Journal of Medicinal Chemistry

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b00082 • Publication Date (Web): 13 Apr 2018

Just Accepted

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Highlights of the Structure Activity Relationships of Benzimidazole Linked Pyrrolidines Leading to the Discovery of the HCV NS5A Inhibitor Pibrentasvir (ABT-530)

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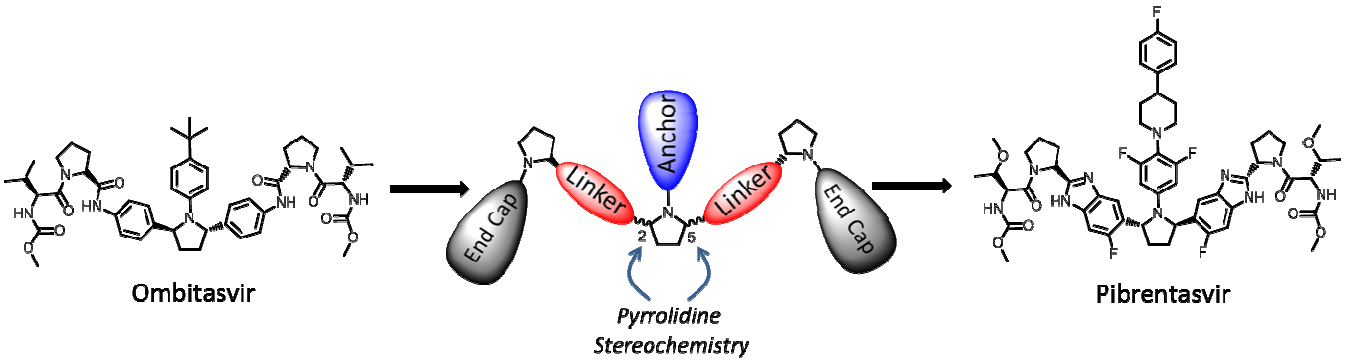
ABSTRACT



Curative interferon and ribavirin sparing treatments for HCV-infected patients require a combination of mechanistically orthogonal direct acting antivirals. A shared component of these treatments is usually an HCV NS5A inhibitor. First generation FDA approved treatments, including the component NS5A inhibitors, do not exhibit equivalent efficacy against HCV virus genotypes 1-6. In particular, these first generation NS5A inhibitors tend to select for viral drug resistance. Ombitasvir is a first generation HCV NS5A inhibitor included as a key component of Viekira Pak for the treatment of patients with HCV genotype 1 infection. Since the launch of next generation HCV treatments, functional cure for genotype 1-6 HCV infections has been achieved, as well as shortened treatment duration across a wider spectrum of genotypes. In this paper we show how we have modified the Anchor, Linker and Endcap architecture of our NS5A inhibitor design template to discover a next generation NS5A inhibitor, pibrentasvir (ABT-530), which exhibits potent inhibition of the replication of wild-type genotype 1-6 HCV replicons, as well as improved activity against replicon variants demonstrating resistance against first generation NS5A inhibitors.

INTRODUCTION

Hepatitis C virus (HCV) was unambiguously characterized in 1989 as the agent responsible for most cases of non-A and non-B hepatitis infection.[1] With current estimates ranging from 71-80 million people worldwide chronically infected with HCV, this viral infection represents a global public health problem.[2] HCV genotype 1, predominant in North America, Europe, and Japan, accounts for over half of the global infections. Genotype 2 infections are most prevalent in North America, Europe and Japan, while genotype 3 and 6 infections are predominant within various parts of Southeast Asia. In Egypt, HCV infections are almost exclusively genotype 4, while genotype 5 is common in South Africa.

Following initial acquisition of the virus, HCV-infected individuals undergo an acute phase, followed in most cases by development of chronic infection.[3] The majority of infected individuals remain asymptomatic for many years after the acute phase, with hepatic fibrosis progressing as the main complication of chronic HCV infection. While there is a tremendous variation in the rate of progression from fibrosis to cirrhosis, with no treatment, the median progression to cirrhosis is 30 years. Of this population, a substantial fraction will continue to experience progression of disease into hepatocellular carcinoma.[4] Complications due to chronic HCV infection have now become the predominant driver for liver transplantation.[5] Additionally, the leading cause of death upon kidney transplantation, which is the most commonly performed organ allograft, is due to chronic liver disease, most commonly precipitated by HCV infection.[6] In 2004, 2.44 deaths per 100,000 people could be attributed to complications due to chronic HCV infection in the U.S.[7] In 2015, it has been reported that 19629 deaths have been attributed to complications related to HCV infection.[8] According to one predictive model, this number is expected to peak in 2030 at ~39,800 deaths per year,[9] though a more conservative prediction places the peak mortality level in 2030 at 12,900 deaths.[10] The CDC In the U.S., of those who do not receive treatment for HCV infection, it is estimated 1 million will die as a consequence of this disease.[10]

Although population genetics studies of HCV indicate an extremely diverse collection of genotypes (1-6) worldwide, HCV genotype 1 comprises ~75% of HCV infections in the U.S., Western Europe, and Japan, and ~60% of HCV infection in Latin America.[11] While treatment of HCV has been

revolutionized with the approval of direct acting antivirals (DAAs), pegylated-interferon/ribavirin therapy was the preferred treatment for genotype 1a/b until recently. Usual therapy required 48 weeks of treatment that was poorly tolerated and provided limited functional cure rates.[12] This functional cure is considered to be achieved when virus RNA levels in blood remain undetectable for 12 weeks after completion of treatment, commonly noted as a sustained viralogic response at 12 weeks (SVR_{12}). It should be noted that while undetectable, sufficient viable virus may still be present to allow for relapse in rare instances. For HCV genotype 1-infected individuals with additional risk factors, such as cirrhosis, HIV co-infection, dialysis treatment, or organ transplants, functional cure are more difficult to achieve.[13] Several teams have successfully conducted clinical studies of multiple DAA-only cocktails for well tolerated curative treatments of this disease, subsequently bringing them to market for genotype 1 HCV infected patients.[14,15,16] Additionally, two of these earlier generation treatments have each been approved for treating HCV patients beyond genotype-1. The combination sofosbuvir/ledipasvir gained additional approval for genotypes 4-6, and ombitasvir/paritaprevir/ritonavir as well as elbasvir/grazoprevir, were approved for genotype 4.[17] Next generation treatment required the ability to achieve SVR_{12} in HCV genotypes 1-6 infections, as well as the ability to inhibit viral replication of those strains that demonstrated resistance against first generation treatments. Since that time, two next generation treatments, sofosbuvir/velpatasvir and sofosbuvir/velpatasvir/voxilaprevir, have been approved by the FDA for the treatment of HCV infections encompassing genotypes 1-6.[18]

The RNA genome of HCV encodes several non-structural (NS) enzymatic and non-enzymatic proteins which enable formation of the poly-protein replication complex prior to the production of new HCV virions. One of these replication complex proteins is NS5A, and although no enzyme activity is associated with this protein it is recognized as essential for viral replication.[19] As already described, several NS5A inhibitors have received FDA marketing approval for use in combination therapy of HCV in recent years, since these inhibitors demonstrate limited utility as single agents due to the emergence of drug resistant HCV variants encountered with mono-therapy.[20] It should also be noted that, although nearly every approved IFN-sparing HCV therapy shares the distinction of including an NS5A inhibitor

as part of its drug combination cocktail, clinical studies need to be conducted in order to assess compatibility between any two agents, as unforeseen drug-drug interactions may either decrease or increase the combination's effectiveness and tolerability. Additional limitations of several current regimens include significant drug-drug interactions, especially in HCV-HIV coinfected patients being treated for HIV, [21] limited options for subjects with renal insufficiency,[22] reduced efficacy in patients with baseline amino acid polymorphisms associated with reduced susceptibility to the HCV NS5A inhibitors or NS3/4A protease inhibitors (PIs).[23]

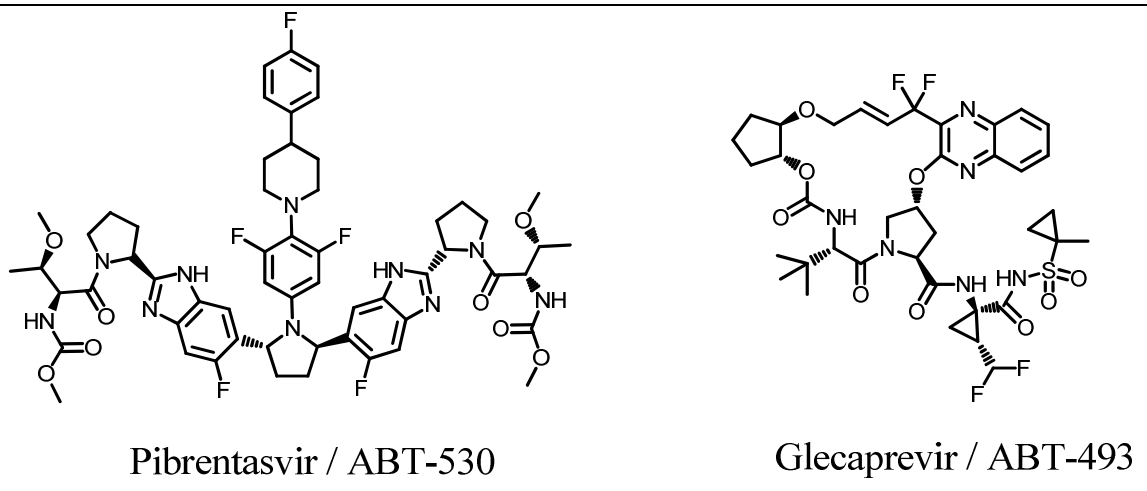


Figure 1. Chemical Structure of Pibrentasvir and Glecaprevir

This paper will describe the discovery of pibrentasvir (ABT-530) which suppresses replication of wild type HCV genotype 1-6 replicons, with a much reduced propensity to elicit drug resistance when compared to other first generation NS5A inhibitors. Pibrentasvir, when combined with glecaprevir (ABT-493) resulted in AbbVie's next generation IFN- and RBV-sparing HCV treatment for the curative treatment of HCV genotypes 1-6 infection.[24]

RATIONALE

Our prior efforts resulted in the discovery of ombitasvir (OMB, Figure 2),[25] the NS5A inhibitor included as a component of the DAA cocktail in AbbVie's first generation IFN-sparing HCV treatment. First generation NS5A inhibitor ombitasvir has been shown to exhibit potent 50% effective concentrations inhibiting replication (EC_{50}s) (ranging 0.82-19.3 pM) against HCV genotypes 1-5 and an EC_{50} of 366 pM against genotype 6a in the replicon assays. *In vitro* resistance selection experiments in genotype 1-6 replicons selected variants which demonstrated reduced susceptibility to the actions of ombitasvir, by factors often greater than 1000-fold.[26] The medicinal chemistry strategy for the discovery of a next generation NS5A inhibitor was to retain the potent antiviral properties of OMB, while introducing significant improvements to genotype coverage, and most significantly, to improve the genotype 1 resistance profile relative to OMB. Replacement of OMB with a compound possessing an improved resistance profile could potentially translate into requiring fewer DAAs in the curative combination.

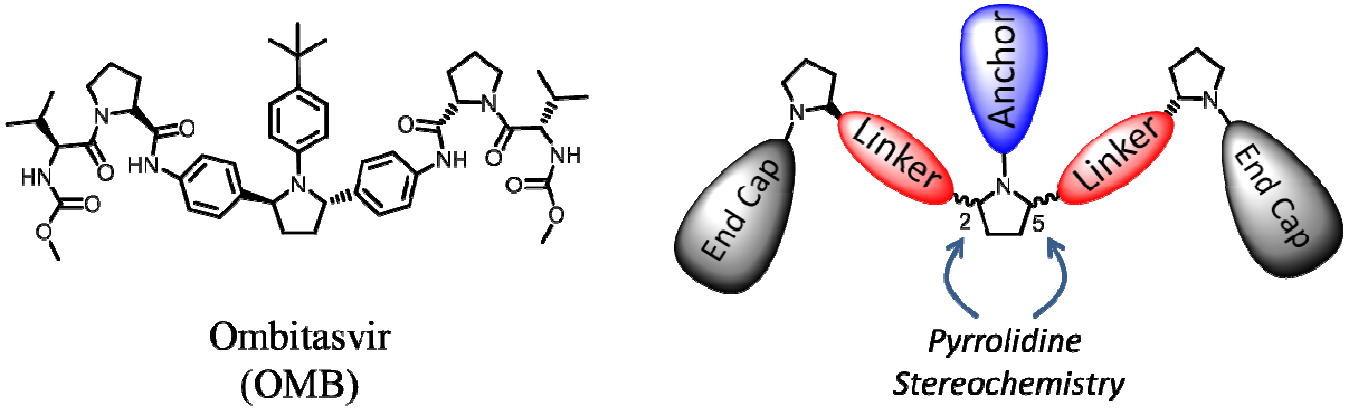


Figure 2. Chemical Structure of ombitasvir and Structural Template Modifications for Next Generation NS5A Inhibitor Design

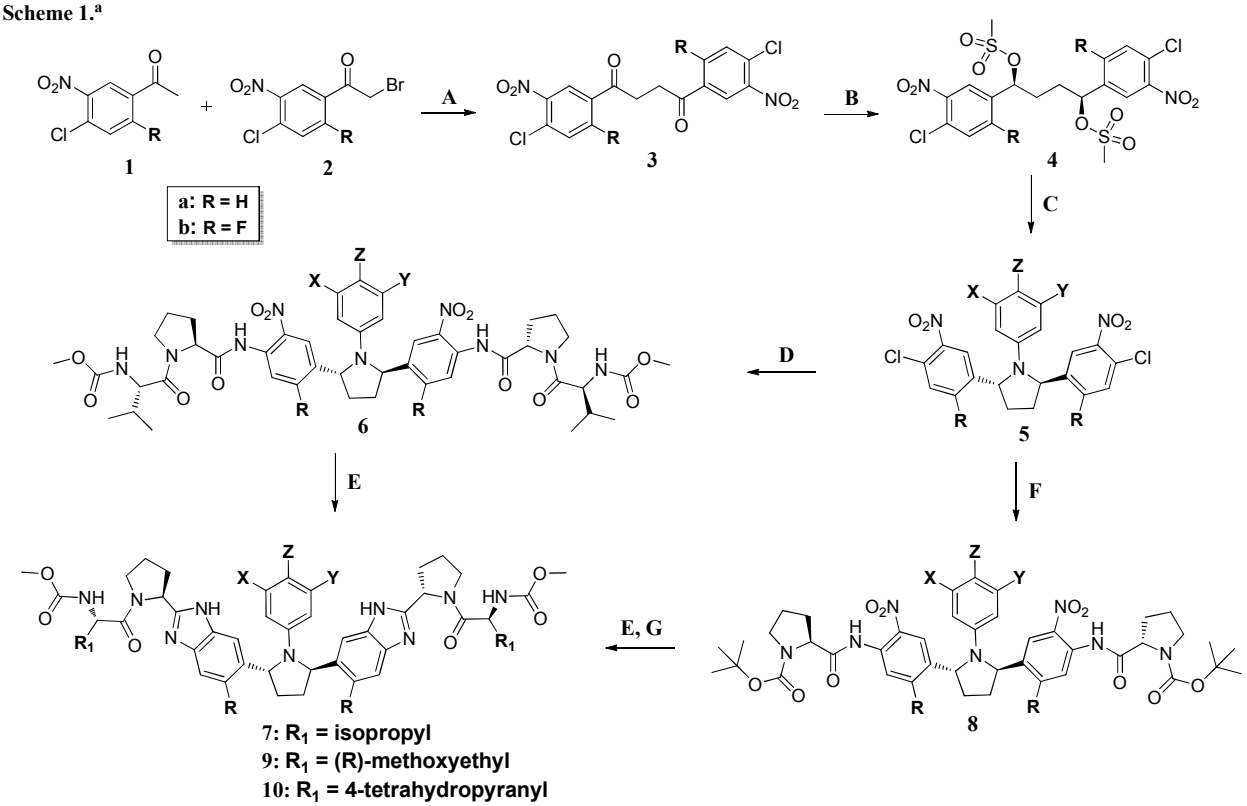
This report will provide an overview of the effects that replacements of the "End Caps," "Linker," and "Anchor Groups," as well as changing the pyrrolidine core stereochemistry (See Figure 2) had on the

virological profile and how these observations provided key insights into crafting the final structure of next generation NS5A inhibitor pibrentasvir.

Chemistry

A stereochemically-controlled method used to synthesize pyrrolidine-core HCV inhibitors targeting the NS5A protein is shown in Scheme 1. Condensation of 4-chloro-3-nitro-phenyl methyl ketone, **1**, with alpha-bromo derivative **2**, gave symmetrical 1,4-diketone **3**.[27] Chiral reduction using (*R*)-(+)-α,α-diphenyl-2-pyrrolidinemethanol, trimethylborate, and diethylaniline borane gave the *S,S*-diol, which was converted to dimesylate **4** and then reacted with the appropriate aniline reagent to give 2,5-*R,R*-pyrrolidine intermediates **5**.[28] Palladium-catalyzed Buchwald coupling of **5** with Moc-L-valine-L-prolinamide provided symmetrically-substituted ortho-nitro acylaniline **6**. Reduction of the nitro groups to give the diamine, followed by heating in acetic acid, formed bis-benzimidazole **7**. In an alternative procedure, compound **5** was coupled with Boc-L-prolinamide to give **8**, which was converted to the bis-benzimidazole intermediate. Removal of the Boc protecting groups with HCl, followed by peptide coupling with Moc-L-valine, gave **7**. Intermediate **8** provided access to HCV inhibitors with various peptide end-capping groups, such as *O*-methyl-threonine analog **9** and 4-tetrahydropyranyl-glycine analog **10**.[29]

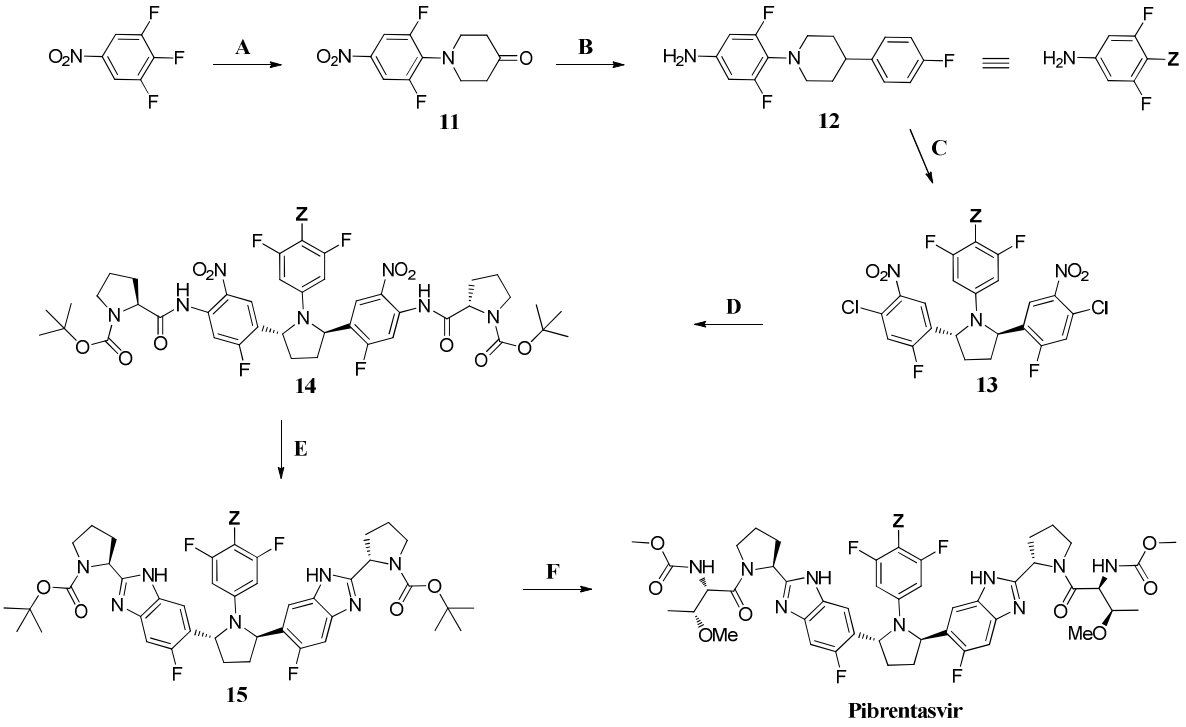
Scheme 1.[a]



[a]**Key:** (**A**) $ZnCl_2$, Et_2NH, *t*-BuOH, benzene. (**B**) i. (*R*)-diphenyl(pyrrolidin-2-yl)methanol, trimethyl borate, *N,N*-diethylaniline borane, THF; ii. MsCl, TEA, CH_2Cl_2, 0 °C. (**C**) $ArNH_2$, Hunig's base, CH_3CN, 60 °C. (**D**) Moc-Val-Pro-NH_2, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, 1,4-dioxane, 100 °C. (**E**) i. PtO_2 or Ra-Ni, H_2, THF; ii. AcOH, 70 °C. (**F**) Boc-Pro-NH_2, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, 1,4-dioxane, 100 °C. (**G**) i. HCl, 1,4-dioxane; ii. Moc-Val-OH or Moc-Thr(OMe)-OH or Moc-2-(*S*)-(4-Thp)Gly-OH, HATU, Hunig's base, DMF.

The method was applied for the synthesis of pibrentasvir as shown in Scheme 2. Heating a DMSO solution of 1,2,3-trifluoro-5-nitrobenzene and dioxolane-protected 4-keto-piperidine, followed by ketal hydrolysis, provided *N*-aryl-piperidinone **11**. The ketone was converted to a vinyl triflate in order to prepare for Suzuki coupling to add the fluorophenyl group. Hydrogenation reduced both the nitro group and olefin to give **12**, which was reacted with *S,S*-dimesylate **4b** (R = F) to give *R,R*-pyrrolidine **13**. Proline amide groups were installed via Buchwald coupling, providing di-nitro intermediate **14**, which was converted to bis-benzimidazole intermediate **15** as described above.

Scheme 2.[a]



[a]**Key:** (**A**) i. 1,4-dioxa-8-azaspiro[4.5]decane, K_2CO_3, DMSO, 100 °C; ii. p-TsOH, 4:1 acetone:water, 50 °C. (**B**) i. LiHMDS, $PhN(Tf)_2$, THF, -78 °C to rt; ii. (4-fluorophenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, LiCl, DME, 100 °C; iii. 10% Pd/C, H_2, THF. (**C**) **7B**, Hunig's base, CH_3CN, 75 °C. (**D**) Boc-Pro-NH_2, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, 1,4-dioxane, 100 °C. (**E**) i. PtO_2, H_2, THF; ii. AcOH, 70 °C. (**F**) i. HCl, 1,4-dioxane; ii. Moc-Val-OH or Moc-Thr(OMe)-OH, HATU, Hunig's base, DMF.

Biology

The primary *in vitro* tool used to screen analogs was the subgenomic HCV cell culture replicon system. Although HCV has a very robust replication rate *in vivo*, efforts to propagate the virus in cell culture have been largely unsuccessful for genotype 1, despite the success of a genotype 2a infectious system.[30] This challenge has led to the development of subgenomic HCV replicons that can be propagated efficiently in the human hepatoma cell line Huh-7.[31] These replicons contain 5' and 3' non-translated regions as well as the HCV non-structural genes. Similar to conventional tissue culture and *in vivo* viral replication, HCV replicon RNA efficiently replicates by undergoing a cycle consisting of translation, polyprotein cleavage (by the NS3/4A protease), RNA synthesis (by the NS5B polymerase), unwinding of the double-stranded RNA (by the NS3 helicase), and assembly of replication complexes containing the HCV nonstructural proteins within Huh-7 cells. The replicon system produces 1,000-10,000 copies

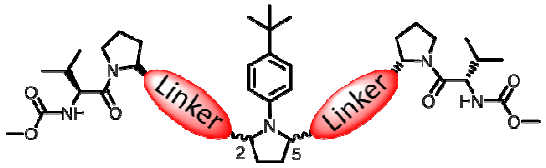
of subgenomic RNA per cell, which is substantially greater than that estimated for *in vivo* infection (50-100 RNA strands/cell). The replicon system lacks assembly and release of virus particles. Therefore, the replicon system is incapable of producing infectious virus, and viral infection of new cells does not occur. Nevertheless, the predictive value of HCV replicons to characterize HCV inhibitors has now long been validated in the clinic, with one of the first instances using the NS3/4A protease inhibitor BILN-2061.[32] Compound potency is reported as EC_{50} against the subgenomic replicons of different genotypes incorporating the firefly luciferase reporter gene. Amino acid positions 24, 28, 30, 31, 32, 58, 92, 93 in NS5A are considered as positions of interest for the NS5A inhibitor class. Of these, variants at positions 28, 30 and 93 were most commonly detected in patients experiencing virologic failure with the first generation NS5A inhibitors, ombitasvir, daclatasvir, and ledipasvir. The resistance profile of compounds was therefore evaluated against representative amino acid substitutions, M28T, Q30E, Q30R, Y93C, Y93H, Y93N in genotype 1a, and Y93H, Y93N in genotype 1b replicons.

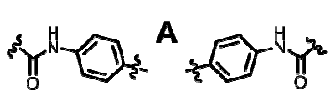
In order to establish the effect of plasma protein binding on the antiviral activity, we tested these compounds in the presence of 5% fetal calf serum (FCS) with or without 40% human plasma. Although it is recognized that the shift in EC_{50} value due to protein binding is static *in vitro*, versus the dynamic conditions encountered *in vivo*, and that individual patient plasma protein binding shows variability, it has nevertheless been established that evaluating protein shifting effects *in vitro* can help to prioritize the advancement of compounds and de-risk hydrophobic chemical compounds as they advance into more costly experiments. This prioritization allows one to roughly estimate the effect of plasma proteins on the potency of compounds and target therapeutic plasma concentrations in the clinic.[33]

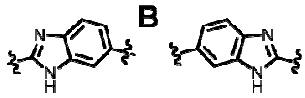
RESULTS AND DISCUSSION

Table 1

Antiviral Activity (EC_{50}, pM) of NS5A Inhibitors in HCV Stable Replicons







cmpd	Chirality Carbons 2,5	Linker Pair	Inhibition of HCV Stable Replicons Containing NS5A from Genotypes 1-6 EC_{50} (pM)									
			1a	1b	40% H. Plasma		2a	2b	3a	4a	5a	6a
					1a	1b						
OMB	S,S	A	14	5	186	56	12	4.3	19	1.7	3.2	366
16	R,R	A	136	9	986	90	NT	NT	NT	NT	NT	NT
17	S,S	B	71	13	1980	354	NT	NT	NT	NT	NT	NT
18	R,R	B	9	13	148	268	152	10	6	6	NT	NT

H. Plasma: human plasma; NT: not tested; pM: picomolar

While seeking to improve upon the virological properties of ombitasvir, whose pyrrolidine core-phenyl amide linker had already served as an exhaustive platform to search for optimal anti-HCV activity, we entertained the notion that perturbing this core structure could open new opportunities for improved virology, that were not otherwise attainable with the original core. An alternative inhibitor scaffold (Table 1), investigated the impact of the "linker" moieties A and B, as well as the absolute stereochemistry at carbons 2 and 5. While the phenyl amide linker presented the desired broad genotype coverage with the chirality at carbon's 2 and 5 being *S*,*S*; altering the stereochemistry *R*,*R*, attenuated the activity of **16** against genotype 1a. 2*S*,5*S*-Benzimidazole analog **17** was weakened in genotype 1a in the presence of 40% human plasma, despite being an isosteric replacement for the linker found in

ombitasvir. Surprisingly, the 2*R*,5*R* isomer **18**, provided potent activity against all the genotypes tested. The most compelling data that indicated a promising path for the benzimidazole lead **18**, came from comparing the lower fold resistance of single point resistant NS5A variants in the 1a and 1b transient replicon experiment against **18** when compared with ombitasvir (Table 2).

Table 2

Fold Resistance of HCV Genotype 1a/1b NS5A Transient Replicon Variants vs. Wild-Type Against NS5A inhibitors **3** and **21**

cmpd	HCV Genotype 1a/1b NS5A Variants vs. Wild Type (Fold Resistance)						
	1a*						1b*
	M28T	Q30E	Q30R	Y93C	Y93H	Y93N	Y93H
OMB	8965	NT	800	1675	41383	66740	77
18	4	61	14	7	216	510	2

*Observed as resistant variants in ombitasvir single agent clinical studies

In mouse PK experiments, **18** demonstrated inferior PK to ombitasvir (Table 3) postulated to result from elevated metabolism, as judged by the lower IV half-life, IV AUC (despite the higher dose), and higher IV clearance measurements (volumes of distribution were similar) vs. ombitasvir. These

Table 3

Comparison of PK Parameters of **3** and **21** in Mouse

cmpd	IV						PO					
	Dose (mg/kg)	$T_{1/2}$ (h)	Vβ (L/kg)	Vss (L/kg)	$AUC_{0\text{-}inf}$ (μg*h/mL)	CLp (L/h/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (nM)	C_{24h} (nM)	$AUC_{0\text{-}inf}$ (μg*h/mL)	F (%)
OMB	3	11	1.8	1.67	26.44	0.11	11.7	7	636	205	11.03	41.7
18	5	2	1.11	0.65	13.13	0.38	2.61	1.67	301	0	1.39	10.6

Formulation: **3** only PO 50:20:10:20 (w/w) PEG-400:Tween 80:Ploxamer 124:Vit.E TPGS; see Experimental Section for additional details

data then set the stage for subsequent program development, as 2,5-R,R-pyrrolidine core, benzimidazole linkers were viewed as a structural platform in which broad HCV genotype coverage might be attainable along with an improved resistance profile. The ultimate objective was to further improve on the initial virological observations seen with **18**, while significantly improving the PK profile of emerging analogs.

Our initial mission sought to achieve greater potencies with comparable activities across the various HCV genotypes, as well as retaining activity against amino acid substitutions commonly selected as resistant variants by first generation NS5A inhibitors. Table 4 summarizes the anti-viral activity of analogs resulting from modifying substituents X, Y and Z in wild-type HCV genotypes 1-4, as well as genotype 6 for selected analogs. A comparison of smaller or comparably sized groups to the *t*-Bu borne by OMB or **18** in the "Z" position revealed that F, Cl, cyclopropyl, cyclohexyl and phenyl all provided picomolar inhibition of wild-type genotype 1-4 HCV replicons, and demonstrated a significant improvement relative to OMB in genotype 6a. This was surprising, as groups such like F, Cl, and cyclopropyl lost significant genotype 1a activity in the prior bis-anilide series relative to genotype 1a and larger groups such as cyclohexyl and phenyl lost significant potency in the presence of 40% human plasma.[25] In the presence of 40% human plasma, the genotype 1a and 1b EC_{50}s remained below 500 pM. Introduction of an oxygen-linking atom (eg. Z = phenoxy in **24**; Z = trifluoromethyloxy in **25**) resulted in analogs possessing similar "antiviral" potency to what we observed for simple halogen, alkyl and aryl substituents. By contrast, introduction of a basic morpholino group (**26**) resulted in much weaker analogs, even upon introduction of a fluorine atom at position "Y" to attenuate basicity.[34] Surprisingly, piperidinyl analogs **28**, **29** and **30** gained potency, especially with the introduction of halogens at both positions X and Y. The unanticipated potency enhancing effect of fluorine at positions X and Y is especially dramatic when comparing analog **31** with **32** and **33**. For example, **32** provides single digit picomolar potency across all tabulated wild-type HCV genotypes as well as 3 pM inhibitory potency for genotype 5a (data not in table).

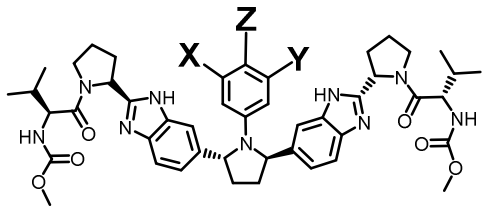


Table 4

SAR of (R,R)-Benzimidazole Linker NS5A Inhibitors

Inhibition of HCV Stable Replicons

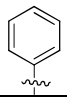
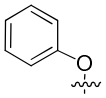
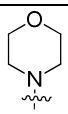
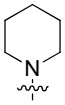
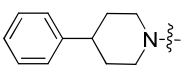
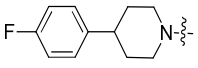
cmpd	X	Y	Z	Inhibition of HCV Stable Replicons Containing NS5A from Genotypes 1-4, and 6, EC_{50} (pM)								
				1a	1b	40% H. Plasma		2a	2b	3a	4a	6a
						1a	1b					
19	H	H	F	38	25	490	427	28	21	31	14	14
20	H	H	Cl	6	7	240	330	12	7	12	5	8
21	H	H		12	23	161	354	41	20	26	20	NT
22	H	H		4	9	107	178	10	7	8	9	NT
23	H	H		4	3	75	108	11	9	16	6	11
24	H	H		12	20	155	279	12	8	11	5	NT
25	H	H		19	12	276	230	43	12	18	13	NT
26	H	H		379	382	902	2070	598	960	1200	316	NT
27	H	F		189	196	474	915	307	229	355	170	NT
28	H	F		18	29	199	383	48	30	44	17	NT
29	F	F		5	6	151	241	13	7	8	5	7
30	Cl	Cl		5	3	213	132	44	5	11	6	10
31	H	H		324	325	1510	1510	468	452	481	243	402
32	F	F		2	6	76	183	8	6	6	4	8
33	F	F		3	8	78	126	20	13	13	9	21

Table 5

SAR of (R,R)-Benzimidazole Linker NS5A Inhibitor - Physical Properties, Metabolism and Mouse PK

cmpd	LogD (HPLC)	PAMPA ($\times 10^{-6}$ cm/s)	CLND Solubility (μM)	Human μ-somal Intrinsic Clearance (L/h/kg)	Rat μ-somal Intrinsic Clearance (L/h/kg)	Mouse PK (3 mg/kg) PO AUC (μg*h/mL)
18	5.3	0.14	1.6	7.3	10	1.39 (5 mg/kg)
26	3.2	0.73	14.2	6.8	6.5	0.002
28	4.7	0.01	2.5	8.4	9.7	2
29	NT	0	0.7	10.5	11.2	1.79
30	5.9	0.03	<0.6	<1.5	3	0.96
31	5.4	NV	0.82	<1.5	2.8	NT
32	NV	0	1.1	<1.5	3.2	6.83
33	5.8	0	0.9	<1.5	3.2	4.59

The physical properties of multiple analogs were measured, trying to establish a correlation between *in vitro* ADME characteristics and AUCs determined after oral administration (Table 5). As all of the molecules fall outside of the boundary of Lipinski's Rule of 5, an effort was extended to improve the water solubility of these molecules by replacing the lipophilic *t*-butyl "R" substituent with moieties expected to lower the logD, increase solubility (CLND) and decrease rat microsomal clearance. Morpholinyl analog **26** showed that as the LogD decreased, the solubility improved, although permeability (desired PAMPA values >2 x 10^{-6} cm/s) did not improve to the required extent, nor was rat microsomal clearance effected substantially. Furthermore, this less lipophilic analog unexpectedly demonstrated even poorer plasma exposure than **18** upon oral administration. Ortho substituted aryl halides **28**, **29** and **30** were synthesized to measure the effect of attenuating piperidine basicity. While higher LogD values, lower aqueous solubility, and poor permeability remained an issue *in vitro*, these

compounds demonstrated improved oral AUCs, but did not achieve the exposures required for additional development. Despite the deleterious effects of lowering the logD upon the oral AUCs, we nevertheless decided to test larger and more lipophilic analogs *in vivo*. When measurable, phenyl or 4-fluoro-phenyl substituted piperidines, **31**, **32** and **33**, demonstrated LogDs greater than 5, with no significant ability to provide intrinsic permeability in the PAMPA assay or solubility in the CLND measurements, yet, for reasons that were not elucidated at that time, oral exposures were unexpectedly improved. It should, however, also be noted that intrinsic permeability measurements in the PAMPA assay are likely misleading due to the very low solubility of these compounds.

Of particular note, in 2016 Randolph et al. reported that 5-fluoro substitution of the benzimidazole linker of analogs **18** and **29**, provided compounds of nearly identical potencies in genotypes 1-4, and 6, while also increasing drug plasma levels after oral dosing in mice.[35] The hypothesis underlying the improvement of oral PK upon benzimidazole fluorination predicted that the halogenation would decrease the basicity of the benzimidazole nitrogens.[36] In order to verify that sufficient antiviral activity of this potentially promising modification extended to other analogs with respect to virology and PK, additional compounds were synthesized. We were gratified to find similar activity across all genotypes for **34** when compared with **32**, but were surprised by the improvement observed for **35** when compared with **33** (see Table 4 and Table 6). While broad genotype *in vitro* activity was achieved in the series, a second requirement of a next generation NS5A inhibitor was effectiveness against replicon variants which confer resistance to first generation NS5A inhibitors.

Table 7 illustrates the SAR of a variety of substituent patterns that, while found to show promise across wild type genotypes 1-6, resulted in significant variation in their ability to suppress the replication of clinically relevant ombitasvir-resistant variants.[26] Examination of the data reveals that the greatest resistance emerged with the Q30E, Y93H and Y93N variants in genotype 1a. Y93 variants of genotype 1b, where tested, were susceptible to all analogs in Table 7. There appears to be a general trend that increased size at position "Z" correlates with an improved ability to suppress replication with lower

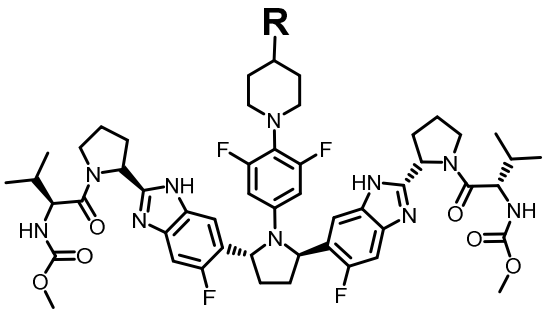


Table 6

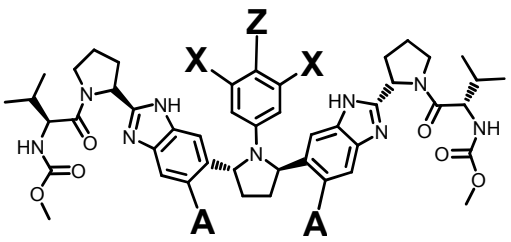
SAR of 6-Fluoro-(R,R)-Benzimidazole Linker NS5A Inhibitors- Antiviral Activity in HCV Stable Replicons

cmpd	R	Inhibition of HCV Stable Replicons Containing NS5A from Genotypes 1-6, EC_{50} (pM)									
		1a	1b	40% H. Plasma		2a	2b	3a	4a	5a	6a
				1a	1b						
34	Phenyl	2	6	58	135	8	3	4	4	2	9
35	4-F-Phenyl	1	3	70	172	3	2	1	1	1	3

multiples of the wild-type EC_{50}. One particular exception to this trend is found for **22**, whose wild-type genotype 1a EC_{50} was lower than the lowest concentration tested. Compounds emerging from these studies, which came close to fulfilling the virology requirements for a next generation analog, are **32**, **33** and **34**. All three compounds deliver a profound improvement in performance against resistant variants when compared to the first generation NS5A inhibitor OMB (ref. 25) or **18**, and maintain reasonable potency against genotypes 1-6. Analog **35** was not characterized against these variants.

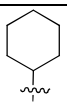
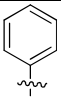
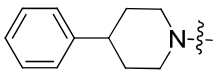
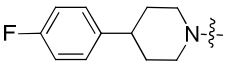
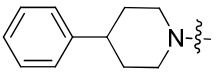
Compounds **34** and **35** were evaluated in the mouse at 3 mg/kg to determine whether or not oral exposures could be improved vs. the unfluorinated benzimidazole analog **32** and **33** (Table 8). It was gratifying to discover that in both cases, the AUC increased by several multiples, thus underscoring the importance of the fluorinated-benzimidazoles towards improving the oral PK properties of resulting analogs, while maintaining virological potency.

Table 7



SAR of NS5A Inhibitors - Fold Resistance of HCV

Genotype1a/1b NS5A Transient Replicon Variants vs.Wild-Type

cmpd	A	X	Z	HCV Genotype 1a/1b NS5A Variants vs. Wild Type (Fold-Resistance)							
				1a*						1b*	
				M28T	Q30E	Q30R	Y93C	Y93H	Y93N	Y93H	Y93N
20	H	H	Cl	28	83	44	22	670	1155	1	NT
22	H	H		>17	>90	>30	>30	>577	NT	1	NT
23	H	H		NT	47	10	24	329	827	1	1
29	H	F		5	134	13	9	241	510	1	NT
32	H	F		3	69	7	6	72	145	1	1
33	H	F		2	20	3	3	20	25	2	NT
34	F	F		1	12	2	6	49	50	1	1

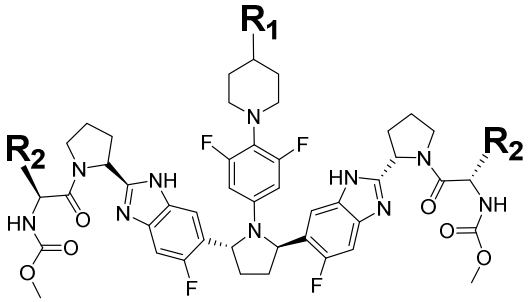
* Observed as resistant variants in ombitasvr single agent clinical studies

Table 8

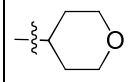
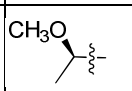
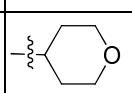
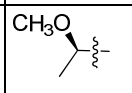
Oral PK Parameters of **37** and **38** in Mouse

cmpd	PO dosing (3 mg/kg)				
	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (nM)	C_{24h} (nM)	$AUC_{0\text{-}inf}$ (μg*h/mL)
34	Unable to determine	24.0	754	754	14.0
35	Unable to determine	24.0	1708	1708	26.1

Table 9



SAR of Endcap Replacements of 6-Fluoro-(*R,R*)-Benzimidazole Linker NS5A Inhibitors Antiviral Activity in HCV Stable Replicons

cmpd	R_1	R_2	Inhibition of HCV Stable Replicons Containing NS5A from Genotypes 1-6, EC_{50} (pM)									
			1a	**1b**	40% H. Plasma		**2a**	**2b**	**3a**	**4a**	**5a**	**6a**
					1a	**1b**						
36	Phenyl		4	8	136	337	9	8	11	6	6	12
37	Phenyl		1	3	43	138	3	3	3	3	2	4
38	4-F-Phenyl		3	7	220	522	8	8	7	6	5	8
PIB	4-F-Phenyl		1.8 ±0.86	4.3 ±1.7	64 ±14	200 ±54	2.3 ±0.65	1.9 ±0.59	2.1 ±0.66	1.9 ±0.61	1.4 ±0.36	2.8 ±0.67

As the fluorobenzimidazole-linked compounds were previously shown to improve oral PK characteristics,[35] final synthetic efforts sought to examine the effects of varying the Moc-Val- "end capping" groups, with the aim of further improving the virological profile, while conserving the central core structure of **33** and **34**. Table 9 summarizes the antiviral activity of the best of these compounds against replicons of wild-type genotypes 1-6. All of these compounds show very potent picomolar EC_{50}s across all genotypes in the absence of 40% human plasma. Indeed, the EC_{50}s of **1** maintain levels below 10 pM in genotypes 1-6.[37] Addition of 40% human plasma attenuated potencies by ca. 30-80 fold, and the best overall profile with respect to EC_{50} potencies across genotypes and the least protein binding attenuation effects were assigned to **37** and pibrentasvir (PIB), both of which contained the Moc-Thr(OMe)- capping group.

Table 10

SAR of End Cap Replacement Analogs

Fold Resistance of Genotype1a/1b NS5A Transient Replicon Variants vs.Wild-Type

cmpd	HCV Genotype 1a/1b NS5A Variants vs. Wild Type (Fold-Resistance)							
	1a*						**1b***	
	M28T	**Q30E**	**Q30R**	**Y93C**	**Y93H**	**Y93N**	**Y93H**	**Y93N**
36	NT	1	NT	NT	3	2	NT	NT
37	1	2	1	1	6	5	1	0.3
38	NT	1	NT	NT	2	2	NT	NT
PIB	2.1	2.4	1.7	1.7	6.7	6.7	0.6	0.6

* Observed as resistant variants in ombitasvr single agent clinical studies

Table 10 provides an overview of the fold-resistance of HCV genotype 1a/1b NS5A variants relative to wild-type in the transient replicon of the analogs resulting from these end capping variations.

When compared to the best analogs present in Table 7 (i.e. **33** and **34**), **37** and PIB provide a significantly improved resistance profile. Indeed, all clinically relevant single amino acid NS5A substitutions confer less than 10-fold resistance relative to wild-type. More details on the virological profile of PIB, including resistant variant "fitness", activity against double/triple amino acid substitutions, the compound's much lower potential than other approved NS5A inhibitors to elicit resistance across all genotypes, and *in vitro* comparisons to daclatasvir, ledipasvir, ombitasvir, elbasvir and velpatasvir, are described by Ng, et al.[37]

Table 11

Pharmacokinetics of PIB in Mouse, Rat, Dog and Monkey at Low Doses

Species	IV					PO						
	Dose (mg/kg)	$T_{1/2}$ (h)	Vc (L/kg)	AUC_{last} (µg*h/mL)	CLp (L/h/kg)	Dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (nM)	C_{24h} (nM)	AUC_{last} (µg*h/mL)	F (%)
mouse	3	2	0.09	252	<0.003	3	ND	11	1140	467	20	7.9
rat	3	6.2	-	-	0.07	3	7.0	5.3	250	41	3.6	9.9
dog	1	7.1	0.1	10.55	0.097	2.5	8.3	3.67	562	82	5.99	29.8
monkey	1	8.3	0.07	6.47	0.15	2.5	5.69	4	260	17	2.25	14.1

Dog and Monkey Formulations: IV 10:90 (v/v) DMSO:PEG-400; PO 70:20:10 (w/w) PEG-400:Tween 20:Poloxamer 124 acidified with 2 eq 1N HCl

Pharmacokinetic characterization of PIB in mouse, rat, dog and monkey is summarized in Table 11. The oral bioavailability observed in all four species is low to moderate. On a per milligram basis, plasma concentrations at the 24 hour time point in rat, dog and monkey are lower than mouse, but are still in the nanomolar range. Adjusted EC_{50}s of PIB due to plasma protein binding against genotype 1a and 1b replicons were shown to be subnanomolar and resistance-fold changes to common NS5A amino acid substitutions were less than 10-fold. Consequently, suppression of HCV replication in patients by PIB was considered achievable, especially if used in combination with another mechanistically

orthogonal agent. Towards that end, *in vitro* synergy with GLE was demonstrated[38] with later clinical studies showing this combination to be effective in patients.[24]

CONCLUSIONS

These studies have examined the effectiveness of altering the linker region of NS5A inhibitors from bis-anilide structures to bis-benzimidazoles. This alteration, in combination with the unexpected finding that reversing the stereochemical linkage at the pyrrolidine central core, improved suppression of the replication of HCV replicons in genotypes 1-4, prompted further structural investigations for more clinically promising inhibitors. These improvements stressed potency across multiple genotypes and an ability to retain activity against resistance-associated amino acid substitutions commonly selected by the first generation NS5A inhibitors. Structural modifications that allowed us to leverage the initial findings into a potent next generation pan-genotypic NS5A inhibitor drove toward larger and more lipophilic anchor substituents, as well as selective fluorination of the anchor and benzimidazole linker groups.

Significant pharmacokinetic challenges in mice were encountered in the early use of the benzimidazole linker in that plasma exposure of the drugs could not be detected upon oral administration. Attempts to improve the physical properties of the agents by the introduction of hydrophilic substituents on the anchor portion of the molecule were not successful. Surprisingly, plasma exposures were achieved upon fluorination of the benzimidazole, as well as increasing the lipophilicity and size of the anchor group.

Pibrentasvir (PIB) was the molecule that emerged from this study due to its possession of the attributes necessary for a successful next generation NS5A inhibitor, namely 1) potent activity across all common genotypes, 2) activity against HCV viral variants demonstrating resistance to OMB and other first generation NS5A inhibitors, 3) high genetic barrier to development of drug resistance in all common genotypes,[37] 4) sufficient plasma exposures upon oral administration to suppress HCV replication, and 5) the ability to be combined with another mechanistically orthogonal antiviral agent.[37]

EXPERIMENTAL SECTION

Replicon assays with laboratory strains.

Compounds were tested against eight stable subgenomic replicon cell lines using the luciferase reporter activity assay as described previously by Ng and Krishnan.[26,37] These include genotype 1a-H77 (GenBank accession number NC004102), genotype 1b-Con1 (GenBank accession number AJ238799), and six chimeras of genotype 1b-Con1 containing the N-terminal region of NS5A from genotype 2a, 2b, 3a, 4a, 5a, or 6a HCV. All replicon constructs were bicistronic subgenomic replicons similar to those described by Bartenschlager and coworkers, and the stable replicon cell lines were generated by introducing these constructs into the Huh-7 human hepatoma cell line. The inhibitory effect of compounds on HCV replication in replicon cells was determined in DMEM containing 5% fetal bovine serum (FBS) with or without 40% human plasma (Bioreclamation, Westbury, NY). The cells were incubated for 3 days and were subsequently lysed and processed according to the manufacturer's instructions (Promega, Madison, WI) to measure the luciferase reporter activity using a Victor II luminometer (Perkin-Elmer, Waltham, MA). Resistance-associated substitutions in NS5A were each introduced into the genotype 1a-H77 or 1b-Con1replicons using the Change-IT Multiple Mutation Site Directed Mutagenesis Kit (Affymetrix, Santa Clara, CA). After the presence of the substitution was confirmed by sequence analysis, the plasmid was linearized and the TranscriptAid T7 High Yield Transcription Kit (Fermentas, Glen Burnie, MD) was used to transcribe the HCV subgenomic RNA from the plasmid. In a transient assay, the replicon RNA containing the substitution was transfected *via* electroporation into a Huh-7 cell line. The cells were incubated for 4 days in the presence of the compounds. The EC_{50} was calculated using nonlinear regression curve fitting to the 4-parameter logistic equation in the Graph-Pad Prism 4/5 software. The protein binding effect on inhibitor potency was assessed by including 40% human plasma in culture media containing 5% FBS during the three day incubation period.

Pharmacokinetic profiles. Unless otherwise noted, compounds were formulated in 2:5:20:73 (v/v) DMSO:Tween 80:PEG-400:D5W (5% dextrose in water) for single 3 mg/kg IV dosing and 40:20:20:20 (w/w) Phosol 53 MCT:PEG-400:Ploxamer 124:Cremophor RH40 for single 3 mg/kg PO dosing in mouse or rat.[39] Heparinized plasma samples were withdrawn at 0.1 (IV only) 0.25, 0.5, 1, 2, 4, 6, 9, 12, and 24 hours post-dosing. Plasma drug concentrations were determined by a liquid chromatography-mass spectrometry (LC-MS) assay as follows. Compounds were separated from plasma samples using protein precipitation with acetonitrile. A 250 μL aliquot of each sample or spiked standard was combined with 25 μL of internal standard and 500 μL of acetonitrile. The samples were vortexed vigorously for one minute followed by centrifugation for 10 minutes at 4 °C. Each supernatant was transferred to a well of a 96-well plate and evaporated to dryness with a gentle stream of nitrogen. The samples were reconstituted with sequential aliquots of acetonitrile and 20 mM ammonium acetate. Compounds and the internal standard were separated from each other and co-precipitated contaminants on a 50 x 3 mm Clipeus 5μm column (Higgins Analytical, Inc.) with a 1:1 acetonitrile:20 mM ammonium acetate mobile phase at a flow rate of 0.3 mL/min. Analysis was performed on a Sciex API2000™ Biomolecular Mass Analyzer with a turbo-ionspray interface, in the negative ion mode. Detection was in the multiple reaction monitoring (MRM) mode at *m/z* 516.2 - >437.0. Compound and internal standard peak areas were determined using Sciex TurboQuan™ software. The drug concentration of each sample was calculated by least squares linear regression analysis (non-weighted) of the peak area ratio (parent/internal standard) of the spiked standards versus concentration. The method, generally evaluated over the concentration range 0-6.4 μg/mL, was linear (correlation coefficient >0.999), with mean accuracy values from 96-107% of theoretical for the analysis of triplicate standards at seven separate concentrations. The limit of quantitation was estimated to be ~20 ng/mL from a 0.25 mL plasma sample.

Chemistry. General Procedures. Reagents and solvents, including anhydrous solvents, were obtained from commercial sources and used as supplied. Column chromatography was carried out on silica gel. ^{1}H NMR spectra were measured using either a Brucker AMX 300 MHz or a Varian Inova 400 MHz spectrometer. Chemical shifts are reported in ppm (δ) and referenced to an internal standard of tetramethylsilane (δ 0.00 ppm). ^{1}H – ^{1}H couplings are assumed to be first-order, and peak multiplicities are reported in the usual manner. MS analysis was conducted using a Finnigan SSQ7000 (ESI) mass spectrometer. All final compounds were purified to >95% purity as determined by reverse phase HPLC performed on a Waters 2695 Separation Module / Waters 2489 UV/Visible Detector equipped with a YMC ODS-A, 5.5 μm, 120Å, 4.6 X 150 mm column using a solvent gradient of 10-100% acetonitrile in water (0.1% TFA).

1-(4-Chloro-2-fluoro-5-nitrophenyl)ethanone (1b). To a solution of 4-chloro-2-fluoro-5-nitrobenzoic acid (16.0 g, 72.9 mmol) in anhydrous dichloromethane (400 mL) was added oxalyl chloride (9.57 mL, 109 mmol) and DMF (2 drops), and the resulting mixture was stirred at room temperature until gas evolution ceased. The mixture was concentrated and dried under vacuum. In a separate, heat-dried reaction flask, a mixture of $ZnBr_2$ (24.6 g , 109 mmol) in anhydrous THF (300 mL) was stirred at -78 ^{o}C while a solution of CH_3MgBr (29.1 mL, 3.0 M in Et_2O, 87 mmol) was added dropwise. The resulting mixture was stirred at -78 ^{o}C for 15 min, and then allowed to warm to rt and stirred for 30 min. The mixture was cooled to -78 ^{o}C, and a solution of the previously prepared acid chloride in anhydrous THF (100 mL) was added dropwise, followed by $Pd(PPh_3)_4$ (1.68 g, 1.46 mmol). The resulting mixture was stirred at -78 ^{o}C for 10 min, and was allowed to warm to rt and stirred for 16 h. The reaction mixture was quenched by adding 1 N aq HCl, and was partitioned between water (100 mL) and dichloromethane (3×300 mL). The combined organic extract was dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica

gel, eluting with 5% ethyl acetate in hexanes, to afford 4**b** (11.79 g, 74%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 6.9 Hz, 1H), 8.04 (d, J = 10.4 Hz, 1H), 2.61 (d, J = 4.0 Hz, 3H).

2-Bromo-1-(4-chloro-2-fluoro-5-nitrophenyl)ethanone (2b). To a solution of **1b** (11.0 g, 50.6 mmol) in THF (400 mL) was added pyridinium bromide perbromide (17.0 g, 53.1 mmol) portion-wise over 10 min. The resulting orange solution was stirred at rt for 20 min, during which time the color changed to light yellow and a precipitate formed. The precipitate was filtered off, and the solution was concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-25% ethyl acetate in hexanes to give **2b** as a light yellow solid (14.39 g, 96%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 6.7 Hz, 1H), 8.10 (d, J = 10.4 Hz, 1H), 4.89 (d, J = 2.1 Hz, 2H).

1,4-Bis(4-chloro-2-fluoro-5-nitrophenyl)butane-1,4-dione (3b). A mixture of $ZnCl_2$ (11.0 g, 81.0 mmol), anhydrous benzene (80 mL), diethylamine (6.34 mL, 60.7 mmol), and *tert*-butanol (5.81 mL, 60.7 mmol) was stirred at rt for 2 h to give a homogeneous mixture. Compounds **3b** (12.0 g, 40.5 mmol) and **1b** (13.2 g, 60.7 mmol) were added, and the mixture was stirred at rt under N_2 for 5 days. A solution of 5% aq H_2SO_4 (100 mL) was added, and the mixture was vigorously stirred for 40 min. The resulting solid product was collected by filtration and washed with benzene, and then water. The collected solid was suspended in 2:1 methanol:water, stirred for several minutes, and collected by filtration and dried to give **3b** as an off-white solid (13.47 g, 77%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 6.8 Hz, 2H), 8.07 (d, J = 10.4 Hz, 2H), 3.40 (s, 4H).

(1*S*,4*S*)-1,4-Bis(4-chloro-2-fluoro-5-nitrophenyl)butane-1,4-diyl dimethanesulfonate (4b). A solution of (*R*)-(+)-α,α-diphenyl-2-pyrrolidinemethanol (0.876 g, 3.46 mmol) and trimethyl borate (0.502 mL, 4.49 mmol) in anhydrous THF (80 mL) was stirred at rt for 1 h and then cooled to 0 ^{o}C. *N,N*-Diethylaniline borane (6.15 mL, 34.6 mmol) was added, and the mixture was stirred at 0 ^{o}C for 20 min before a solution of **3b** (3.74 g, 8.64 mmol) in anhydrous THF (10 mL) was added dropwise. The resulting solution was allowed to slowly warm to rt and stirred overnight. The reaction was quenched with 1 N aq HCl and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered and

concentrated under vacuum, and the crude product was purified by column chromatography on silica gel using a solvent gradient of 0-50% ethyl acetate in hexanes to give the *S,S*-diol as a colorless solid (3.66 g, 97%). A portion of this solid (1.00 g, 2.29 mmol) in anhydrous dichloromethane (20 mL) was cooled to 0 ^{o}C, and triethylamine (0.956 mL, 6.86 mmol) was added, followed by methanesulfonyl chloride (0.446 mL, 5.72 mmol). The resulting mixture was stirred at 0 ^{o}C for 90 min, and was then concentrated under vacuum (avoid heating) to ~1/4 volume. Hexanes was added to give a solid that was collected by filtration, washed with water and air dried to provide **4b** as a colorless solid (1.34 g, 98%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 6.7 Hz, 2H), 7.93 (d, J = 9.8 Hz, 2H), 5.88 - 5.81 (m, 2H), 3.22 (s, 6H), 2.21 - 1.88 (m, 4H).

1-(2,6-Difluoro-4-nitrophenyl)piperidin-4-one (11). A mixture of 1,4-dioxa-8-azaspiro[4.5]decane (8.24 mL, 64.3 mmol), 1,2,3-trifluoro-5-nitrobenzene (5.00 mL, 42.8 mmol), and potassium carbonate (7.10 g, 51.4 mmol) in anhydrous DMSO (35 mL) was stirred for 4 h while heating at 100 °C. The reaction mixture was cooled to rt and partitioned between ethyl acetate (200 mL) and water (2 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give a yellow solid (13 g). The solid was dissolved in a mixture of acetone (100 mL) and water (20 mL), and concentrated aq HCl (18 mL) was added. The resulting solution was stirred for 16 h while heating at 50 ^{o}C. The solution was cooled to rt, and water was added to give a precipitate that was collected and dried to give **11** (8.2 g, 74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.80 (m, 2H), 3.67 (t, J = 6.1 Hz, 4H), 2.63 (t, J = 6.1 Hz, 4H).

1-(2,6-Difluoro-4-nitrophenyl)-1,2,3,6-tetrahydropyridin-4-yl trifluoromethanesulfonate. A solution of **11** (5.00 g, 19.5 mmol) in anhydrous THF (50 mL) was cooled to -78 ^{o}C and a 1.0 M solution of LiHMDS in THF (29.3 ml, 29.3 mmol) was added dropwise over 10 min. The resulting deep red solution was stirred at -78 ^{o}C for 5 min and 1,1,1-trifluoro-*N*-phenyl-*N*-(trifluoromethylsulfonyl)methanesulfonamide (7.67 g, 21.5 mmol) was added. The mixture was stirred at -78 ^{o}C for 1 h and then allowed to warm to rt. TLC (3:1 hexanes:ethyl acetate) showed the reaction to be complete. The mixture was diluted with ethyl acetate and washed with 1 N aq NaOH, water, and

dried over Na_2SO_4. The drying agent was filtered off, and the solution was concentrated under vacuum to give a crude product that was purified by column chromatography on silica gel using a solvent gradient of 0-40% ethyl acetate in hexanes. The title compound was obtained as a yellow oil that crystallized under vacuum (6.12 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.75 (m, 2H), 7.45 – 7.28 (m, 1H), 4.08 – 4.01 (m, 2H), 3.62 – 3.53 (m, 2H), 2.67 – 2.58 (m, 2H).

1-(2,6-Difluoro-4-nitrophenyl)-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine. A mixture of 1-(2,6-difluoro-4-nitrophenyl)-1,2,3,6-tetrahydropyridin-4-yl trifluoromethanesulfonate (1.55 g, 3.99 mmol), 4-fluorophenylboronic acid (0.614 g, 4.39 mmol), 2.0 M aq Na_2CO_3 (6.0 mL, 12.0 mmol), and LiCl (0.507 g, 12.0 mmol) in 1,2-dimethoxyethane (20 mL) was sparged with N_2 for 30 min. Tetrakis(triphenylphosphine)palladium(0) (0.23 g, 0.20 mmol) was added, and the mixture was sparged with N_2 for 10 min more. The reaction container was sealed, and the mixture was stirred for 24 h while heating at 100 ^{o}C. The mixture was cooled to rt and partitioned between ethyl acetate and water. The aq layer was washed with ethyl acetate, and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-20% ethyl acetate in hexanes. The title compound was obtained as an orange solid (0.716 g, 54%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.79 (m, 2H), 7.42 – 7.36 (m, 2H), 7.08 – 7.02 (m, 2H), 6.11 – 6.05 (m, 1H), 4.15 – 4.09 (m, 2H), 3.66 – 3.58 (m, 2H), 2.73 – 2.65 (m, 2H); MS (ESI) *m/z* 335 $(M+H)^+$.

3,5-Difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)aniline (12). To a solution of 1-(2,6-difluoro-4-nitrophenyl)-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine (0.716 g, 2.14 mmol) in THF (20 mL) was added 10% palladium on carbon (114 mg, 0.107 mmol). The reaction container was flushed with N_2, and the mixture was stirred under 1 atm H_2 overnight. The mixture was filtered through Celite, and the filtrate was concentrated under vacuum to give **12** as a colorless solid (0.651 g, 99%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (dd, J = 8.7, 5.6 Hz, 2H), 7.10 (t, J = 8.9 Hz, 2H), 6.14 (d, J = 11.8 Hz, 2H), 5.42 (s, 2H), 3.13 - 2.94 (m, 4H), 2.65 - 2.54 (m, 1H), 1.79 - 1.65 (m, 4H); MS (ESI) *m/z* 307 $(M+H)^+$.

1-(4-((2*R*,5*R*)-2,5-Bis(4-chloro-2-fluoro-5-nitrophenyl)pyrrolidin-1-yl)-2,6-difluorophenyl)-4-(4-fluorophenyl)piperidine (13). To a solution of **4b** (0.60 g, 1.0 mmol) and **12** (0.31 g, 1.0 mmol) in anhydrous acetonitrile (5 mL) was added Hunig's base (0.176 mL, 1.01 mmol), and the resulting solution was stirred at 75 ^{o}C for 36 h. The mixture was cooled to rt and was partitioned between water and ethyl acetate (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-20% ethyl acetate in hexanes to give **13** as a colorless solid (0.364 g, 51%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 9.7 Hz, 2H), 7.76 (d, J = 7.1 Hz, 2H), 7.29 – 7.22 (m, 2H), 7.10 – 7.03 (m, 2H), 5.98 (d, J = 11.8 Hz, 2H), 5.55 (d, J = 6.8 Hz, 2H), 3.08 – 2.90 (m, 4H), 2.61 – 2.36 (m, 5H), 1.86 – 1.57 (m, 4H).

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((((2*R*,5*R*)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrrolidine-2,5-diyl)bis(5-fluoro-2-nitro-4,1-phenylene))bis(azanediyl))-bis(carbonyl))bis(pyrrolidine-1-carboxylate) (14). A mixture of **13** (0.360 g, 0.509 mmol), (*S*)-*tert*-butyl 2-carbamoylpyrrolidine-1-carboxylate (0.273 g, 1.27 mmol), Cs_2CO_3 (0.497 g, 1.53 mmol), and Xantphos (53 mg, 0.092 mmol) in 1,4-dioxane (6 mL) was degassed by sparging with N_2 for 20 min. Tris(dibenzylidineacetone)dipalladium(0) (0.014 g, 0.015 mmol) was added, and the mixture was sparged with N_2 for 5 min. The reaction container was sealed, and the mixture was stirred for 90 min while heating at 100 ^{o}C. The mixture was cooled to rt and partitioned between water and ethyl acetate (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-40% ethyl acetate in heptanes to give **14** (0.29 g, 54%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 – 10.38 (m, 2H), 8.02 – 7.58 (m, 4H), 7.29 – 7.20 (m, 2H), 7.12 – 7.00 (m, 2H), 6.05 (d, J = 12.2 Hz, 2H), 5.56 – 5.47 (m, 2H), 4.34 – 4.18 (m, 2H), 3.45 – 3.32 (m, 4H), 3.06 – 2.89 (m, 4H), 2.59 – 2.39 (m, 3H), 2.26 – 2.10 (m, 2H), 1.97 – 1.75 (m, 8H), 1.73 – 1.57 (m, 4H), 1.41 – 1.24 (m, 18H); MS (APCI) *m/z* 1064.2 $(M+H)^{+}$.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(6,6'-((2*R*,5*R*)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrrolidine-2,5-diyl)bis(5-fluoro-1*H*-benzo[d]imidazole-6,2-diyl))bis(pyrrolidine-1-carboxylate) (15): To a solution of **14** (0.38 g, 0.357 mmol) in THF (2.5 mL) and ethanol (2.5 mL) was added Pt_2O (0.030 g, 0.132 mmol). The reaction container was flushed with N_2, and the mixture was stirred under 1 atm H_2 for 90 min. The mixture was filtered through Celite and concentrated under vacuum. To the residue was added toluene (4 mL) and acetic acid (0.20 mL, 3.5 mmol), and the resulting mixture was stirred for 3 h while heating at 70 ^oC. The mixture was allowed to cool to rt and partitioned between saturated aq $NaHCO_3$ and ethyl acetate (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-10% methanol in dichloromethane to give **15** (0.22 g, 64%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 – 12.06 (m, 2H), 7.46 – 7.37 (m, 1H), 7.30 (dd, *J* = 10.3, 3.1 Hz, 1H), 7.27 – 7.13 (m, 3H), 7.09 – 6.89 (m, 3H), 5.95 – 5.76 (m, 3H), 5.63 – 5.44 (m, 2H), 4.89 (d, J = 8.0 Hz, 1H), 4.85 – 4.77 (m, 1H), 3.58 – 3.43 (m, 2H), 3.40 – 3.31 (m, 2H), 3.02 – 2.77 (m, 5H), 2.56 – 2.49 (m, 2H), 2.29 – 2.10 (m, 1H), 2.02 – 1.71 (m, 8H), 1.70 – 1.52 (m, 4H), 1.41 – 0.89 (m, 18H); MS (ESI) *m/z* 967.2 $(M+H)^+$.

(2*S*,3*R*)-3-Methoxy-2-((methoxycarbonyl)amino)butanoic acid. To a solution of *O*-methyl-L-threonine (1.01 g, 7.59 mmol) in saturated aq $NaHCO_3$ (93 mL) was added methyl chloroformate (0.90 mL, 11.61 mmol) dropwise, and the resulting mixture was stirred at rt for 24 h. The mixture was extracted with methyl t-butyl ether, and then cooled to 0 ºC. The mixture was adjusted to pH 1-2 by addition of concentrated aq HCl, and extracted with ethyl acetate (3x). The combined organic extract was dried over Na_2SO_4, filtered, and concentrated under vacuum to afford the title compound as a colorless solid (1.31 g, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (d, *J* = 8.7 Hz, 1 H), 4.40 (dd, *J* = 8.7, 2.3 Hz, 1 H), 4.01 (dd, *J* = 6.2, 2.4 Hz, 1 H), 3.72 (s, 3 H), 3.37 (s, 3 H), 1.23 (d, *J* = 6.2 Hz, 3H).

(*S*)-6,6'-((2*R*,5*R*)-1-(3,5-Difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrrolidine-2,5-diyl)bis(5-fluoro-2-((*S*)-pyrrolidin-2-yl)-1*H*-benzo[d]imidazole). A solution of **15** (2.46 g, 2.54 mmol) in 2 N HCl in 1,4-dioxane (25.4 mL) was stirred at rt for 2 h. The solution was concentrated

under vacuum, and the residue was partitioned between 3:1 dichloromethane:isopropanol and 1 N aq NaOH. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give the title compound as a colorless solid (1.78 g, 91%).

Dimethyl ((2*S*,2'*S*,3*R*,3'*R*)-((2*S*,2'*S*)-2,2'-(6,6'-((2*R*,5*R*)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrrolidine-2,5-diyl)bis(5-fluoro-1*H*-benzo[d]imidazole-6,2-diyl))bis(pyrrolidine-2,1-diyl))bis(3-methoxy-1-oxobutane-2,1-diyl))dicarbamate (PIB). To a solution of (2*S*,3*R*)-3-methoxy-2-((methoxycarbonyl)amino)butanoic acid (0.33 g, 1.72 mmol) in anhydrous DMF (2.92 ml) was added HATU (0.600 g, 1.58 mmol) and Hunig's base (0.125 mL, 0.717 mmol). The resulting mixture was stirred at rt for 10 min, and a solution of (*S*)-6,6'-((2*R*,5*R*)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)pyrrolidine-2,5-diyl)bis(5-fluoro-2-((*S*)-pyrrolidin-2-yl)-1*H*-benzo[d]imidazole) (0.55 g, 0.72 mmol) and Hunig's base (0.125 mL, 0.717 mmol) in anhydrous DMF (2.92 mL) was added. The resulting solution was stirred at rt for 15 min. Water was added to give a precipitate that was collected by filtration and dried under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-3.5% methanol in dichloromethane to give PIB as a colorless solid (0.538 g, 98%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 – 12.00 (m, 2H), 7.41 – 7.34 (m, 2H), 7.34 – 7.28 (m, 2H), 7.27 – 7.16 (m, 3H), 7.13 – 6.96 (m, 5H), 5.93 – 5.79 (m, 2H), 5.61 – 5.41 (m, 2H), 5.12 – 5.03 (m, 2H), 4.21 (q, J = 8.0 Hz, 2H), 3.85 – 3.70 (m, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 3.47 – 3.36 (m, 1H), 3.29 – 3.05 (m, 4H), 3.04 – 2.83 (m, 6H), 2.59 – 2.49 (m, 2H), 2.23 – 1.52 (m, 14H), 1.27 – 1.16 (m, 1H), 1.06 – 0.86 (m, 6H); MS (ESI) *m/z* 1113.4 $(M+H)^+$.

ASSOCIATED CONTENT

Supporting Information

Additional syntheses, analytical information and SMILES strings for the compounds which are the subject of this publication are available free of charge via the Internet at http://pubs.acs.org.

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NOTES

The authors declare the following competing financial interest(s): R.W., J.T.R., L.N., M.A.M., J.K.P., D.L., A.C.K., P.L.D., T.I.N., P.K., T.P-M., C.C. N.P., T.R., T.D., D.F.S., Y.G., W.G. and W.M.K. are employees of AbbVie. S.V.P., R.K., D.K.H., C.F., D.B., T.R., C.J.M., R.M. and D.A.B. are former Abbott/AbbVie employees. This study was sponsored by AbbVie. AbbVie contributed to the study design, research, interpretation of data, writing, reviewing, and approval of the publication.

ABBREVIATIONS USED

CLND, chemiluminescent nitrogen detection; cmpd, compound; DAA, direct acting antiviral; FCS, fetal calf serum; GLE, glecaprevir; IFN, interferon; H. plasma, human plasma; NS, non-structural; NS3, nonstructural 3 helicase protein; NS3/4A, nonstructural 3/4A protein; NS5A, non-structural 5A protein; NS5B, non-structural 5B protein; NT, not tested; OMB, ombitasvir; PI, protease inhibitor; PIB, pibrentasvir; pM, picomolar; RBV, ribavirin; SVR_{12}, sustained viralogic response at 12 weeks post dosing

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39) All animal studies were approved by the AbbVie Institutional Animal Care and Use Committee (IACUC) and conducted in an AAALAC accredited facility to ensure high standards of animal care and use.

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