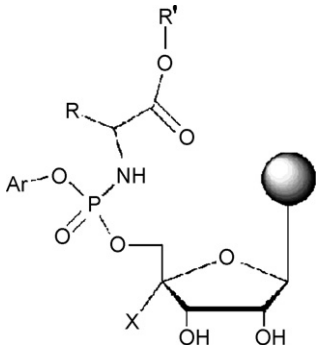

Fig. 1.

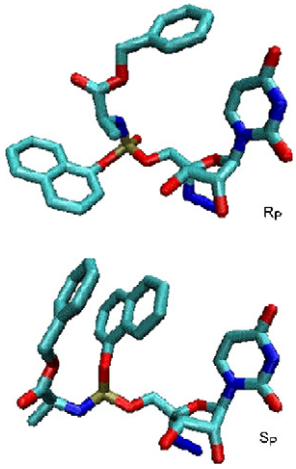

Fig. 2.

tive in the assay. In some cases, we were able to separate, and separately evaluate the phosphate stereoisomers generated in the synthesis; in a few cases the absolute phosphate stereochemistry was solved (Fig. 2). The generic message is that ProTide synthesis from inactive parent nucleosides may be a warranted drug discovery strategy.

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GL59728: A Potent Allosteric Inhibitor of the HCV NS5b RNA Dependent RNA Polymerase with Excellent Pharmacokinetic Properties

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The HCV RNA dependent RNA polymerase NS5b is a virally encoded enzyme responsible for HCV RNA replication and is essential for viral replication. A class of non-nucleoside compounds that target an allosteric site in the finger-loop region of the polymerase was identified. Multiple rounds of optimization led to compounds with nanomolar potencies against both the purified NS5b enzyme and the subgenomic HCV replicon. In vivo DMPK profiling and optimization led to GL59728, which displays excellent cross-species pharmacokinetic properties. Finally, GL59728 displays outstanding in vitro antiviral efficacy when combined with other classes of potential anti-HCV agents.

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Substituted Imidazopyridines as Potent Inhibitors of Hepatitis C Virus Replication that Target the Viral Polymerase

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Following lead optimization, a series of substituted imidazopyridines was identified as potent and selective inhibitors of in vitro HCV replication, as was determined using various HCV subgenomic replicon systems. The activity was also assessed in hepatoma cells infected with an infectious full-length chimeric HCV construct. The particular characteristics and mechanism of action of one of the most potent congeners in this series (GS-327073) was studied. Typically, 50% effective concentrations (EC_{50}) for inhibition of replicon or viral replication ranged between 2.4 and 22 nM; 50% cytostatic concentrations (CC_{50}) were $\geq$16 μM, thus resulting in selectivity indices of >800 to >8000. GS-327073 remained active against HCV replicons that were resistant to various HCV polymerase or protease inhibitors. When GS-327073 was combined with either interferon 2α or several polymerase or protease inhibitors, an additive antiviral activity was obtained. Several months of selective in vitro pressure with GS-327073 were required before drug resistant variants were obtained. Genotyping of the GS-327073res replicons resulted in the identification of mutations C316C/Y, C445F and Y452H in the polymerase gene. Transfection of naïve cells with RNA isolated from GS-327073res replicons transferred drug resistance, indicating that resistance is associated with the viral genome and not with the host cell. Reintroduction of the mutations in wild-type replicons resulted in a drug-resistant phenotype, whereby the total number of mutations correlates with the degree of resistance. Although the viral polymerase is obviously the target for the antiviral activity, GS327073 was not able to inhibit the activity of recombinant HCV polymerase in initiation or elongation assays. Further studies are ongoing to determine by which mechanism the compound inhibits the viral polymerase in infected cells.

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