Preclinical Profiles of IDX136 and IDX316, Two Novel Macrocyclic HCV Protease Inhibitors

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INTRODUCTION

- Last year data were presented on the Idenix protease inhibitor program, which showed better selectivity and activity as compared to first generation protease inhibitors[1]. The compound series also exhibited a favorable early PK profile that suggested once- or twice-daily dosing.
- IDX136 and IDX316 are novel and specific macrocyclic HCV protease inhibitors that lack a covalent binding moiety and bind with low nanomolar potencies.
- This study evaluated the *in vitro* biochemical and cell-based activities of IDX136 and IDX316, as well as their pharmacokinetic profile in the mouse, rat and monkey.

METHODS

IC_{50} (50% inhibitory concentration) determination: Cleavage of a synthetic peptide by purified, recombinant HCV NS3/4A protease from genotypes 1a, 1b, 2a, 3a and 4a was measured in the presence of compound.

Binding kinetics: The binding kinetics of IDX316 to NS3/4A (Con1) were determined by surface plasmon resonance.

HCV replicon assay: Activity or cytotoxicity was measured by 1b replicon luciferase assay or MTS, respectively, after 3 day treatment with compound.

HCV *in vitro* infection assays: HPC cells were infected with JFH-1 (genotype 2a) and treated with compound for 4 days (virus inoculum was removed after 16 hours); remaining virus was measured by an anti-HCV core ELISA.

Long-term treatment assay: A replicon cell line was treated with compound, in the absence of G418, for 14 days and the level of replicon RNA was measured at multiple time points. At the end of the 14-day treatment, cells were cultured in the absence of compound ± G418 in 10 cm dishes for 21 days, whereupon the cells were stained and multicellular colonies counted.

HCV transient transfection assay: Protease inhibitor-associated resistance mutations were introduced into a luciferase-replicon by site-directed mutagenesis. The activity of compound was measured in cells transiently transfected with *in vitro* transcribed wild-type or mutant luciferase-replicon RNA via luminometry after 3-day treatment.

PK methods: Male CD-1 mice, Sprague-Dawley rats and rhesus monkeys were given a single intravenous (IV) or oral (PO) dose of compound. Plasma samples were analyzed by reverse-phase HPLC-MS/MS using a protein precipitation or liquid-liquid extraction method.

***In vitro* cytotoxicity assays:** Intracellular ATP content was measured (CellTiter-Glo luminescent cell viability assay) in freshly isolated hepatocytes treated with compound for 48 h.

CYP inhibition assays: CYP450 cDNA-expressed isozymes were incubated with IDX136 or IDX316 and the respective substrate, per the manufacturer's protocol (BD Bioscience).

RESULTS

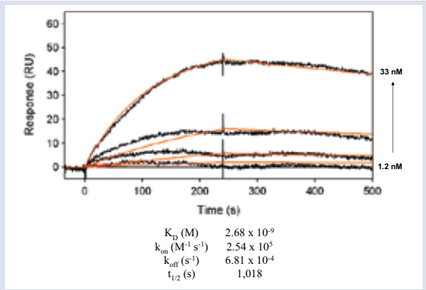
IDX316 is a potent and selective inhibitor of HCV NS3/4A protease (genotypes 1a, 1b, 2a, and 4a)

Table 1: In vitro activity of IDX PIs against NS3/4A proteases

Protease	Mean IC_{50} (nM) IDX136	IDX316	VX-950
Genotype 1a	2.9	2.0	34.6
Genotype 1b	2.8	1.3	22.1
Genotype 2a	72.0	13.1	24.3
Genotype 3a	721.4	206.7	42.1
Genotype 4a	1.8	1.3	252.6

Data derived from at least four experiments.

Figure 1: Sensorgram of IDX316 binding to genotype 1b (Con1) NS3/4A



- IDX316 binds to protease tightly, with an equilibrium constant (K_D) less than 3 nM, as determined by surface plasmon resonance. Association (k_{on}) was fast, and dissociation (k_{off}) was very slow, with a dissociation half-life ($t_{1/2}$) of nearly 17 minutes.
- IDX316 did not significantly inhibit (IC_{50} > 24 μM) eight cellular proteases.

IDX316 is a potent inhibitor of genotype 1b HCV replicon replication in cell culture with a high selectivity index

Table 2: In vitro activity of IDX PIs in a HCV genotype 1b replicon assay

	IDX136	IDX316
Mean EC_{50} (nM)[a]	12.1	5.3
Mean CC_{50} (μM)	27	> 100
Selectivity Index	2,231	> 18,868

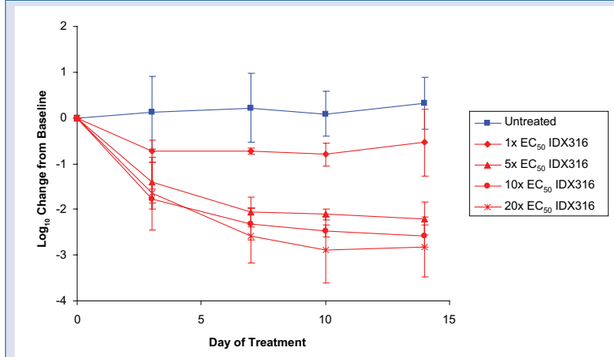
[a]Luciferase reporter-based assay
Data derived from at least 15 experiments.

- Similar CC_{50} values were obtained using HepG2 cells: >100 μM for IDX316.

Long-term treatment of replicon cells with IDX316 results in a multilog reduction in replicon RNA

The activities of IDX136 and IDX316 were examined in 14-day treatment assays in the absence of G418. Similar results were obtained for IDX136 (data not shown).

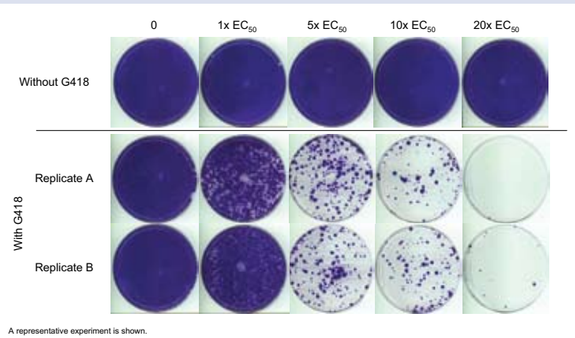
Figure 2: Long-term inhibition of replicon replication by IDX316



Data was derived from three independent experiments. Log_{10} change from baseline values were calculated from log_{10} copies HCV/GAPDH RNA.

- Long-term treatment with IDX316 at concentrations above the EC_{50} effectively suppressed RNA replication compared to the untreated control (blue line).
- Concentrations of 5x to 20x EC_{50} gave high levels of suppression (approximately 3 log_{10} at 20x EC_{50}).
- Suppression was maintained over the 14 days with no evidence of RNA rebound or visible cytotoxicity.
- The treated cells shown in **Figure 2** were further cultured without compound ± G418 to quantitate the remaining replicon-bearing cells; only these cells survive G418 selection.

Figure 3: Confirmation of replicon reduction after long-term treatment with IDX316



A representative experiment is shown.

- As expected, in the absence of G418 selection pressure, cells remained viable after treatment with 0 to 20x EC_{50} of drug (top row).
- In the presence of G418, a dose-dependent reduction in replicon-bearing colonies was observed after IDX316 treatment from too many to count (> 600; no drug) to ≤10 (20x EC_{50}).

Resistance profile

The activity of IDX PIs was evaluated against replicons bearing a single protease inhibitor-associated resistance mutation and compared to their activity against the wild-type replicon.

Table 3: In vitro resistance profile of IDX PIs in a transient transfection assay

Mutant replicon	Mean EC_{50} fold-change IDX136	IDX316
T54A	0.7	0.9
Q80R	9.2	10.3
R155K	28.9	49.4
R155Q	0.4	0.9
A156S	0.1	0.2
A156T	6.0	241.2
D168A	> 314	667.4
D168E	37.5	47.4
D168V	> 459	> 2242
D168Y	> 314	721.4

Data derived from at least three experiments.

- Replicons bearing T54A, R155Q, and A156S mutations in NS3 remained susceptible to IDX316.

IDX316 exhibits additive activity in combination with other HCV drug classes

The activities of IDX136 or IDX316 together with standard-of-care agents or Idenix polymerase inhibitors in development were tested.

Table 4: Combination effects of IDX316 with other agents

IDX316 +	Bliss Independence	Loewe Additivity	Combination Index
IDX375 (NNI)	Additive	Additive	Additive (0.93)
IDX184 (NI)	Additive	Additive	Additive (0.92)
IFN	Additive	Additive	Additive (0.95)
RBV	Additive	Additive	Additive (1.00)

Data obtained from 3-day replicon assays.
No cytotoxicity was observed.
CI values between 0.90 and 1.10 = additive; CI values under 0.90 = synergy

- Enhanced activity corresponding to additivity was observed when IDX316 was combined with IFN-α, RBV, IDX184 or IDX375.
- Similar combination effects were observed with IDX136 (data not shown).

Favorable preclinical pharmacokinetics of IDX316

The pharmacokinetic parameters of IDX136 and IDX316 were studied in rodent and non-human primate species.

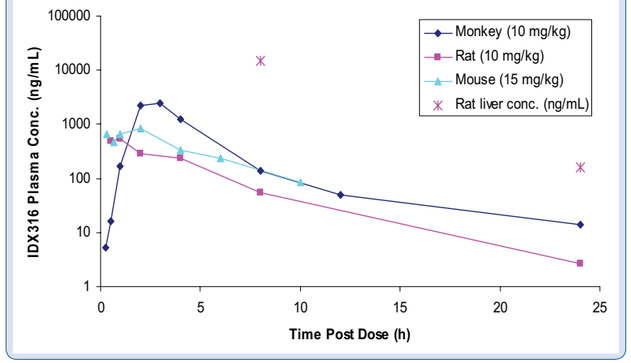
Table 5: Summary of mean pharmacokinetic parameters for IDX PIs

Compound	Species	Dose (mg/kg)	Cl (L/h/kg)	V_d (L/kg)	$t_{1/2}$ (h)	C_{max} (ng/mL)	T_{max} (h)	F (%)
IDX136	rat	2 (IV) 10 (PO)	1.80	3.5	2.0	1532	1.0	45%
	monkey	2 (IV) 10 (PO)	0.73	1.1	2.0	509	2.5	11%
	mouse	15 (IV) 15 (PO)	0.70	5.5	5.2	834	2.0	19%
IDX316	rat	2 (IV) 10 (PO)	0.79	1.7	4.0	551	1.0	17%
	monkey	2 (IV) 10 (PO)	0.20	0.4	4.8	3023	2.0-3.0	18%

Cl and V_d from animals dosed IV; $t_{1/2}$, C_{max}, T_{max} and F from animals dosed orally

- The plasma half-life of IDX316 ranged from 4.0 to 5.2 h in mice, rats and monkeys.
- The oral bioavailability of IDX316 was nearly 20% in mice, rats and monkeys.

Figure 4: Mean plasma profiles of oral IDX316 in three species



- IDX316 was selectively concentrated in the liver in rats given a single oral dose of 10 mg/kg; liver levels at 8 and 24 h were 280- and 60-times the corresponding plasma concentrations.

Favorable cytotoxicity and CYP450 interaction profile for IDX PIs

- IDX316 was not cytotoxic to freshly isolated mouse, rat, monkey or human hepatocytes *in vitro* (CC_{50} > 10 μM).
- No significant inhibition of human CYP450 by IDX316 was observed.

Favorable *in vivo* toxicology profile for IDX316

- Monkeys given 10 or 100 mg/kg oral doses of IDX316 for 7 days showed no adverse effects, including no meaningful changes in clinical chemistries and no histological abnormalities.

CONCLUSIONS

- IDX136 and IDX316 exhibited potent and selective *in vitro* activity against HCV NS3/4A protease and replicons.
- Effective *in vitro* HCV suppression can be maintained for 14 days with IDX PIs.
- Our results support the concept of combining IDX PIs with standard-of-care agents or other classes of direct-acting HCV antivirals.
- IDX316 demonstrated favorable pharmacokinetic profiles in rodent and non-human primate species, suggesting the potential for once- or twice-daily dosing in humans.
- No *in vitro* cytotoxicity in freshly isolated hepatocytes across species or significant human CYP450 inhibition was observed with IDX316.
- No adverse effects were observed in monkeys dosed daily with 10 or 100 mg/kg of IDX316 for 7 days.
- IND-enabling pharmacology and toxicology studies are ongoing.

Acknowledgments

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References

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Disclosures: All authors are current employees of Idenix Pharmaceuticals, Inc.

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