

Pharmacological Properties and *In Vitro* Characterization of INX-189, a Liver Targeted Phosphoramidate Nucleoside Analogue Inhibitor of NS5b

#1611

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Introduction

2'-C-methyl guanosine has been shown to possess activity against HCV in the replicon, however its cellular uptake and intracellular metabolism to its pharmacologically active antiviral triphosphate are inefficient, thereby limiting its potential as a therapeutically useful compound. To overcome these biochemical limitations we have utilized an aryloxy-phosphoramidate approach to modify the 2'-substituted nucleoside analogue. INX-189 has been selected as the clinical candidate from this medicinal chemistry program.

Methods

The antiviral activity of INX-189 was determined using the genotype 1b subgenomic replicon assays. Cell cytotoxicity was measured using the CellTiter-Glo luminescent assay. Mitochondrial genome copy number was quantitated by Q-PCR in human lymphoblastic leukemic cells (CEM). Carcinogenicity was evaluated with the AMES II test. The levels of intracellular 2'-C-MeGTP produced in primary, plated hepatocytes was measured using LC-MS/MS. The pharmacokinetics of INX-189 was evaluated in rats and monkeys following 14 days of oral dosing. The levels of intracellular 2'-C-methyl guanosine triphosphate in liver samples and plasma concentrations of 2'-C-methyl guanosine were measured using LC-MS/MS.

Results

INX-189 exhibited potent anti-HCV activity with a EC_{50} and EC_{90} of 10 nM and 40 nM, respectively. 14 day treatment of replicon cells with $2X EC_{50}$ of INX-189 resulted in complete clearance of the replicon. Combination of INX-189 with ribavirin was highly synergistic. INX-189 \pm S9 activation was negative in the AMES II test. Conversion of INX-189 in hepatocytes to the triphosphate was rapid and the level of the NTP at 24 hrs was 52 pmol/ 10^6 cells, representing approximately a 27-fold increase over the EC_{90} and a 26 hour half-life. INX-189 did not reduce the mitochondrial copy number in CEM cells after continuous treatment for 14 days at 0.1, 0.5, or 1 μ M. Pharmacodynamic studies in rats and primates indicated that levels of the triphosphate exceeding the EC_{90} were achieved for ≥ 24 hours post oral dosing at human equivalent doses of 100 mg.

Figure 1: 14 Days of Treatment with INX-189 Clears Replicon

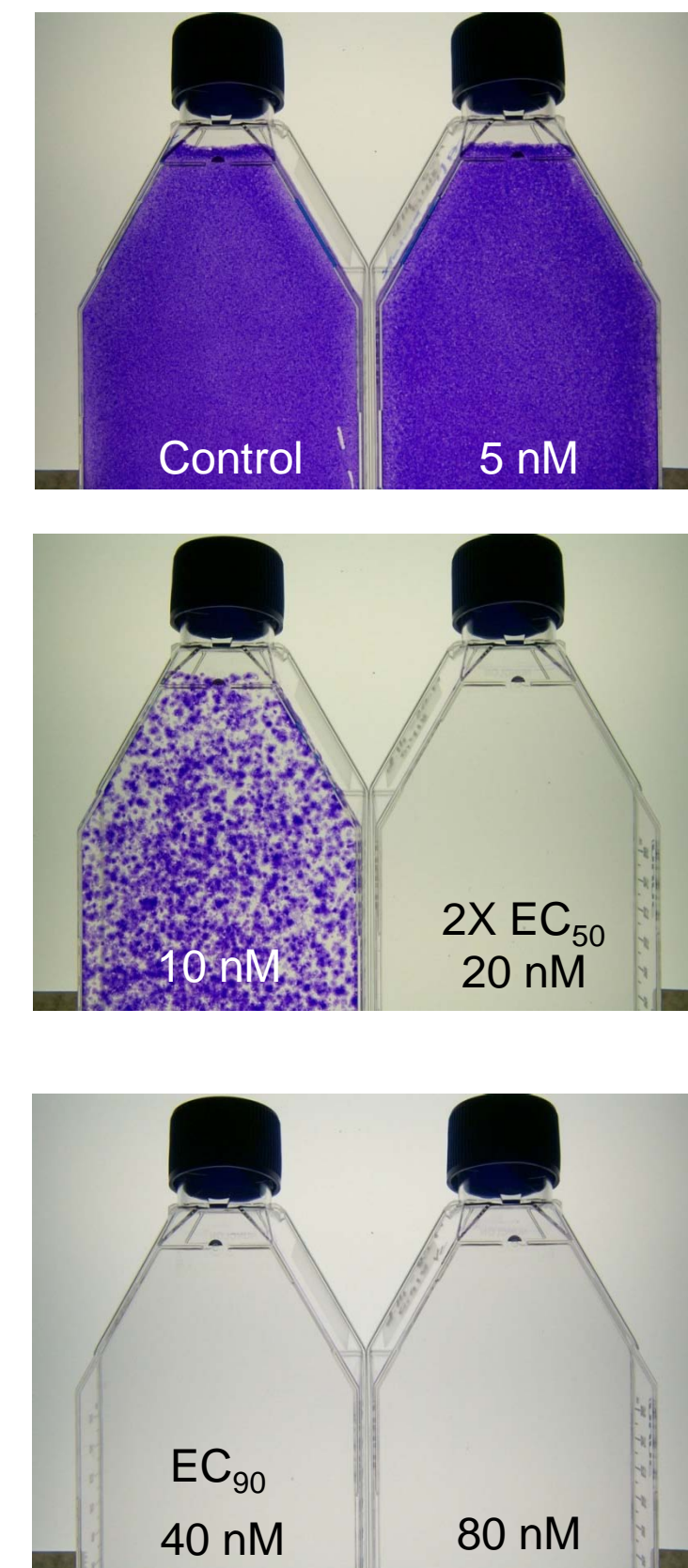


Figure 2: INX-189 is Synergistic with Ribavirin

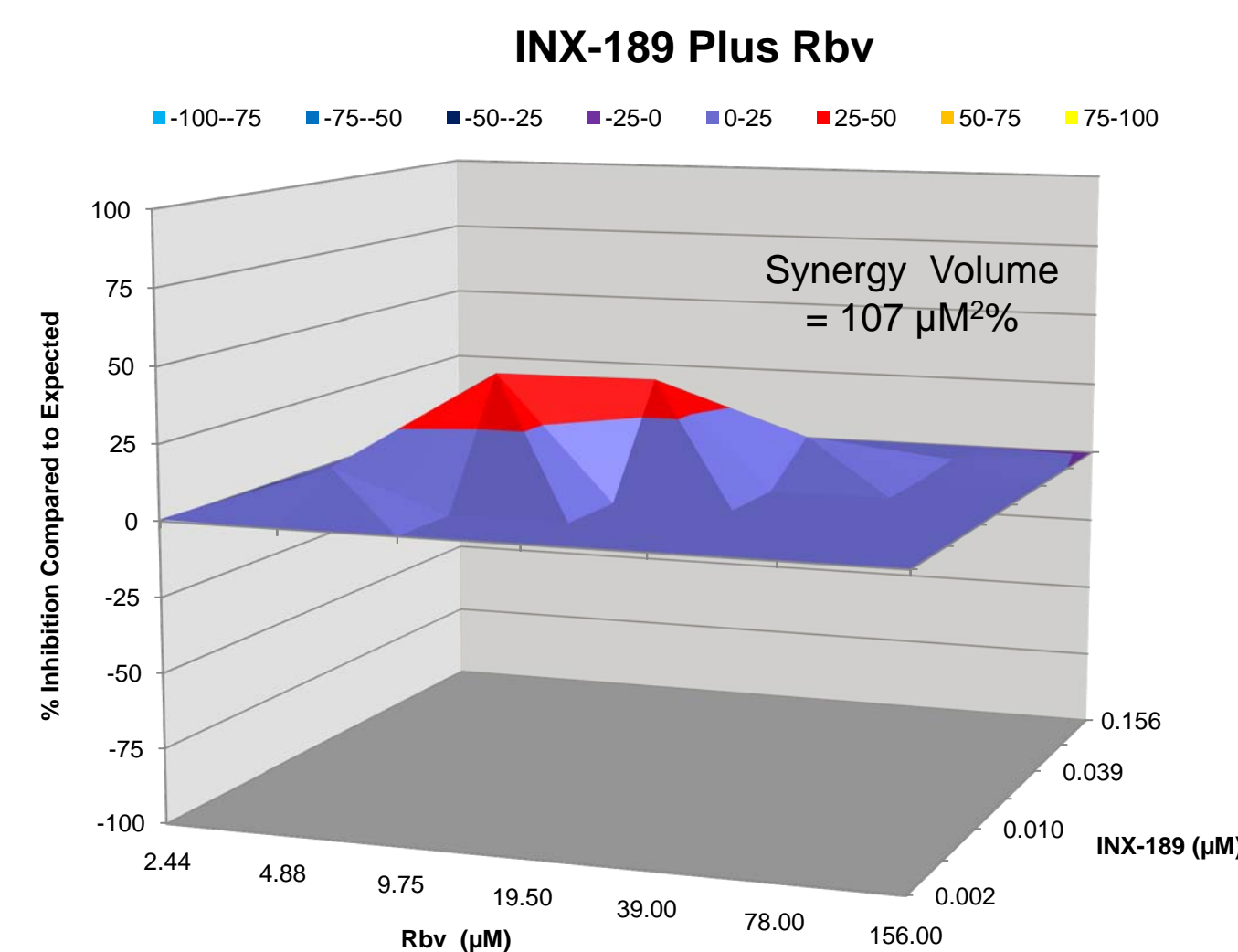


Figure 3: Mitochondrial Toxicity Study

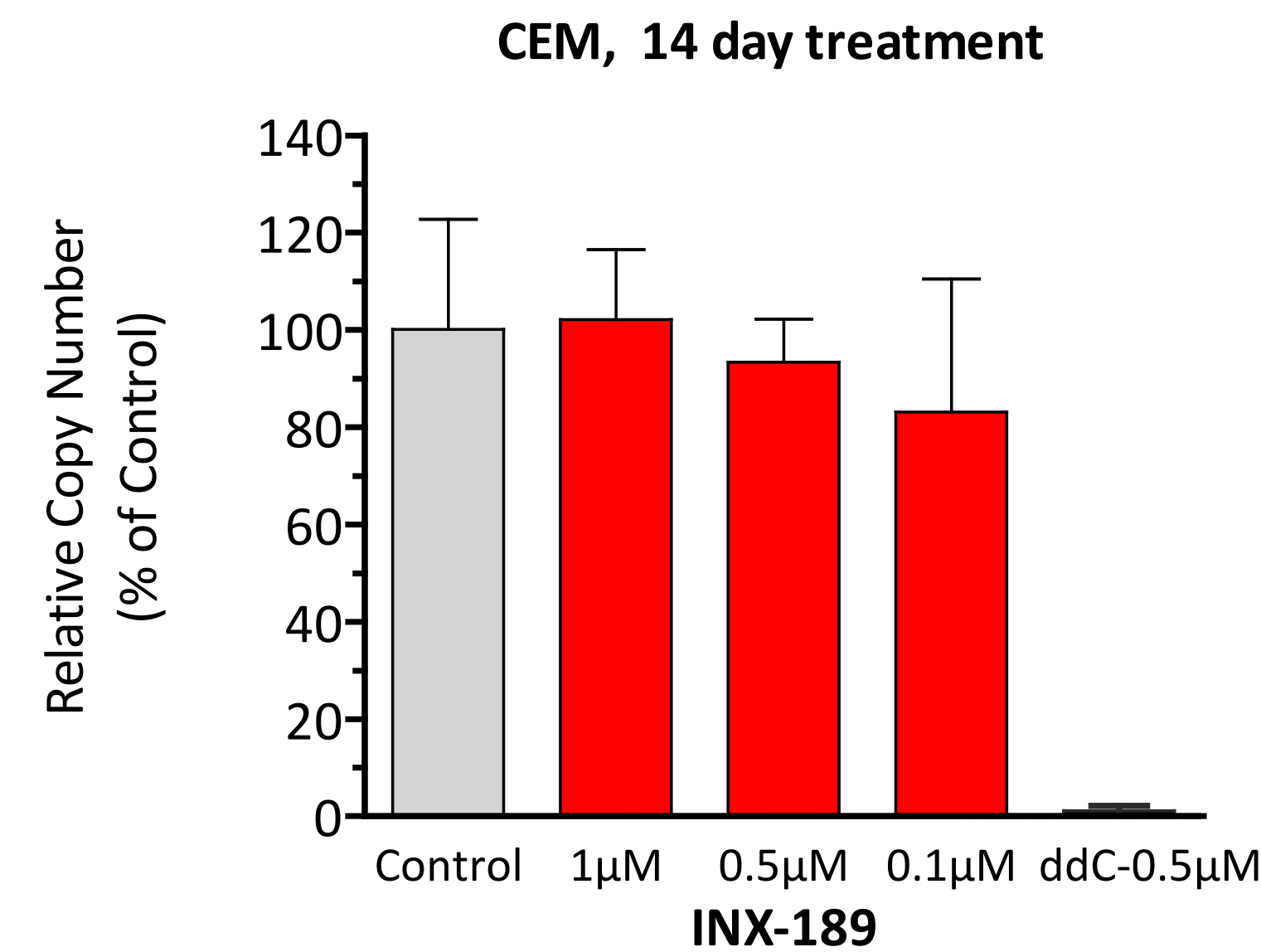


Figure 4: 14-Day Oral Dosing Pharmacodynamics in Rats

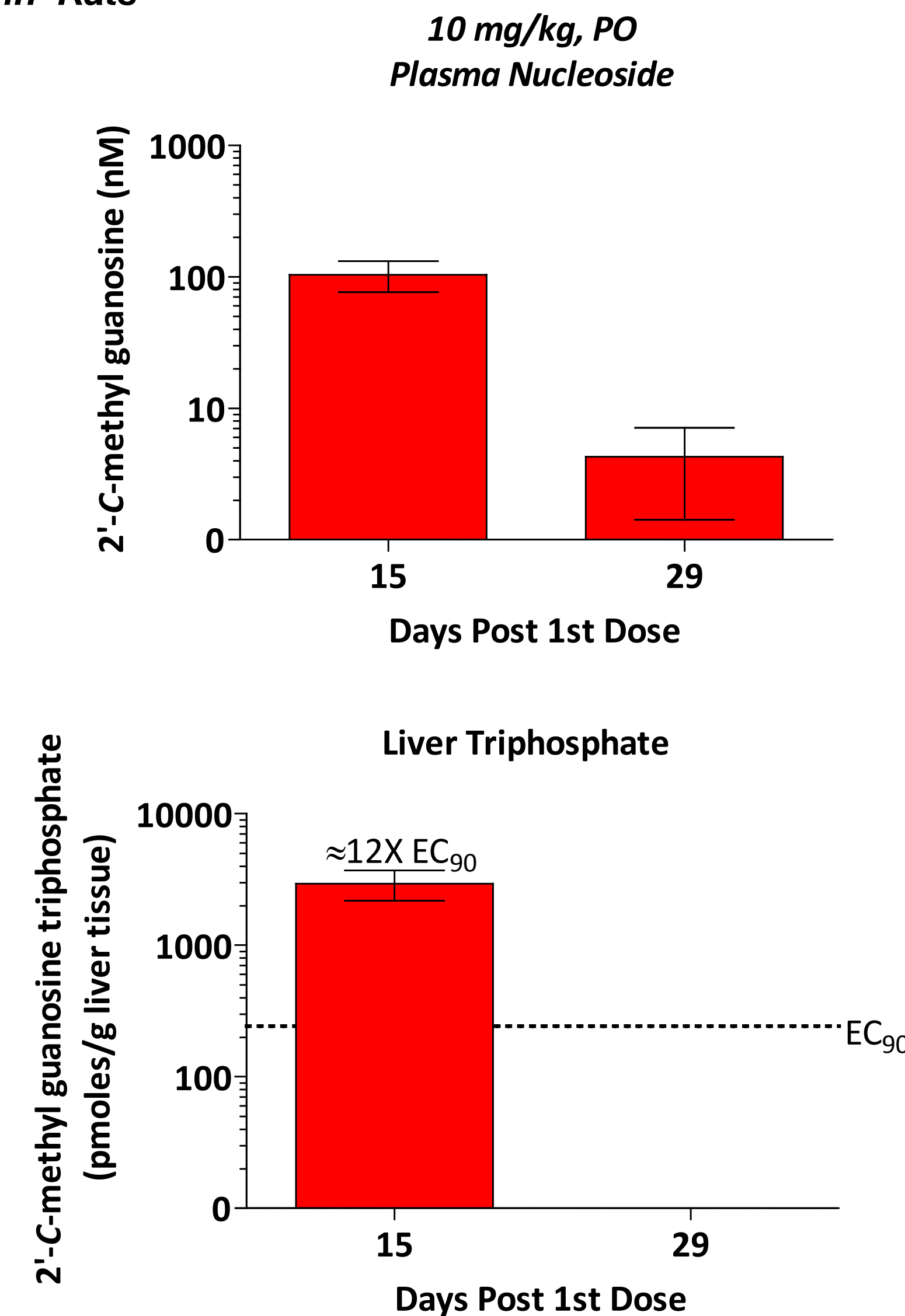
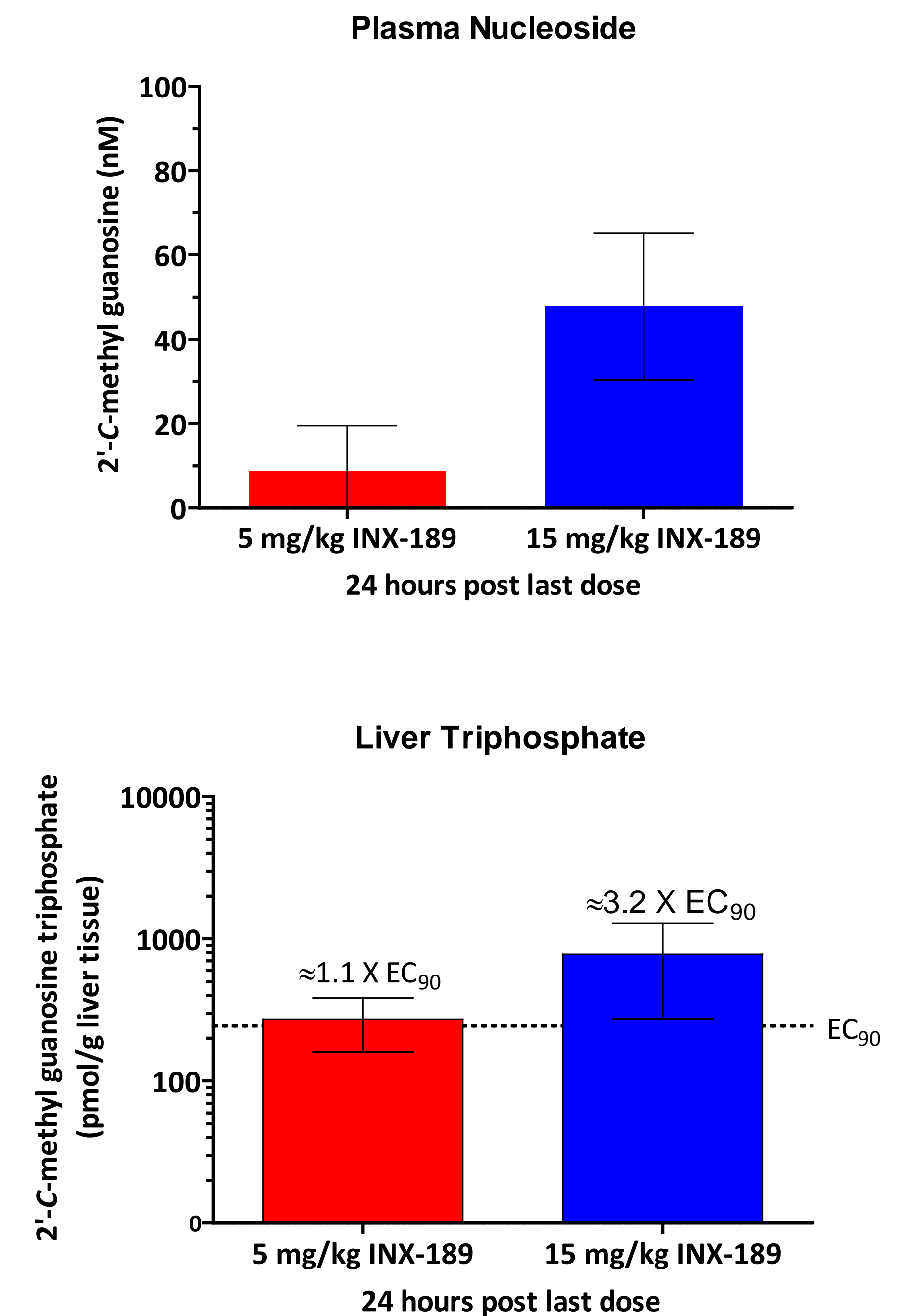


Figure 5: 14-Day Oral Dosing Pharmacodynamics in Non-Human Primates



Conclusions

INX-189 demonstrated potent HCV antiviral activity in cell based assays, a favorable therapeutic index, and extensive delivery of the triphosphate to the liver of orally dosed rats and primates. Taken together, these data support the selection of INX-189 as a candidate for clinical studies.