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.

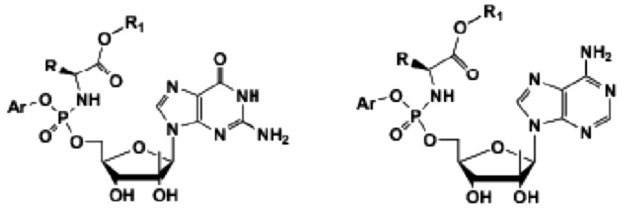

Figure1. Structure of 2'-C-Methylguanosine and 2'-C-Methyladenosine phosphoramidates.