ACH-2684: HCV NS3 PROTEASE INHIBITOR WITH POTENT ACTIVITY AGAINST MULTIPLE GENOTYPES AND KNOWN RESISTANT VARIANTS

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ACHILLION

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ABSTRACT
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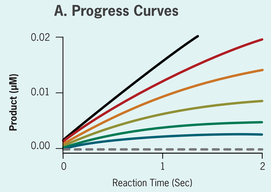
Background

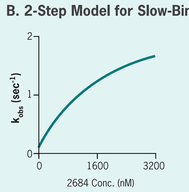
HCV NS3 protease is a clinically validated target. Most NS3 protease inhibitors in clinical development are ineffective against some non-genotype 1 HCV and HCV variants carrying NS3 protease inhibitor-resistant mutations. These NS3 protease inhibitor-resistant variants preexist in HCV-infected patients, a factor that could contribute to virologic breakthrough (1). ACH-2684 is derived from our efforts to design NS3 protease inhibitors with potent activity against different genotypes and against known resistant variants.

Methods and Results

1 ACH-2684 FORMS A LONG-LIVED HIGH-AFFINITY COMPLEX WITH NS3 PROTEASE

FIGURE 1. ACH-2684 Binding Kinetics to NS3 Protease





K_i^* (nM)	0.0102 ± 0.0015
K_i (nM)	466 ± 75
k_5 (sec^{-1})	2.17 ± 0.13
k_6 (sec^{-1})	4.76 x 10^{-5} ± 5.2x10^{-6}
$T_{1/2}$ (h)	4.0

$$E + I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EI \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EI^*$$

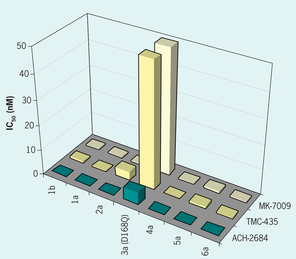
Initial affinity: $K_i = k_4 / k_3$
Steady state: $K_i^* = K_i\, k_6 / (k_5 + k_6)$

ACH-2684 kinetics from reactions (GT-1b NS3 protease) without pre-incubation were monitored by plate reader or stopped-flow spectrometer. Steady state affinity K_i^* was determined from reactions with 1 h preincubation to achieve steady-state Enzyme Inhibitor complex binding. Complex $T_{1/2}$ was derived from the two-step slow-binding model using parameters obtained from progress curves.

- ACH-2684 binds the enzyme slowly and very tightly ($T_{1/2}$ of the enzyme and inhibitor complex = 4 hours).
- ACH-2684 is a highly potent inhibitor (Ki^* = 5.9 and 10 pM against 1a and 1b NS3 protease, respectively).

2 ACH-2684 POSSESSES POTENT ACTIVITY AGAINST GT 1-6 NS3 PROTEASES

FIGURE 2. Activity against NS3 Proteases of Multiple Genotypes



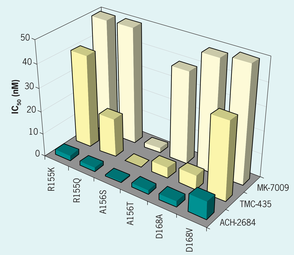
Reactions were monitored after 15 minutes pre-incubation of the enzyme (GT 1a, 1b, 2a, 3a, 4a, 5a and 6a NS3 protease) and inhibitor by plate reader. IC_{50} values were calculated from initial velocities for each inhibitor concentration.

Note: IC_{50} > 50 nM is shown as 50 nM.

- ACH-2684 is a pan-genotypic inhibitor that displays potent activity against all genotypes tested.

3 ACH-2684 RETAINS POTENT ACTIVITY AGAINST NS3 PROTEASE RESISTANT MUTANTS

FIGURE 3. Activity Against NS3 Protease Resistant Mutants



Reactions were monitored after 15 minutes pre-incubation of the enzyme (GT-1b carrying the mutation indicated) and inhibitor by plate reader. IC_{50} values were calculated from initial velocities for each inhibitor concentration.

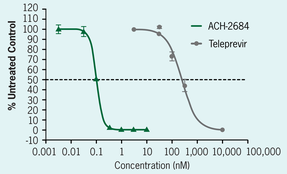
Note: IC_{50} > 50 nM is shown as 50 nM.

- ACH-2684 retains its activity against the most common NS3 protease mutants that are highly resistant to multiple NS3 protease inhibitors.

4 ACH-2684 POTENTLY INHIBITS HCV REPLICONS

TABLE 1. Potency and Selectivity

Compound	Potency					Toxicity	SI
	EC_{50} (nM)	EC_{90} (nM)	EC_{95} (nM)	EC_{90}/ EC_{50}	EC_{95}/ EC_{50}	CC_{50} (nM)	
ACH-2684	0.18	0.28	0.31	1.6	1.7	16000	88889
Telaprevir	282	805	1294	2.9	4.6	ND	ND



Inhibition of HCV replication was assessed by measuring reporter luciferase activity in Huh-luc/neo cell line (GT 1b,Strain Con-1).

ND, not done.

SI, selective index and calculated as CC_{50} / EC_{50}.

- High potency against NS3 protease of ACH-2684 is translated into high potency against HCV replicon.
- The hill slope of ACH-2684 dose-response curve is higher. Hence, < 2 fold increase in ACH-2684 concentration above EC_{50} is needed to achieve 95% inhibition.

TABLE 2. Comparison of Potency between GT 1a and GT 1b

Compound	ACH-2684 EC_{50} (nM)		
	GT 1a (Strain H77)	GT 1b (Strain Con-1)	
	HSG/1a	Huh-9-13	Huh-luc/neo
ACH-2684	0.59	1.5	0.32
Telaprevir	450	610	680

Inhibition of HCV replication was assessed by measuring HCV RNA amount in stable replicon cells.

- ACH-2684 displays a similar potency against both genotype-1 and 1b replicons.

TABLE 3. Activity against GT 1a And GT 1b Clinical Variants

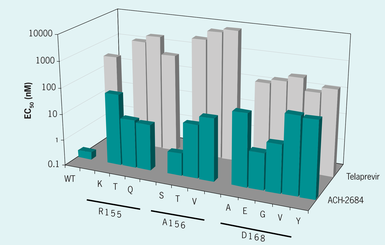
GT	Compound	EC_{50} (nM)					
		REF	P1	P2	P3	P4	P5
1a	ACH-2684	0.071	0.067	0.19	0.58	0.27	0.34
	Telaprevir	213	133	63	178	246	63
1b	ACH-2684	0.12	0.15	0.76	0.079	0.14	0.044
	Telaprevir	262	355	7225	82	84	400

Chimeric replicons were engineered to incorporate GT 1a and GT 1b NS3 protease from reference strains (1a, H77; 1b, Con-1) and patient isolates (1a and 1b, P1 — P5) into a GT 1b Con-1 replicon backbone. Compound activity was assessed in a transient replication assay by luciferase readout.

- The high potency of ACH-2684 is retained against HCV replicons carrying NS3 protease from patient isolates

5 ACH-2684 RETAINS ACTIVTY AGAINST RESISTANCE MUTANT REPLICONS

FIGURE 5. Activity Against Resistant HCV Replicons



HCV inhibition was assessed in a transient replication assay by luciferase readout. The indicated substitutions were engineered into the NS3 sequence of the GT 1b HCV replicon.

- ACH-2684 remains active against replicon variants carrying the most common mutations that confer high resistance to covalent and/or noncovalent NS3 protease inhibitors.

6 ACH-2684 IS A HIGHLY SPECIFIC PROTEASE INHIBITOR

TABLE 4. Inhibition of Human Proteases

% inhibition @ 10 μM ACH-2684									
Serine Protease								Cysteine Protease	
Cathepsin G	19	Factor Xa	30	Factor VIIa	11	Urokinase	-22	Cathepsin L	1
Chymase	7	Kallikrein	0	Thrombin	3	Tryptase	7	Cathepsin S	26
Chymotrypsin	46	Plasmin	10	tPA	–3				

- ACH-2684 shows < 50% inhibition of a variety of human proteases at a concentration >10^6 fold over its Ki^* against HCV NS3 serine protease.

7 ACH-2684 IS NOT A CYP ISOENZYME INHIBITOR NOR INDUCER

TABLE 5. Cytochrome P450 (CYP) Inhibition

CYP ISOFORM	ACH-2684 IC_{50} (μM)		CYP ISOFORM	ACH-2684 IC_{50} (μM)	
	ENZ	HLM		ENZ	HLM
1A2	5.9	>50	2C19	4	>50
2B6	>100	ND	2D6	>100	ND
2C9	38	ND	3A4	20.8	>50

ACH-2684 was screened against recombinant CYP with fluorescent substrate. Selected isoforms were assessed further in pooled human liver microsomes (HLM) with isoform-specific substrates.

- The lowest IC_{50} of ACH-2684 against CYP enzymes is > 10^5 fold over its Ki^* against HCV NS3 protease.
- ACH-2684 was also shown to be negative for induction of CYP 1A2 and CYP 3A4 (data not shown).
- These data demonstrate low potential for ACH-2684 interaction with other drugs.

REFERENCE

Kronenberger B, Zeuzem S. Current and future treatment options for HCV. Ann Hepatol. 2009 April – June; 8(2):103-12.

8 ACH-2684 IS METABOLICALLY STABLE IN MULTIPLE SPECIES IN VITRO

TABLE 6. Metabolic Stability of ACH-2684 in Liver Microsomes

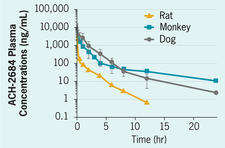
Species	In Vitro Half-life (min)	Intrinsic Clearance (mL/min/kg)	Predicted Plasma Hepatic Clearance (mL/min/kg)	Predicted Hepatic Extraction (%)
Rat	>600	<4.16	<3.87	<7.0
Dog	98.0	20.4	12.3	40
Human	144	11.3	7.3	35
Monkey	123	15.2	11.3	26

ACH-2684 metabolic stability was determined by incubation with hepatic microsomes followed by LC/MS-MS quantitation. Data were fitted to exponential decay to derive $T_{½}$.

- Stability of ACH-2684 in S9 fractions is similar to that observed in microsomes.
- These data show that ACH-2684 is metabolically stable.

9 ACH-2684 SHOWS FAVORABLE PHARMACOKINETICS IN PRECLINICAL SPECIES

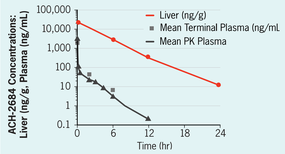
FIGURE 6. Pharmacokinetics after IV Administration



Species	CL (L/h/kg)	Vdss (L/kg)	$T_{1/2}$ (h)	MRT (h)
Rat	1.37	1.09	1.64	0.76
Monkey	0.22	0.9	5.49	2.6
Dog	0.14	0.57	3.54	1.14

ACH-2684 was administered as a single IV bolus dose (1 mg/kg) followed by LC/MS-MS quantitation. Non-compartmental pharmacokinetic analysis was performed using WinNonlin™ Professional.

FIGURE 7. Concentration in Liver vs. Plasma (Rat)



Time (hr)	Liver (ng/g)	Plasma (ng/mL)	Liver: Plasma
0.083	23262	1506	14.6
2	12049	33	365
6	2929	6.5	477
12	316	BLQ	>526
24	11.5	BLQ	>11

ACH-2684 was administered as a single IV bolus dose (1mg/kg) followed by LC/MS-MS quantitation. Non-compartmental pharmacokinetic analysis was performed using WinNonlin™ Professional. In addition to collecting plasma from PK group, plasma and liver were collected from 3 additional rats at time points of 0.083, 2, 6, 12, and 24 h post dose respectively.

- Pharmacokinetics after IV administration display low to moderate clearance and volume of distribution.
- ACH-2684 displays significant partitioning to the liver.
- These data, along with good oral bioavailability in preclinical species (data not shown), suggest that once daily dosing of ACH-2684 in human is achievable.

Conclusions

- ACH-2684 is a potent NS3 protease inhibitor against genotypes 1 to 6.
- ACH-2684 retains its activity against the most common NS3 protease mutants that are highly resistant to multiple NS3 protease inhibitors.
- ACH-2684 forms a long-lived complex with NS3 enzyme that may prolong suppression of HCV replication.
- ACH-2684 potently inhibits HCV replication in genotype 1a and 1b replicon systems, with broad coverage of natural variants and resistant mutants.
- ACH-2684 shows low potential for off-target activity and drug-drug interaction.
- ACH-2684 is metabolically stable and shows favorable pharmacokinetic properties including preferential distribution into the liver.
- ACH-2684 has been selected for clinical development for hepatitis C treatment.

PRESENTED AT THE LIVER MEETING,® BOSTON, MA, USA, OCTOBER 29 – NOVERMBER 2, 2010 © 2010 ACHILLION PHARMACEUTICALS, INC.