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Discovery of a Novel HCV Helicase Inhibitor by a De Novo Drug Design Approach

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Structure-based drug design methods utilize knowledge of a three-dimensional structure of an enzyme/receptor to develop small molecules able to bind to the desired target, generating a specific biological response. These computer-based methodologies are now becoming an integral part of the drug discovery process and, although the principles of molecular recognition are far from being completely understood, some marketed compounds (i.e. influenza neuraminidase inhibitors and HIV protease inhibitors) have been developed with a successful application of structure-based design techniques.

In this presentation we are reporting a successful application of a computer-aided design approach to identify and synthetize a series of novel HCV helicase inhibitors. Initially a putative binding site was identified on the enzyme surface, then a de novo drug design software package was used to generate an initial set of structures that could potentially bind to it. A further structure refinement was carried out by docking a series of virtual libraries derived from the de novo procedure. The best structure identified in silico (AB100) was then prepared and it exhibits a submicromolar inhibition of the HCV helicase. The results of the replicon assay as well as the enzymatic assay for AB100 and a series of related analogues will be also presented.

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PG 301029 Inhibits HCV Replication Through a Novel Late Stage Mechanism of Action

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PG 301029 was identified from a large structure–activity relationship evaluation as a highly effective small molecule inhibitor of the replication of the hepatitis C virus. Primary screening was performed using bovine viral diarrhea virus (BVDV), and the activity was confirmed using the HCV replicon system. Subsequent IND-directed development of the compound has demonstrated a novel mechanism of antiviral action of PG 301029 and the possibility of using the compound in combination with current therapies. PG 301029 exhibited an EC_{50} value of 0.65 μg/mL against BVDV, was 100–200-fold less toxic than ribavirin to cells infected with BVDV, and was nontoxic to fresh human hepatocytes at the highest concentration tested (316 μg/mL). Mechanism of action studies revealed that PG 301029 inhibited BVDV replication through suppression of viral RNA synthesis that was not related to inhibition of virus entry, translation initiation, or inhibition of the NS2/3 viral proteinase. Additionally, in combination anti-BVDV assays, the addition of PG 301029 to ribavirin or ribavirin plus interferon yielded synergistic anti-HCV activity and also resulted in a reduction of the toxicity of ribavirin. PG 301029 is efficacious in an HCV replicon system, yielding an EC_{50} concentration of 0.38 μg/mL. We hypothesize that PG 301029 inhibits HCV replication through a novel late stage mechanism of action which results in significant reductions in the accumulation of viral RNA in infected cells. Confirmation of the antiviral mechanism of action has been performed using the HCV replicon system as well as from the selection and characterization of PG 301029-resistant HCV replicons. Additionally we have examined the effect of PG 301029 on HCV protein processing and stability, on viral RNA synthesis and stability, and on the differential expression of IFN-regulated genes in treated Huh-7 cells and Huh-7 cells transfected with HCV replicons. Results of *in vitro* combination studies for the inhibition of HCV replicons by PG 301029 with ribavirin and IFN-α will also be presented. PG 301029 exhibits a novel antiviral mechanism of action distinct from that of existing anti-HCV therapies and clinical use of PG 301029 in combination with existing agents may provide significant therapeutic benefits.

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Different Incorporation Efficiencies for Nucleotide Analogs During HCV Polymerase RNA Synthesis Initiation and Elongation Phases

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Background: Most antiviral nucleotide analogs function as alternative substrates for the viral polymerase and result in chain termination after incorporation. Potential differences in incorporation efficiency relative to natural NTPs and the effect of the S282T resistance mutation during initiation and elongation phases of RNA synthesis by HCV polymerase are not well understood.

Methods: Gel-based assays were developed to determine the kinetic parameters of nucleotide incorporation by NS5B wild-type and S282T mutant NS5B at initiation or elongation phase. Incorporation kinetics were determined for nucleotide analogs including development candidates R1479-TP, NM107-TP, RO-9187-TP and RO-0622-TP.

Results: The elongation complex of NS5B showed significantly higher incorporation efficiency of the natural CTP substrate as compared to the initiation complex. The elongation complex was not inhibited by non-nucleoside inhibitors of HCV polymerase or by heparin. In contrast, nucleotide analogs were incorporated during initiation and elongation phases of RNA synthesis and could inhibit RNA synthesis during both phases. Among the CTP analogs tested, 4′-azido-CTP (R1479-TP) was the most efficient alternative substrate for NS5B during both initiation and