# Preclinical Characterization of SCH 900518, A Novel Mechanism-Based Inhibitor of HCV NS3 Protease

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## Abstract

**Background:** Small molecule hepatitis C virus (HCV) nonstructural protein 3 (NS3) protease inhibitors have shown antiviral activity as monotherapy and in combination with pegylated interferon-alfa and ribavirin in clinical trials; however, clinical efficacy can be limited by inadequate drug exposure and development of viral resistance. Improvement in inhibitor potency and pharmacokinetic properties offers opportunities to overcome such limitations and to further increase SVR.

**Methods:** Inhibition of HCV NS3 protease was measured using a single-chain NS3 (3-181)/4A protease. The antiviral effect and resistance study of protease inhibitors were evaluated using genotype 1b HCV replicon cells.

**Results:** Combination of medicinal chemistry and structure-based design has led to the discovery of SCH 900518, a novel ketoamide protease inhibitor which forms a reversible covalent bond with the active site serine with an inhibition constant ($K_i$*) of 7 nM. SCH 900518 showed a 10-fold improvement in replicon potency ($EC_{90}$ = 40 nM) compared to boceprevir and telaprevir. In biochemical assays, SCH 900518 was active against proteases of genotypes 1a, 1b, 2, and 3. A 2-week treatment with 5× $EC_{90}$ of the inhibitor reduced replicon RNA by 3-log. High exposures of SCH 900518 had minimal effects on a variety of human normal and tumor cell lines. Selection of replicon cells with SCH 900518 resulted in outgrowth of several major resistant mutants (T54A/S, A156S/T/V). Preclinical cross-resistance studies demonstrated that the majority of mutations against boceprevir and telaprevir showed similar fold loss of activity against all three inhibitors; however, SCH 900518 retained more activity against these mutants due to its higher intrinsic potency. Combination treatment with interferon-alfa enhanced inhibition of replicon RNA and suppressed emergence of resistant replicon colonies, supporting the use of SCH 900518/peginterferon combination therapy in the clinic.

**Conclusions:** The preclinical characterization of SCH 900518 supports its progression toward clinical evaluation of safety, pharmacokinetic, and pharmacodynamic parameters.

## Background

- Approximately 50% of patients with chronic hepatitis C infection attain sustained virologic response with current standard-of-care therapies.[1,2] An urgent unmet need exists for novel treatments that will improve the rate of sustained virologic response.
- The nonstructural protein 3 (NS3) protease is essential for viral replication.
  - NS3 protease is responsible for processing the nonstructural portion of the hepatitis C virus (HCV) polyprotein and represents a promising target for novel hepatitis C therapies.
  - Ketoamides such as boceprevir bind to the active site on the NS3 protease and have shown promising clinical activity.[3,4]
- Further study of this class of molecule has resulted in the development of the novel ketoamide SCH 900518

## Aim

- To assess the activity of the novel ketoamide SCH 900518 in preclinical models of hepatitis C treatment.

## Methods

### Continuous Spectrophotometry Assay

- Recombinant single-chain HCV NS3/NS4A proteases were prepared as previously described by Taremi et al.[5]
- HCV NS3 protease assays were performed in a 200-µL reaction mixture, as previously described by Zhang et al.[6]
  - Typically, 100 µL protease was added to 100 µL assay buffer containing the chromogenic substrate Ac-DTEDVVP(Nva)-O-PAP.
  - Change in absorbance was monitored over 60 minutes using a microtiter plate reader (Spectromax Plus; Molecular Devices, Eugene, OR).
  - Data were fitted to the 2-step slow-binding inhibition model:

$$P = v_s t + (v_0 - v_s)(1 - e^{-kt})/k$$

  - The overall inhibition constant $K^*$ ($v_s = V_{max}S/(K_m[1 + I/K^*])$) was used to measure inhibitor potency.
- $K^*$ is derived from the velocity at steady state and is used to distinguish the overall inhibition constant, obtained for slow-binding (eg, covalent) inhibitors, from the inhibition constant, $K_i$, used for rapid-binding noncovalent competitive inhibitors.

### Replicon Assay

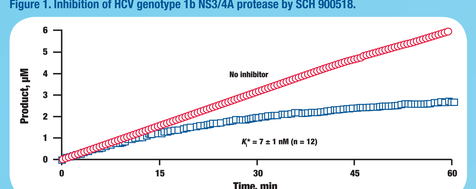
- Replicon construction was performed as previously described.[4,7]
- To measure antireplicon activity, replicon cells were seeded in 96-well, collagen I-coated plates.
- SCH 900518 was added 24 hours after seeding and was incubated for 3 days, at which point cells were lysed and replicon RNA was measured with real-time RT-PCR reaction.
  - Inhibition concentration 50 ($IC_{50}$) was the drug dose necessary to achieve an increase of 1 over the projected baseline in cyclic threshold (ΔCT).
  - $IC_{90}$ was the drug dose necessary to achieve an increase of 3.2 over the projected baseline in ΔCT.

## Results

### Inhibition of HCV NS3/4A Protease In Vitro

- When tested in a continuous spectrophotometry assay, the inhibition constant $K^*$ of SCH 900518 for genotype 1b HCV NS3/4A was 7 ± 1 nM (n = 12) (**Figure 1**).
- SCH 900518 was active against proteases from genotypes 1a, 2a, 3a, and 4 ($K^*$ = 0.7 nM, 3 nM, 7 nM, and 16 nM, respectively).

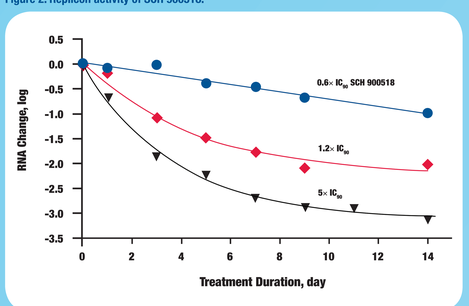
Figure 1. Inhibition of HCV genotype 1b NS3/4A protease by SCH 900518.




### Replicon Activity

- Antiviral activity of SCH 900518 was evaluated in the HCV replicon system.
- $IC_{50}$ and $IC_{90}$ values for SCH 900518 were 20 ± 6 nM (n = 49) and 40 ± 10 nM (n = 63), respectively, in a 72-hour assay.
  - Prolonged exposure of SCH 900518 at 1× $IC_{90}$ resulted in a 2-log decrease in RNA levels by day 14 (**Figure 2**).
  - Dosing with 5× $IC_{90}$ resulted in a 3-log decrease in RNA level by day 14.
  - SCH 900518 showed minimal effects on cell viability in a variety of human cell lines and primary cell cultures using standard MTS or CTB assays (data not shown).

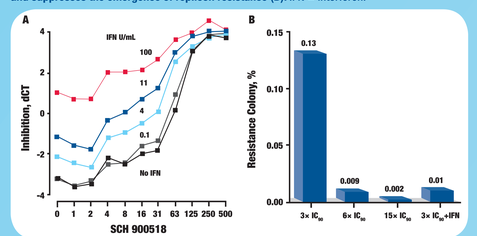
Figure 2. Replicon activity of SCH 900518.




### Combination Studies With Interferon alfa-2b

- When interferon (IFN) alfa-2b was coadministered with SCH 900518, there was a dose-dependent, enhanced inhibition of replicon RNA (**Figure 3A**).
- Combination with IFN alfa-2b suppressed the emergence of replicon-resistant colonies (**Figure 3B**).
  - Culturing of replicon-bearing cells in the presence of 3× $IC_{90}$ SCH 900518 resulted in the emergence of resistant colonies at approximately the 0.13% level.
  - Increasing the concentration of SCH 900518 to 0.4 µM and 1.0 µM reduced the number of resistant colonies that emerged by approximately 14-fold and 100-fold, respectively.
  - The emergence of resistant colonies was reduced approximately 10-fold when using 3× $IC_{90}$ SCH 900518 with IFN alfa-2b (30 IU/mL; approximately 10× $IC_{90}$) compared with SCH 900518 alone.
  - This suggests that SCH 900518 could be used in combination with IFN-based therapy to minimize the emergence of resistant mutants.

Figure 3. Combination treatment with IFN alfa-2b enhances the inhibition of replicon RNA (A) and suppresses the emergence of replicon resistance (B). IFN = interferon.




### Resistance Studies

- Sequence analysis of replicon clones recovered from the SCH 900518 de novo resistance studies identified mutations at 2 different loci (amino acids 54 and 156) in the protease domain (**Table 1**).
  - After selection with 0.4 µM (6× $IC_{90}$) of SCH 900518, T54A/S was the predominant mutation, and it conferred an approximate 10-fold increase in $IC_{90}$ values.
  - Increasing the initial selection dose to 15× $IC_{90}$ (1.0 µM SCH 900518) led to the emergence and overgrowth of mutations at the A156 locus, which conferred a higher level of resistance to the compound.
  - A double mutant (T54A/A156T) was also observed in 1 clone at the higher selecting dose, which rendered the replicon cells insensitive to the compound, even at 40 µM.
- Importantly, replicon cells resistant to SCH 900518 remained sensitive to IFN alfa-2b (data not shown).

Table 1. Resistance Mutations Selected by SCH 900518

| Mutation | Frequency at Drug Level | | Increase in $IC_{90}$, fold |
|---|---|---|---|
| | 6× $IC_{90}$ | 15× $IC_{90}$ | |
| T54A/S | 4/6 | 0/5 | 10 |
| A156S | 1/6 | 3/5 | 30-60 |
| A156T/V | 1/6 | 1/5 | ~500 |
| T54A, A156T | 0/6 | 1/5 | >600 |

- SCH 900518 was cross-resistant to mutations raised against boceprevir in enzymatic and replicon assays but retained more activity on many of the mutants as a result of its higher intrinsic potency (**Tables 2** and **3**).[7]

Table 2. Enzyme Activity of SCH 900518 Against Mutations Conferring Resistance to Boceprevir

| | $K^*$, nM (fold increase relative to WT) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | V36M[a] | V36A[a] | F43C[a] | T54A[a] | T54S[a] | R155K[a] | A156S[b] | A156T[b] | D168V[b] | V170A[b] | V170T[b] | V36M+ R155K[c] | T54S+ R155K[c] |
| SCH 900518 | 12 (3) | 20 (5) | 33 (8) | 40 (8) | 11 (3) | 31 (8) | 500 (100) | 15,000 (3000) | 5 (1) | 30 (6) | 9 (2) | 650 (160) | 470 (58) |
| Boceprevir | 35 (2) | 39 (2.5) | 110 (7) | 110 (4) | 39 (2.5) | 56 (3.5) | 460 (17) | 8000 (300) | 16 (0.6) | 300 (10) | 35 (2) | 230 (14) | 330 (12) |

$K^*$ = overall inhibition constant; WT = wild-type.
[a] WT $K^*$ for SCH 900518 = 4 nM; for boceprevir = 16 nM.
[b] WT $K^*$ for SCH 900518 = 5 nM; for boceprevir = 27 nM.
[c] WT $K^*$ for SCH 900518 = 8 nM; for boceprevir = 27 nM.

Table 3. Replicon Activity of SCH 900518 Against Mutations Conferring Resistance to Boceprevir

| | $IC_{50}$ nM (fold increase relative to WT) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | V36M[a] | T54A[b] | R155K[a] | A156S[c] | A156T[c] | D168V[c] | V170A[c] | V36M+ R155K[a] |
| SCH 900518 | 430 (8) | 400 (11) | 810 (15) | 2200 (70) | >40,000 (>1000) | 40 (1) | 300 (10) | 6900 (130) |
| Boceprevir | 530 (3) | 820 (6) | 650 (4) | 4300 (16) | 23,000 (85) | 310 (1) | 3400 (12) | 1800 (10) |

IC = inhibition concentration; WT = wild-type.
[a] WT replicon $IC_{50}$ for SCH 900518 = 50 nM; for boceprevir = 180 nM.
[b] WT replicon $IC_{50}$ for SCH 900518 = 40 nM; for boceprevir = 130 nM.
[c] WT replicon $IC_{50}$ for SCH 900518 = 30 nM; for boceprevir = 270 nM.

## Conclusions

- In the enzymatic assay, SCH 900518 is active against proteases from genotypes 1 to 4.
- In the replicon assay, the $IC_{50}$ and $IC_{90}$ values for SCH 900518 were 20 nM and 40 nM, respectively.
  - Coadministration of IFN alfa-2b and SCH 900518 resulted in dose-dependent, enhanced inhibition of replicon RNA.
- The emergence of resistant colonies was reduced 10-fold when SCH 900518 was used in combination with IFN alfa-2b.
- Mutations at amino acid 54 (T54A/S) and 156 (A156) in the protease domain were observed in replicons selected against SCH 900518.
  - Replicon cells resistant to SCH 900518 remained sensitive to IFN alfa-2b.
- SCH 900518 was cross-resistant to mutations raised against boceprevir.
- The enhanced inhibition of replicon RNA and the suppressed emergence of resistant colonies suggest the potential for clinical use of SCH 900518 in combination with pegylated interferon therapy.

## References

1. Manns MP et al. *Lancet*. 2001;358:958-965.
2. Fried MW et al. *N Engl J Med*. 2002;347:975-982.
3. Schiff E et al. 43rd Annual Meeting of the European Association for the Study of the Liver; April 23-27, 2008; Milan, Italy.
4. Malcolm BA et al. *Antimicrob Agents Chemother*. 2006;50:1013-1020.
5. Taremi SS et al. *Protein Sci*. 1998;7:2143-2149.
6. Zhang R et al. *Anal Biochem*. 1999;270:268-275.
7. Tong X et al. *Antiviral Res*. 2008;77:177-185.

### Disclosures

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