



NOVEL HEPATITIS C VIRUS NS5A INHIBITORS WITH IMPROVED POTENCY AGAINST GENOTYPE-1A REPLICONS AND REPLICONS CARRYING MUTATIONS ASSOCIATED WITH VIRAL RESISTANCE TO 1ST GENERATION NS5A INHIBITORS

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ABSTRACT P357
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BACKGROUND

HCV NS5A has emerged as an important target for treatment of HCV. The 1st generation NS5A inhibitors display picomolar potency against genotype-1b replicons but are less active against genotype-1a and especially genotype-2 replicons because of the existence of an L31M polymorphism in NS5A of genotype-2 HCV. Consequently, the clinical efficacy of the 1st generation NS5A inhibitors is less in genotype-1a patients than in genotype-1b patients, and is expected to be rather limited in genotype-2 patients. Here, we report novel NS5A inhibitors that exhibit improved potency against genotype-1a replicons and replicons carrying mutations associated with viral resistance to the 1st generation NS5A inhibitors.

METHODS

Compounds

- ACH-NS5A inhibitors with 3 novel core structures were synthesized by Achillion Pharmaceuticals. The control NS5A inhibitor BMS-790052 (BMS-052)¹ was also synthesized by Achillion Pharmaceuticals

HCV Replicons

- Stable Replicon Cells: five cell lines were used, including 1) Huh-9-13, harboring a genotype-1b/strain Con-1 subgenomic replicon that carries a NPTII gene; 2) Huh-Luc/neo, harboring a genotype-1b/strain Con-1 subgenomic replicon that carries both NPTII and luciferase genes; 3) Huh-Luc/neo-1a chimera, derived from Huh-Luc/neo with NS5A from genotype-1a/strain H77; 4) H/SG-neo, harboring a genotype-1a/strain H77 subgenomic replicon that carries a NPTII gene; and 5) Huh-JFH-neo, harboring a genotype-2a/strain JFH-1 subgenomic replicon that carries a NPTII gene
- Transiently Replicating Replicons: all transiently replicating replicons were constructed on the backbone of PI-Luc/ET replicon, a genotype-1b/strain Con-1 subgenomic replicon encoding a luciferase reporter, with the exception of 2a/JFH-1 which is a genotype-2a/strain JFH-1 subgenomic replicon that encodes a luciferase reporter. Two categories of chimeric transiently replicating replicons were used: 1) sequences encoding the entire NS5A in the PI-Luc/ET were replaced with NS5A from genotype-1a/strain H77 or from genotype-1 patient isolates; 2) sequences encoding the 1-100 amino acids of NS5A in the PI-Luc/ET were replaced with the 1-100 amino acids of NS5A from other genotypes.

Potency Assay

- Assessment of the potency of compounds in stable replicon cell lines was conducted as follows: 1) seed stable replicon cells in 96-well plates; 2) Add compounds on the second day in 6 half-log serial dilutions; 3) 3 days after addition of inhibitors, quantify the level of HCV RNA replication with either a luciferase assay or an RNA dot blot assay
- Assessment of the potency of compounds for transiently replicating replicons was conducted as follows: 1) synthesize replicon RNAs *in vitro* from either pooled plasmid clones (patient isolates) or a plasmid clone (all the others); 2) transfect replicon RNA into Huh-Luc/neo cells and seed the transfected cells in 96-well plates; 3) add compounds on the second day in 6 half-log serial dilutions; 4) 3 days after addition of inhibitors, quantify the level of HCV RNA replication with a luciferase reporter assay

Cell Viability Assay

- Cells were plated into 96-well plates and were treated with compounds for 3-5 days. Cell viability was then evaluated with either a CellTiter 96[®] AQueous One Solution kit or a CellTiter-Glo[®] Luminescent Cell Viability kit.

Pharmacokinetic Studies in Animals

- A representative compound was administered to male beagle dogs intravenously at a dose level of 1 mg/kg or orally with a capsule at a dose level of 4 mg/kg. Plasma concentration of the compound was measured at indicated time points by LC-MS/MS. Noncompartmental pharmacokinetic analysis was performed using WinNonlin Professional.

REFERENCE

1. Gao, M., et al., Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature*, 2010, 465(7294): 96-100.

DISCLOSURES

GY, YZ, DP, JF, SW, CM, JR, KS, JW, VG, AH, DC, QW, GP, XW, MD, AP, and MH are employees of Achillion Pharmaceuticals

RESULTS

1 POTENCY COMPARISON: GENOTYPE-1A VERSUS GENOTYPE-1B

Table 1. EC₅₀ Values (pM) in 2 Cell Lines (Luciferase Endpoint)

Compound	Huh-Luc/neo (NS5A: GT-1b)			Huh-Luc/neo-Chimera (NS5A: GT-1a)			Ratio in EC ₅₀ 1a Versus 1b
	Mean	SD	N	Mean	SD	N	
A	3.7	1.7	3	9.8	0.33	2	3
B	2.1	0.57	2	4.7	0.11	2	2
C	1.6	NA	1	1.7	0.13	2	1
D	4.1	8.4	4	267	0.40	2	65
E	5.1	8.4	4	>1000	0.40	2	>195
F	1.6	>50	>50	17	>50	>50	10
G	2.7	>50	>50	230	>50	>50	85
H	4.4	>50	>50	720	>50	>50	162
I	12	>50	>50	78	>50	>50	7
BMS-052	2.9	>50	>50	11	>50	>50	4

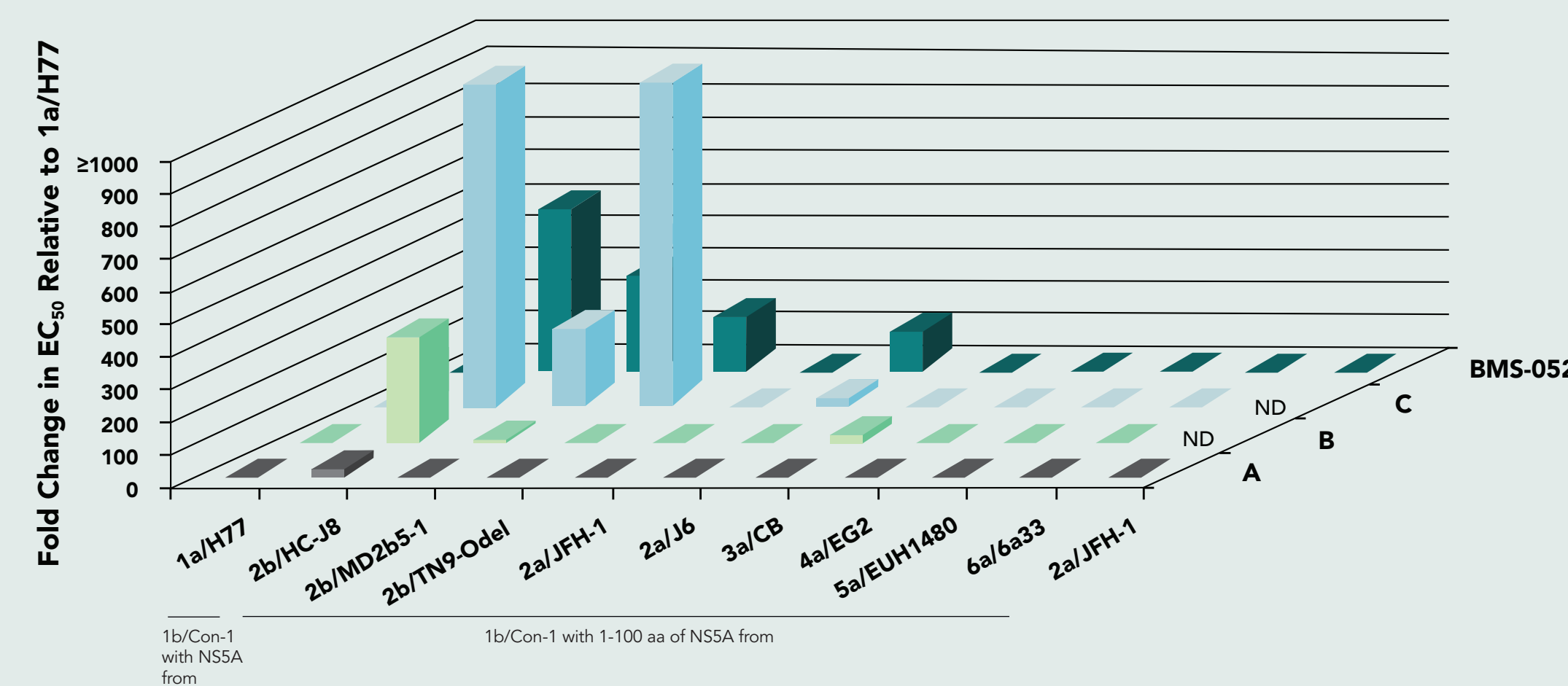
- Representative compounds from each of novel core structures were assessed for their activity against 2 stable replicons: 1 carrying NS5A from genotype-1b; the other carrying NS5A from genotype-1a
- Three compounds (A, B, and C) showed improvement in absolute potency and/or the relative potency against the NS5A genotype-1a stable replicon
- Hence, compounds A, B, and C were chosen for further *in vitro* assessment

2 ANTI-HCV SPECTRUM: GENOTYPE 1-6

Table 2. EC₅₀ Values (pM) in Huh-Luc/neo, H/SG-neo, and Huh-JFH-neo Cell Lines (HCV RNA Endpoint)

Compound	Genotype-1a: H/SG-neo			Genotype-1b: Huh-Luc/neo			Ratio in EC ₅₀ 1a Versus 1b	Genotype-2a: Huh-JFH-neo
	Mean	SD	N	Mean	SD	N		
A	24	1.7	3	5.1	0.33	2	5	28
B	22	0.57	2	2.1	0.11	2	11	17
C	8.5	NA	1	1.7	0.13	2	5	16
BMS-052	56	8.4	4	2.9	0.40	2	20	88

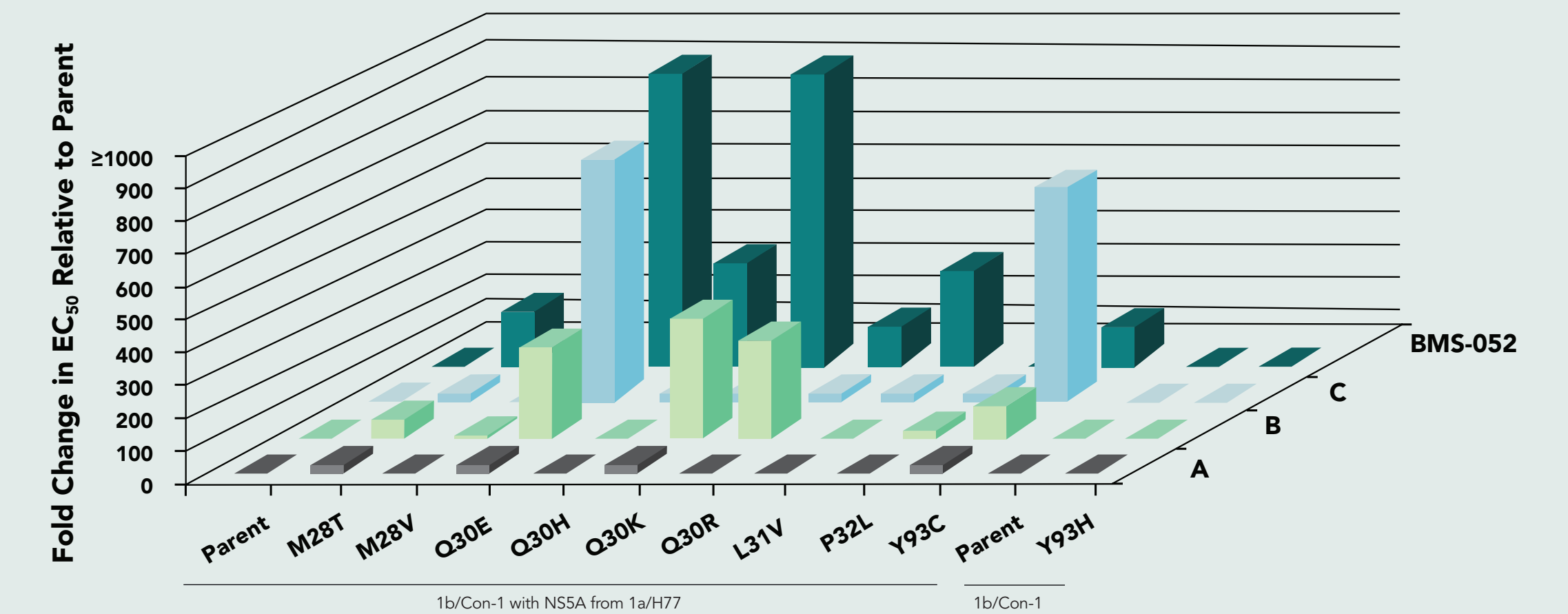
Figure 1. Fold Changes in EC₅₀ Values Against Transiently Replicating Replicons From Genotypes 2-6 (Luciferase Endpoint)



- EC₅₀ values of ACH compounds against genotype-1a replicons are >2-fold lower than that of BMS-052; difference in potency of compounds A and C against genotype-1a and genotype-1b replicon is only 5-fold
- EC₅₀ values of ACH compounds against genotype-2a replicons are >3-fold lower than that of BMS-052
- Improvement in potency against replicons with the 1st 100 amino acids of NS5A from genotype-2a and 2b that carry the L31M polymorphism was observed with ACH compounds; compound A showed the least fold increase against all genotypes/strains tested

3 ANTI-HCV SPECTRUM: RESISTANT VARIANTS

Figure 2. Fold Changes in EC₅₀ Values Against Transiently Replicating Replicons Carrying Mutations (Luciferase Endpoint)



- Significant improvement in potency against replicons carrying various mutations known to confer resistance to 1st generation of NS5A inhibitors was observed with ACH compounds
- Compound A displayed the least fold increase in EC₅₀ against these mutants

4 CELLULAR TOXICITY

Table 3. CC₅₀ Values (μM) in Multiple Cell Lines

Cell Lines	Compound A	Compound B	Compound C	BMS-052
Huh-Luc/neo	>32	>32	>32	23
MT4	>50	>50	>50	26
Hep-2	>50	>50	>50	24
MDBK	>50	>50	>50	20
Vero	>50	>50	>50	3

- No cellular toxicity was observed at the highest concentration tested for all ACH compounds, yielding selectivity indexes of all ACH compounds >3,000,000
- Pharmacokinetic studies in rats and dogs (an example with compound A in dogs is shown in Figure 3) showed a long half-life and high concentration at 24 hours post-administration of the compound

CONCLUSIONS

- Compounds A, B, and C display more potent activity than BMS-052 against genotype-1a as well as less of a difference in potency between genotype-1a and 1b
- Compounds A and B also exhibit less fold changes in potency against a panel of replicons carrying the NS5A target gene from various genotypes/strains
- In addition, compounds A, B, and C demonstrate less fold changes in potency against a panel of replicons carrying the mutations known to confer resistance to 1st generation of NS5A inhibitors
- Among 3 compounds, compound A displays the least variability in potency across all replicons tested
- Compound A possesses a desirable pharmacokinetic profile in preclinical species
- Combined with the data obtained in toxicology studies, compound A (ACH-3102) has been chosen as a clinical development candidate

5 PHARMACOKINETICS

Figure 3. Plasma Concentration Versus Time Profile (Compound A)

