

Discovery and Preclinical Characterization of Novel Inhibitors of the HCV NS3/4 Protease

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ABSTRACT

BACKGROUND: Current standard of care for chronic hepatitis C patients with genotype 1, peg-IFN- α -2 and ribavirin (RBV), results in a sustained virologic response in <50% of patients. Thus, a need exists for the development of novel therapeutic approaches. The HCV NS3/4 protease represents an attractive drug target because it (i) is required for viral replication and (ii) diminishes the host response to viral invasion (via a block to Interferon Regulatory Factor-3 [IRF-3] signaling).

METHODS: The 3-D structure of the HCV NS3/4 protease was used to design inhibitors in a structure-activity relation approach. Compounds were synthesized and tested for potency (EC₅₀) in a fluorogenic NS3/4 protease assay and cell-based HCV replication system. Plasma pharmacokinetic analysis in *Rattus* sp. after IV and oral administration was then used in conjunction with in vitro human liver microsome (HLM) and hepatocyte stability studies to direct further optimization to metabolically stable compounds with <20 nM potency. These leads were then administered in oral doses in *Rattus* sp. to assess liver, heart, and plasma concentrations. Toxicologic assessment of compounds with liver concentrations >100-fold in excess of their replicon EC₅₀ 8 hours after a single 3 mg/kg oral dose were performed at up to a 30 mg/kg BID dose for 7 days.

RESULTS: Several lead compounds were identified with replicon EC₅₀ values of ~2 nM and good in vitro stability following incubation in hepatocytes. These compounds display a high degree of selectivity against a panel of other serine proteases, and no significant inhibition of cytochrome P450 isoforms or hERG channel activity at the highest concentrations tested (10 μ M). Oral dosing in *Rattus* sp. (single 30 mg/kg dose) of these compounds yield 24 hours postdose liver concentrations >200-fold in excess of their replicon EC₅₀ values. Heart and plasma levels of compound from the same animals are up to two orders of magnitude lower than, and correlated with, liver concentrations. At a dose of 3 mg/kg these compounds afford liver concentrations more than 100-fold over their respective replicon EC₅₀ levels 8 hours postdose. On 7-day dosing at 30 mg/kg BID, no mortality, change in weight, or abnormalities in clinical chemistries were observed for the two compounds run in this model.

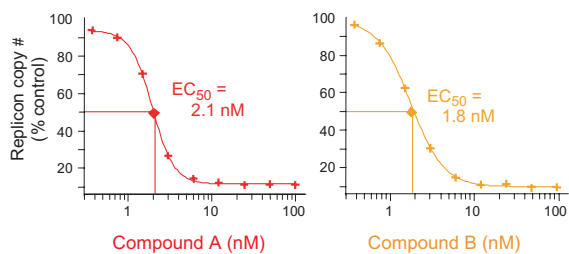
CONCLUSION: We have developed potent, metabolically stable, orally available small molecule inhibitors of the HCV NS3/4 protease. Exposure to plasma and heart is up to two orders of magnitude below that observed in liver, and such low concentrations minimize any potential systemic or cardiac toxicity. These compounds do not display toxicity in *Rattus* sp. when dosed for 7 days at 30 mg/kg BID. These potent compounds have the potential to be used in the treatment of HCV and further study is warranted.

SUMMARY

- Five novel compound estates containing examples with replicon potencies <10 nM
- Extensive knowledge of structure-activity relationships developed
- Lead compounds in final stage of discovery
- Additional compounds provide backups/best-in-class molecules

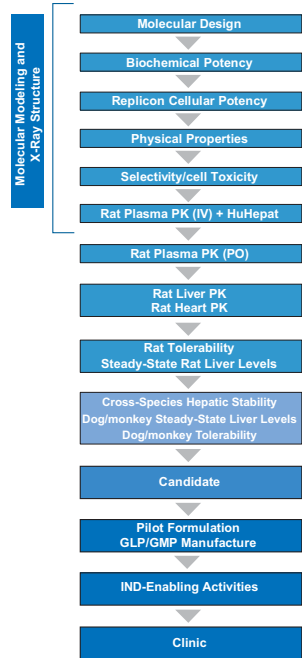
IN VITRO POTENCY

Lead Compounds Are Highly Potent in Replicon Assays



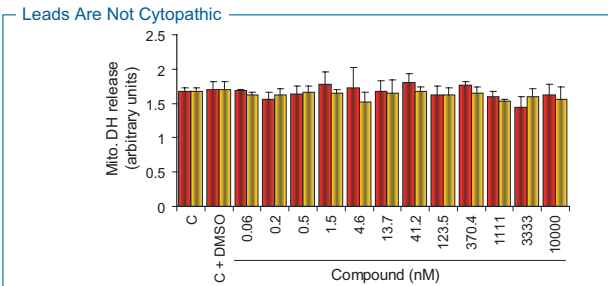
- Patient isolated genotype 1b replicon potencies ~2 nM

DISCOVERY PARADIGM

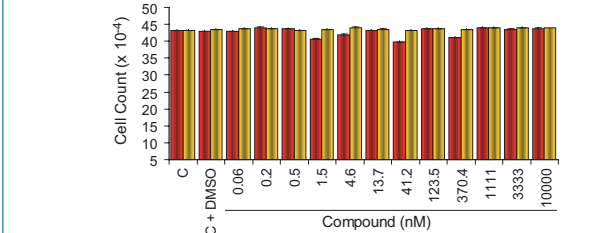


IN VITRO SAFETY

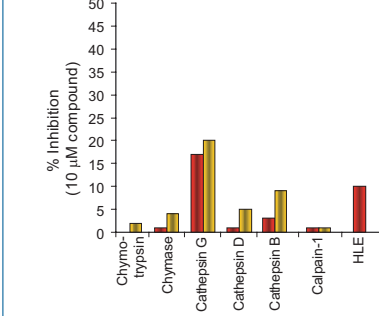
Compounds Display a Promising Profile



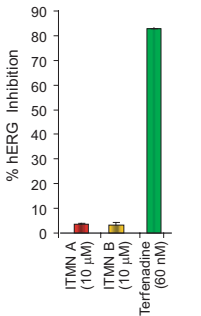
Leads Display No Antiproliferative Effect



No Appreciable Off-Target Effects



No hERG Activity



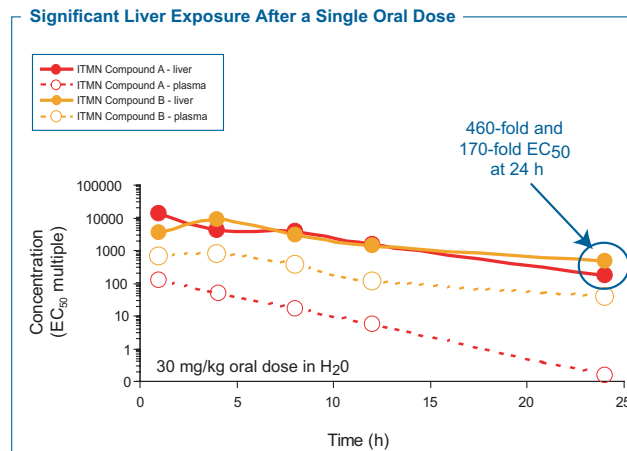
Negligible Inhibition of Cytochrome Isoforms

Compound	1A2	2C19	2C9	2D6	3A4	Replicon EC ₅₀ (nM)	Index
ITMN A	25,000	25,000	25,000	25,000	9,300	2.1	4,428
ITMN B	25,000	25,000	25,000	25,000	5,400	1.8	3,000

- No cytopathic effects observed
- Protease selectivity is high
- No cytochrome P450-related drug-drug interactions expected
- No hERG-related cardiotoxicity expected

PHARMACOKINETICS

Low Doses Afford High Liver Concentrations in Rat



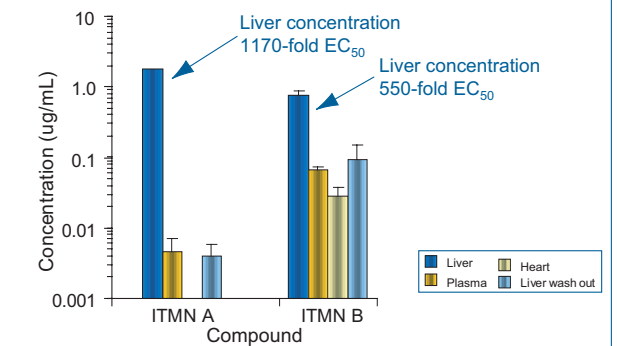
Methods
Male Sprague Dawley rats (n = 3/dose/timepoint)
Time points: 1, 4, 8, 12, 24 h
Blood samples collected, into sodium citrate, via cardiac puncture and processed to plasma
Animals perfused via the left ventricle with heparinized saline
Liver and heart flash frozen in liquid N₂ and stored at -20°C until analysis
Plasma and tissue samples analyzed using a qualified LC/MS/MS method

Plasma Pharmacokinetics After a Single IV Dose

Compound	Dose (mg/kg)	C _{max} (μg/mL)	AUC ₀₋₁ (μg h/mL)	t _{1/2} (h)	C _{obs} (mL/min/kg)	V _d (mL/kg)	V _{dss} (mL/kg)
ITMN A	1.0	0.7	0.18	0.9	90.5	6,990	2,580
ITMN B	1.0	11.1	3.44	1.4	4.8	602	156

Methods
Male Sprague Dawley rats (n = 3/dose)
Time points: 5, 15, 30 min; 1, 2, 4, 8, 12, 24 h
Blood samples collected, into sodium citrate, via tail vein withdrawal or cardiac puncture (terminal time point) and processed to plasma
Plasma samples analyzed using a qualified LC/MS/MS method

Low Doses Afford High Liver Concentrations in Multidose Regimes (3 mg/kg twice daily)



Methods
Male and female Sprague Dawley rats (n = 8/group)
Dosing: 7 days 3 mg/kg twice daily
Compound levels assessed 4 h after dosing on day 7
Liver washout refers to 16 h after last dose
ITMN Compound A not detected in heart at this concentration
Blood samples collected, into sodium citrate, via cardiac puncture and processed to plasma
Animals perfused via the left ventricle with heparinized saline
Liver and heart flash frozen in liquid N₂ and stored at -20°C until analysis
Plasma and tissue samples analyzed using a qualified LC/MS/MS method

- Compounds found in rat liver in concentration well above predicted efficacious amounts when administered in low oral doses
- Liver exposure is proportional to dose administered (data not shown)
- Relative liver/plasma exposure and rapid IV clearance suggest active transport into liver in rat
- Volume of distribution ~10- to 100-fold blood volume
- Heart AUC values ~1/10 to 1/100 of the plasma AUC values (data not shown)

CONCLUSIONS

- Five robust compound estates generated
- Structure-activity relationships for these estates well understood
- ITMN Compound A and ITMN Compound B:
 - 2 nM replicon EC₅₀ in genotype 1b
 - High liver exposure
 - Minimal plasma and heart exposure
 - Excellent in vitro safety profile
 - Possess at least 10-fold safety margin in preliminary rat safety assessment
- Chemical diversity represented in the four estates provides for:
 - Back-ups
 - Non-cross-resistant inhibitors
- PK and safety assessment in second species will inform candidate selection for IND development
- Clinical strategy under consideration