nism of RdRp activity regulation by NS5A was observed for soluble recombinant proteins. In contrast, in infected cells both NS5A and NS5B are bound to ER membrane and lipid rafts by their membrane associating domains and cellular partners, and these interactions might change their orientation or alter position of NS5A C-terminal region not allowing the latter to block RdRp.

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## Comparison of Various Combination Therapies for the Treatment of Yellow Fever Virus

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Yellow fever virus (YFV) continues to be an important health concern, despite the availability of an effective vaccine. There are no approved drugs for the treatment of this acute viral disease, which can have case fatality rates from 20% to 50% in individuals with visceral disease. Several compounds effective against YFV have been discovered using a hamster model of disease, including ribavirin, T-1106, T-705, and interferon alfacon-1. Human cases of acute arboviral disease are likely to present once serious symptoms are manifest, so it is important to have a safe and highly efficacious treatment available for immediate use. Our approach to this problem is the use of combination therapy. Combination treatments were evaluated in cell culture and in a hamster model. Treatment with T-1106, T-705, or ribavirin in combination with interferon alfacon-1 was evaluated in Vero cells at 2-fold dilutions of compound with starting doses of 4000 μM for T-1106, T-705, and ribavirin, and 0.0032 μg/ml for interferon alfacon-1. Combinations were further evaluated in a hamster model of YFV. Suboptimal doses of 3.2, 100, and 10 mg/(kg d) of T-1106, T-705, and ribavirin, respectively, were evaluated alone or in combination with 0.5 μg/(kg d) of interferon alfacon-1. In general, combination therapy significantly improved disease parameters as compared with monotherapy. Disease parameters improved after combination therapy included survival, virus titer in the liver, serum aminotransferase levels, and weight change. In some instances, treatment could also be delayed later with combination therapy than with monotherapy. It appears that combination therapy may be useful in the treatment of human cases of YFV disease, and may also be applicable to other acute arboviral diseases.

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## A New Series of Tricyclic Nucleosides as Potent Inhibitors of Hepatitis C Virus RNA Replication: Design, Synthesis and Structure–Activity Relationships

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From our extensive investigation of 7-deaza-7-substituted-2′-methyladenosine nucleosides, we envisioned the potential synthesis of tricyclic nucleosides that incorporate the active substituents of the 7-deaza position of the base. From this effort, we identified the potent anti-HCV tricyclic nucleoside GL60630, which could be viewed as a cyclized derivative of 2′-C-methylsangivamycin. This compound was characterized as a potent and selective HCV NS5B RNA-dependent RNA polymerase chain terminating inhibitor of HCV replication. GL60630 demonstrated an $EC_{50}$ of 0.5 μM in the replicon cell-based assay and an $IC_{50}$ of 0.32 μM in the NS5B enzyme assay as its corresponding synthetic triphosphate. No concomitant cytotoxicity was observed in Huh-7, MT-4 or HepG2 cell lines. We synthesized multiple analogs of this tricyclic scaffold and found a number of nucleosides that possess anti-HCV activity as the parent nucleoside or as its corresponding nucleotide. The synthesis and SAR of the analog series will be presented.

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## Phosphoramidate Protides Greatly Enhance the Anti-HCV Activity of 2′-Methylguanosine

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2′-C-Methyl purines are recognised inhibitors of HCV replication (Eldrup et al., 2004). As with most bioactive nucleosides their active form is represented by the corresponding 5′-triphosphates, which may inhibit the RNA dependant RNA polymerase (RdRp). From the literature, 2′-C-methyladenosine showed good in vitro activity (replicon $EC_{50}$ = 0.26 μM) while 2′-C-methylguanosine showed an approximately 10-fold lower potency ($EC_{50}$ = 3.5 μM). We hypothesised that this difference may arise from poor initial phosphorylation of the guanine analogue that may be by-passed using the ProTide approach (Perrone et al., 2007). Data will be presented showing a 2-log enhancement in the potency of the guanine analogue.

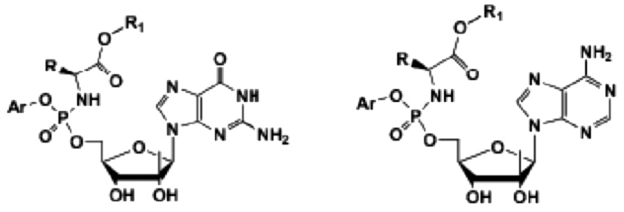

**Figure1.** Structure of 2'-C-Methylguanosine and 2'-C-Methyladenosine phosphoramidates.

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## Use of the BelloCell System to Determine the Optimal Dose of Ribavirin to Inhibit the Expression of an HCV Replicon in 2209-23 Cells

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**Introduction:** To use antiviral compounds effectively for the treatment of hepatitis C virus (HCV) infections in man, one must know the optimal dose and schedule of administration of compounds that will inhibit virus replication without leading to the emergence of resistant viruses. We used the BelloCell system to determine the optimal dose of ribavirin that will inhibit the in vitro expression of an HCV replicon in 2209-23 cells.

**Methods:** HCV replicon containing 2209-23 cells were obtained from Roche Pharmaceuticals Inc., Palo Alto, CA. The replicon contains a neomycin gene for its detection and quantitation by RT-PCR and a luciferase gene for its detection and quantitation using the *Renillin* Luciferase Assay. To determine a dose response of ribavirin, $10^8$ 2209-23 cells were added to each of four BelloCell systems containing DMEM supplemented with 10% fetal bovine serum. The systems were incubated at 37 °C, 5% $CO_2$ for 7–10 days. One unit was continuously infused with medium without ribavirin, the others contained either 10, 20 or 40 μM ribavirin. Fresh ribavirin was added daily. PK/PD analysis of the concentration of ribavirin in each unit was determined daily by LC/MS/MS. Each day, 20 flakes were taken from each BelloCell unit, the cells were removed with trypsin/EDTA, counted, normalized to 5000 cells and the luciferase activity in these cells was determined using a luminonometer. The HCV replicons in the remainder of the cells were quantified by kinetic RT-PCR.

**Results:** The $EC_{50}$ value for ribavirin for this replicon system is about 20 μM when determined in the standard 96 well format. A similar $EC_{50}$ value was found when these replicon containing cells were treated with various concentrations of ribavirin in the BelloCell system.

**Conclusion:** The results of this proof-of-principle study suggests that the BelloCell system can be used to determine the dose of antiviral compounds effective against HCV. With knowledge of the $EC_{50}$ values, the BelloCell system could be used to perform a dose fractionation assay to determine the pharmacodynamically linked variable for antiviral compounds effective against hepatitis C virus.

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## Silibinin (Legalon-SIL) Inhibits HCV Replication In Vitro

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**Background:** Intravenous Legalon-silibinin (SIL) is a potent antiviral agent in HCV infected persons. SIL monotherapy at 20 mg/kg resulted in significant viral load reduction in prior non-responders to pegylated interferon and ribavirin therapy (VL reduction of $3.0 \pm 1.0$ log IU/ml after 7 days). Antiviral potency of SIL was further increased in combination with pegylated interferon and ribavirin (VL reduction of $4.8 \pm 0.9$ log IU/ml after 7 days). The mechanism of action of SIL remained unresolved.

**Methods:** We determined the inhibitory activity of SIL and structurally related analogs in a number of HCV in vitro assay systems including subgenomic replicon, GT-1a infectious virus replication (HCVcc), HCV entry (HCVpp), RNA polymerase, protease and helicase. In addition, in vitro selection of SIL resistant replicons was performed.

**Results:** SIL inhibited HCV replication in the genotype 1a and 1b replicon systems with similar potency and $IC_{50}$ values in the low micromolar range. SIL was not cytotoxic ($CC_{50} > 100$ μM) or cytostatic (3H-Thy incorporation $IC_{50} > 100$ μM) at these concentrations. SIL also inhibited HCV replication in the GT 1a infectious virus assay (H77-HCVcc), but did not inhibit HCV entry in either the genotype 1a or 1b cell HCVpp systems, consistent with SIL targeting HCV replication. Among the HCV replication targets, HCV protease and helicase activity were not affected by SIL, whereas RNA-dependent RNA polymerase NS5B was moderately sensitive to inhibition by SIL ($IC_{50} = 50 \pm 7.6$ μM). SIL interfered with NS5B–RNA interaction, whereas preformed NS5B–RNA complexes were resistant to inhibition by SIL. Characterization of SIL analogs suggested structural features important for HCV replication inhibition. Results from the in vitro selection of SIL resistant replicon variants will be available and discussed at the time of presentation.

**Conclusions:** HCV replication was identified as a target of SIL. The ability of SIL to inhibit HCV RNA synthesis by HCV polymerase NS5B was consistent with inhibition of HCV replicon and HCV infectious virus replication in vitro, whereas HCV entry was not affected by the presence of SIL.

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## Therapeutic Efficacy of the Amphipathic DNA Polymer REP 9AC in the Treatment of Duck Hepatitis B Virus Infection In Vivo

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The amphipathic DNA polymer REP 9AC has demonstrated potent antiviral activity in vitro against DHBV during viral entry and post-entry. A large amphipathic domain related to those targeted by these compounds in type 1 virus fusion proteins is likely targeted by REP 9AC in DHBV. The therapeutic potential of REP 9AC for the treatment of HBV infection in humans was assessed using DHBV-infected Pekin ducks as a surrogate model. REP 9AC (10 mg/kg) was administered 1 day prior to, or from 4 or 14 days