2H)-Pyridazinones: Part 1 Exploration of Pyridazinone 4-Substituent Variation

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Background: Hepatitis C virus (HCV) is a leading cause of chronic liver disease. Current therapies for genotype 1 HCV are associated with sub-optimal response rates and debilitating side effects. There remains an urgent need for the development of more effective HCV treatments.

Methods: As part of our efforts to discover non-nucleoside small molecule inhibitors of genotype 1 HCV polymerase, we investigated a series of 5-hydroxy-3(2*H*)-pyridazinones using a structure-based design approach. We systematically explored variation of the substituents located at the 2-, 4- and 6-positions on the pyridazinone ring (Fig. 1). A number of the analogs we prepared were found to inhibit the NS5B enzyme with low nanomolar potencies.

Results: Described here are the structure-activity relationships observed by varying the 4-substituent present in our 5-hydroxy-3(2*H*)-pyridazinone NS5B inhibitors. Variation of this substituent has a dramatic impact on NS5B binding affinity. We identified the methylsulfonylamino-substituted benzothiadiazine as among the best 4-substituents and its inclusion into our pyridazinones afforded NS5B inhibitors with low nanomolar potencies against both the HCV polymerase and replicon.

Conclusions: Optimization of the 4-substituent present in a series of 5-hydroxy-3(2*H*)-pyridazinones provided NS5B inhibitors with low nanomolar potencies in both biochemical and HCV replicon assays. These inhibitors generally display reasonable solubility properties. Some analogs exhibit very high liver to plasma ratios after oral administration to Sprague–Dawley rats.

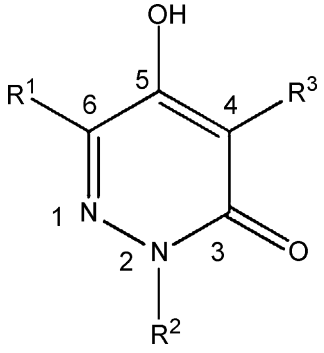

Fig. 1.

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Development of Hepatitis C Virus (HCV) Chimeric Replicons for Identifying Broad Spectrum NS3 Protease Inhibitors

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Background: Given its essential roles in HCV polyprotein processing, replication and host immune evasion, NS3 protease is a prime target for antiviral chemotherapy. However, tremendous genetic diversity exists among HCV isolates and identifying compounds with broad spectrum activity can be challenging. With a limited number of lab strains available for preclinical antiviral testing, new tools are required for predicting clinical dose and efficacy for protease inhibitors (PIs).

Methods: We developed a cell-based chimeric replicon system for evaluating the activity of PIs against diverse natural isolates. NS3/4A genes were cloned from the plasma of patients infected with HCV genotypes 1a, 1b, 2b, 3a and 4. Clonal sequence analysis was performed and the consensus for each clinical isolate was subsequently inserted into existing genotypes 1b and/or 2a lab strain replicons, replacing the native protease sequences. The chimeric replicons were transfected into cells, their replication was monitored, and the susceptibilities of the viable chimeras to HCV PIs were measured.

Results: Most chimeric replicons carrying full-length clinical isolate NS3/4A genes failed to replicate efficiently. In contrast, chimeras carrying only clinical isolate protease domains (N-terminal third of NS3) were replication competent. Viable chimeras expressing genotypes 1a, 1b, 2b, 3a and 4 protease domains were successfully developed and these chimeras exhibited varying degrees of susceptibility to PIs. Consistent with biochemical and clinical results, large peptidomimetic PIs demonstrated potent activity against all genotypes 1a, 1b and 4 clinical isolate chimeras (50% effective concentrations ranging from 5.1 to 750 nM), while genotypes 2b and 3a chimeras were more resistant (50% effective concentrations ranging from 1200 to >32,000 nM). Amino acid identities at NS3 positions 78, 79, 80 and 168 appeared to be key determinants of PI susceptibility.

Conclusions: This novel chimeric replicon system is the first known cell-based assay for characterizing the activities of HCV protease inhibitors against diverse natural isolates and may improve the ability to predict clinical dose and efficacy.

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