

Favorable Cross-Resistance Profile of Two Novel Hepatitis C Virus Inhibitors, SCH 503034 (Boceprevir) and HCV-796, and Enhanced Anti-Replicon Activity Mediated by the Combined Use of Both Compounds

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Background

Cell-culture replicon studies evaluated the combined antiviral effects of two novel inhibitors of hepatitis C virus (HCV): SCH 503034 (Boceprevir) (Schering-Plough), a NS3 protease inhibitor, and HCV-796 (Wyeth/ViroPharma), a non-nucleoside polymerase inhibitor. Each inhibitor demonstrated significant antiviral activity in early clinical studies. Replicon studies demonstrated that genetic variants exhibiting reduced susceptibility can be selected from each compound. Because SCH 503034 and HCV-796 target different viral enzymes, a potential exists for enhanced *in vivo* antiviral effect if the inhibitors are used in combination, as well as the potential for forestalling *in vivo* selection of clinically resistant HCV. The present replicon studies were performed to ascertain the likelihood of achieving these goals.

Figure 1A: SCH 503034

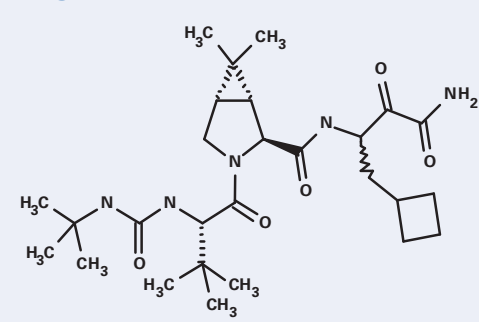
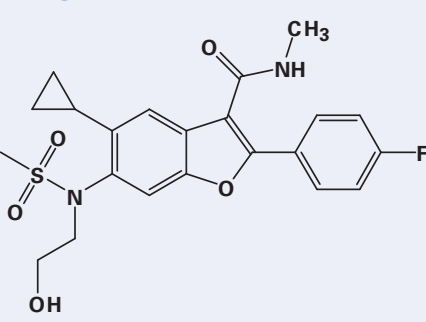


Figure 1B: HCV-796



In vitro Biochemical and Intracellular Activities of SCH 503034 (Boceprevir) and HCV-796

Table 1: In vitro Activities of SCH 503034 and HCV-796

	IC ₅₀ (μM)	
	SCH 503034	HCV-796
NS5B Enzymes		
Genotype 1a and 1b ^a	Ki ^b : 0.014 (1b)	0.01 – 0.16
Genotype 2, 3, and 4 ^b	not available	0.22 – 1.7
Replicon		
HCV RNA	200 (1b)	0.005 (1a); 0.009 (1b)
HCV Protein	not available	0.019 (1a); 0.014 (1b)
Therapeutic Index^c	>200	>5000
Selectivity Index^d	2000	>18 - >5000

a: 3 isolates of genotype 1a and 6 isolates of genotype 1b for HCV-796.
b: 1 isolate each for HCV-796.
c: Based on the HCV and GAPDH RNAs for both HCV-796 and SCH 503034.
d: Enzyme panel for HCV-796 includes human polymerase α, β, γ, HIV RT, calf thymus polymerase α; for SCH 503034 includes human neutrophil elastase.

Cross-Resistance Profiles of SCH 503034 (Boceprevir) and HCV-796

- SCH 503034 exhibited equivalent inhibitory activity against WT and HCV-796 resistant variants.
- HCV-796 inhibited WT and resistant replicons selected by SCH 503034 with similar potency.

Table 2A: Activities of SCH 503034 Against HCV-796 Resistant Replicons

	HCV-796 (nM)	Mean Fold Resistance	SCH 503034 EC ₅₀ (nM) ± SD ^a	Mean Fold Resistance
1b, BB7 WT	1.1 ± 0.2 (n=3)	—	272 ± 27 (n=4)	—
1b, 796R (10 μM) Pool ^b	>88,000 (n=3)	—	169 ± 44 (n=4)	0.6x
1b, C316Y	501 ± 291 (n=4)	166x	215 ± 42 (n=3)	0.8x
1b, C316F	392 ± 209 (n=4)	130x	215 ± 30 (n=3)	0.8x
1b, C316S	30 ± 4 (n=4)	10x	205 ± 70 (n=3)	0.8x
1b, M414I	23 ± 3 (n=5)	8x	69 ± 57 (n=3)	0.3x
1b, S365T	643 ± 168 (n=4)	212x	146 ± 2 (n=3)	0.5x
1b, I363V	16 ± 5 (n=3)	5x	125 ± 90 (n=3)	0.5x
1b, S365A	124 ± 41 (n=4)	41x	113 ± 30 (n=3)	0.4x

a: Based on HCV RNA normalized with rRNA as determined by TaqMan[®] RT-PCR.
b: A pool of resistant replicons selected from 10 μM HCV-796.

Table 2B: Activities of HCV-796 Against SCH 503034 Resistant Replicons

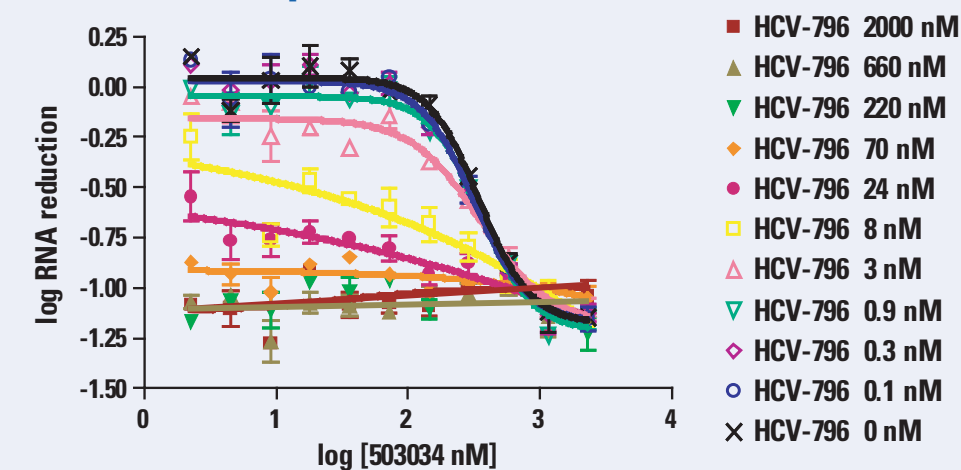
	SCH 503034 (nM)	Mean Fold Resistance	HCV-796 EC ₅₀ (nM) ± SD ^a	Mean Fold Resistance
1b, CL 16 WT	325 ± 35	—	7 ± 2	—
1b, T54A	1250 ± 500	4x	7 ± 2	—
1b, A156S	3050 ± 210	9x	8 ± 1	—
1b, V170A	3050 ± 210	9x	14 ± 4	2x
1b, 2H8 A156T ^b	15500 ± 710	48x	7 ± 3	—

a: Based on HCV RNA normalized with GAPDH RNA as measured by TaqMan[®] RT-PCR.
b: Clonal cells that have greatest resistance to SCH 503034.

3-Day Combination of SCH 503034 (Boceprevir) and HCV-796

- Time- and dose-dependent inhibition of replicon RNA was observed with both inhibitors.
- Combination treatment achieved greater reduction in viral RNA than either agent used alone.

Figure 2A: Titration of SCH 503034 in the Presence of Varying Concentrations of HCV-796 in 1b, CL16 Replicon Cells

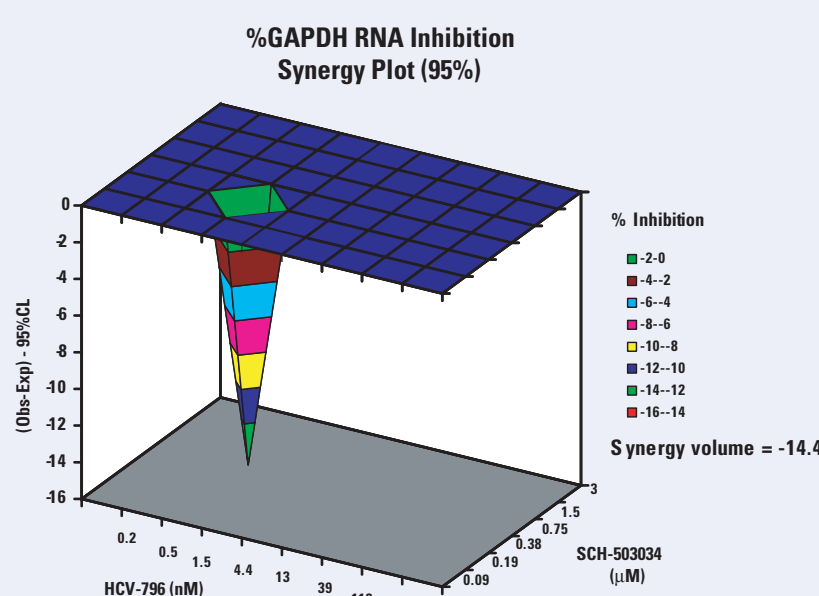
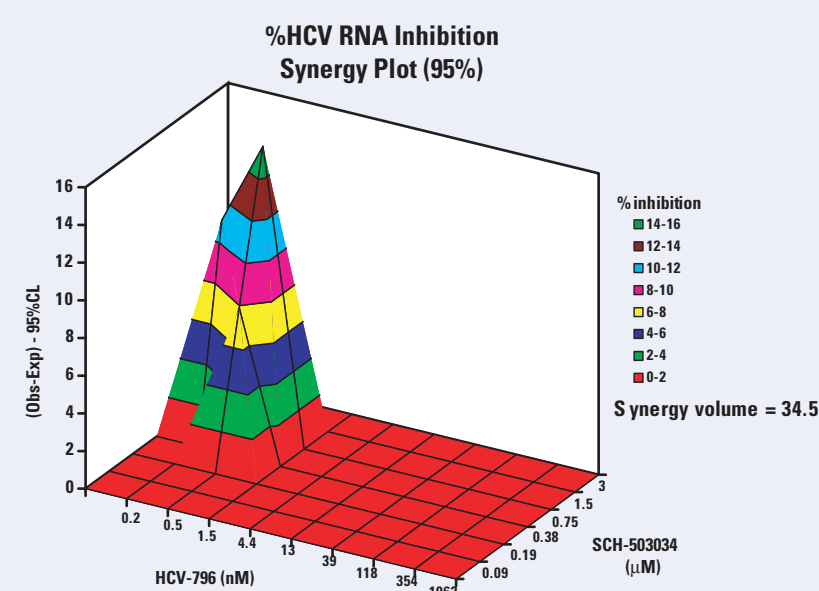


Replicon cells (CL16 genotype 1b) were treated for 3 days with SCH 503034 which was serially diluted at 1:2 for a 10-point titration. To each concentration of SCH 503034, the second inhibitor, HCV-796 was titrated in. The final concentrations of DMSO and fetal bovine serum were 1% and 10%, respectively. The replicon RNA level was measured using real time PCR (TaqMan[®] assay). GAPDH RNA was used as an endogenous control. Replicon RNA reduction was calculated as below:

- dCT=5BCT-gapdhCT
- ddCT=dCT-dCT of no cpd control
- log RNA reduction=log (1/2^{ddCT})

Figure 2B: Analysis of SCH 503034 and HCV-796 in 1b, BB7 Replicon Cells

- No synergy or antagonism was observed in inhibition of HCV RNA or GAPDH RNA



Genotype 1b, BB7 replicon cells were treated with combinations of SCH 503034 and HCV-796 in the presence of 2% FBS without G418 for 3 days. HCV, GAPDH, and rRNA levels were quantified using TaqMan[®] RT-PCR. The amounts of HCV and GAPDH RNAs were normalized with rRNA, and calculated using an external standard curve method. A 3-dimensional graphical representation of these data, at the 95% confidence intervals, is shown. Additive interactions appear as a horizontal plane at 0%. Any peaks above the plane are indicative of synergy, or greater than expected effects. Conversely, peaks below the plane are indicative of antagonism, or less than expected effects. The degree of synergy or antagonism in the 3-dimensional graph can be quantified using the shading scale on the right. (Prichard MN, Aseltine KR, Shipman JC. MacSynergy II. Version 1.0. User's manual. Ann Arbor, MI: University of Michigan, 1993).

2-Week Combination of SCH 503034 (Boceprevir) and HCV-796

Estimation of Efficacy (ε) Using the Perelson Bi-exponential Model

- The data for HCV levels from one of three comparable studies are graphed in Figure 3.

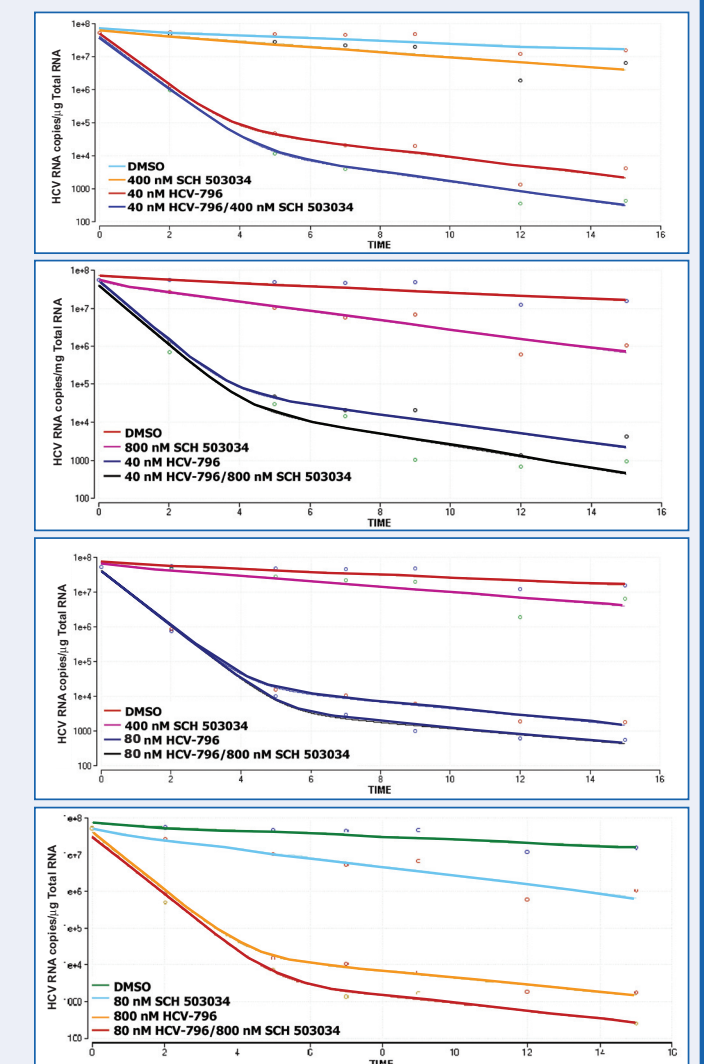
– The impact of combination therapy, throughout the time course, is equivalent to the sum of the impact of each drug independently (within experimental error)
– Comparison of the efficacy parameter estimates (ε) using the Perelson bi-exponential model suggests that the two agents are basically additive i.e. for independent therapies $\epsilon_{\text{comb}} = 1 - [(1 - \epsilon_{\text{HCV-796}})(1 - \epsilon_{\text{SCH 503034}})]$.

Figure 3: (i) 40 nM HCV-796/ 400 nM SCH 503034

(ii) 40 nM HCV-796/ 800 nM SCH 503034

(iii) 80 nM HCV-796/ 400 nM SCH 503034

(iv) 80 nM HCV-796/ 800 nM SCH 503034



Epsilon (ε) Values for HCV-796, SCH 503034 and Combination	
c (mean)	ε (mean)
0.101	0.998
0.996	0.455
0.455	0.710
0.997	0.999
0.999	0.996
0.997	0.997

1b, BB7 cells were treated with the compounds at the specified concentrations under the same conditions as described in Figure 2B. The first (ε) and second (δ) phase slopes as well as initial RNA copy number were estimated by modification of the Perelson bi-exponential model (Neumann, A.U et al., 1998, Science, 282:103-107):

$$RNA(t) = RNA_0 [Ae^{-\lambda_1 t} + (1-A)e^{-\lambda_2 t}]$$

$$\text{where: } \lambda_{1,2} = 1/2\{(c + \delta) \pm [(c - \delta)^2 + 4(1 - \epsilon)c\delta]^{1/2}\}$$

$$A = (\epsilon c - \lambda_2) / (\lambda_1 - \lambda_2)$$

and assuming the half-life of free cytosolic HCV RNA is ~ 9 hr, i.e. c = 1.8 (Dahari H, et al. 2007, J. Virol., 81: 750-760).

Frequency of Resistant Colonies Formation

		HCV-796 (nM)			
		0	30	75	150
SCH 503034 (nM)	0	—	TNTC	TNTC	TNTC
	1000	570	21	3	0
	2000	NA	17	2	0
		97	2	0	0

Number of colonies in each duplicate shown
TNTC: > 800 colonies
NA: not available

Conclusions

- Resistant replicons selected by SCH 503034 were inhibited by HCV-796. Likewise, replicon variants with reduced susceptibility to HCV-796 were inhibited by SCH 503034.
- Time- and dose-dependent inhibition of replicon RNA was observed with both inhibitors.
- An additive intracellular antiviral activity was observed in both 3-day and 2-week combination treatments.
- No cytotoxicity was found in cells treated with combinations of SCH 503034 and HCV-796.
- Frequency of emergence of resistant colonies was significantly reduced by combination treatment.