

Metabolite Characterization of INX-189, a Potent HCV Inhibitor, in Fresh Human Primary Hepatocytes and Human Liver Cell Lines

#1874

Andrea Hall¹, Stanley Chamberlain¹, Babita Ganguly¹, Elena Gorovits¹, Geoffrey Henson¹, Jeff Hutchins¹, Jerry Muhammad¹, Joseph Patti¹, Nicholas Raja¹, John Vernachio¹, Jin Wang¹,

Karolina Madela², Mohamed Aljarah², Sarah Jones², Arnaud Gilles² and Christopher McGuigan²

¹Inhibitex, Inc., Alpharetta, Georgia, USA; ²Welsh School of Pharmacy, Cardiff University, Cardiff, UK

Introduction

INX-189 is a novel potent phosphoramidate based pro-drug of an O6-methyl modified 2'-C-Methyl guanosine monophosphate currently in clinical development for the treatment of HCV. The phosphoramidate is designed to deliver the monophosphate form of the nucleotide intracellularly, by-passing the first rate-limiting phosphorylation step and facilitating rapid conversion to the triphosphate. The O6-methyl is designed to improve uptake of INX-189 into cells. The aim of this study was to identify and quantitate key metabolites generated *in vitro* after incubation of this novel double prodrug in human hepatocytes and other human liver cells using established analytical methods.

Methods

Fresh plated hepatocytes from human donors and cell lines such as human liver cells (Huh7) were incubated at 37°C in the presence of 10 µM INX-189. The cell supernatant was removed and intracellular contents of cells were extracted overnight in 70% methanol. The intracellular and extracellular metabolite intermediates were detected and quantified using LC-MS/MS methodology and synthesized analytical standards.

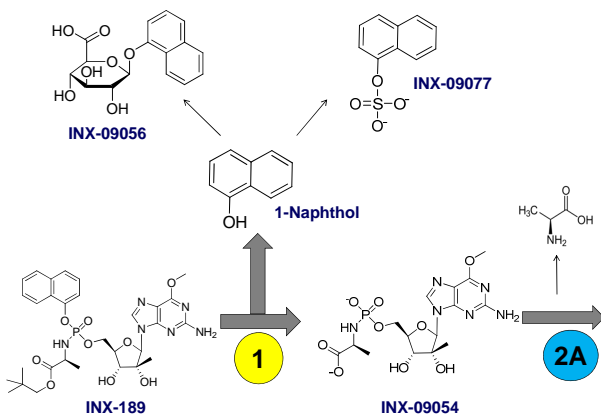
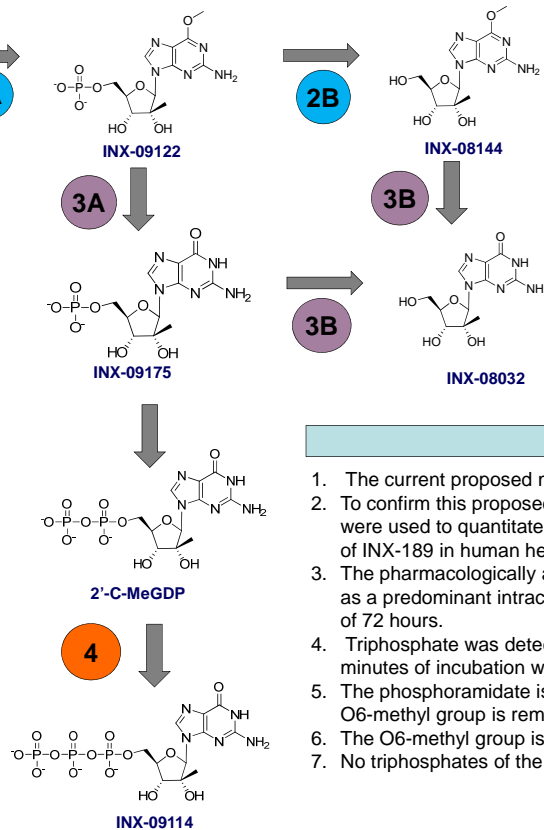
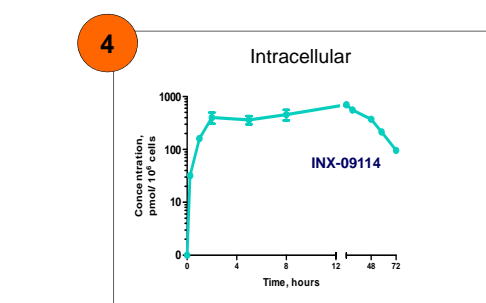
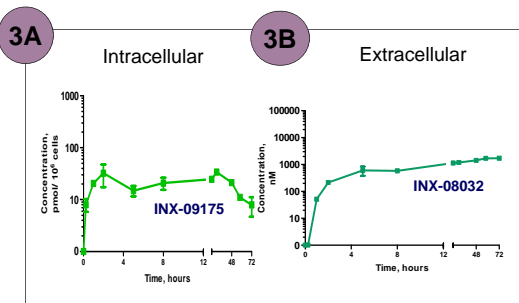
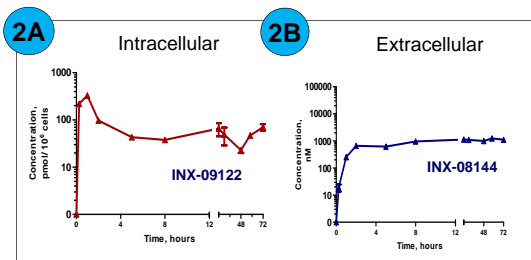
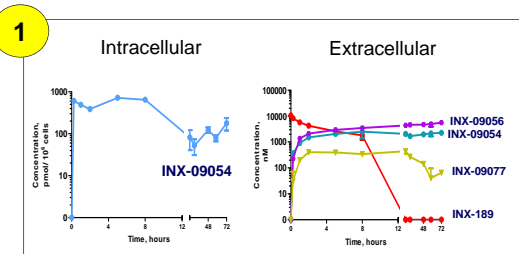


Table 1: Intracellular Triphosphate Levels in Human Hepatocytes and a Human Liver Cell Line

INX-09114 (2'-C-Methyl GTP) Levels (pmol/10 ⁶ cells)	
Primary Human Hepatocytes	761
Huh7 (Liver Cell Line)	455

Table 2: Summary of INX-189 Metabolite Exposure

Metabolite	Intracellular AUC (pmol-h/1.0x10 ⁶ cells)	Extracellular AUC (nM-h)
INX-09056	ND	315123
INX-09077	ND	16442
INX-09054	15175	145281
INX-09122	3732	ND
INX-09175	1496	ND
INX-08144	ND	74567
INX-08032	ND	87316
INX-09114	29245	ND



Conclusions

1. The current proposed metabolic pathway of INX-189 is shown.
2. To confirm this proposed pathway, LC-MS/MS analytical methods were used to quantitate the key intermediates in *in vitro* metabolism of INX-189 in human hepatocytes and human liver cell lines.
3. The pharmacologically active triphosphate INX-09114 was identified as a predominant intracellular metabolite throughout the time course of 72 hours.
4. Triphosphate was detected early in the time course (within 15 minutes of incubation with cells) and peaked within 24 hours.
5. The phosphoramidate is cleaved to the mono-phosphate before the O6-methyl group is removed
6. The O6-methyl group is removed at the monophosphate stage
7. No triphosphates of the O6-methylated nucleoside were observed