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Article

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Synthesis and Biological Characterization of Aryl Uracil Inhibitors of Hepatitis C Virus NS5B Polymerase: Discovery of ABT-072, a Trans-Stilbene Analog with Good Oral Bioavailability

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KEYWORDS. Hepatitis C, antiviral, NS5B, ABT-072

abstract

ABT-072 is a non-nucleoside HCV NS5B polymerase inhibitor that was discovered as part of a program to identify new direct-acting antivirals (DAAs) for the treatment of HCV infection. This compound was identified during a medicinal chemistry effort to improve on an original lead, inhibitor **1,** which we described in a previous publication. Replacement of the amide linkage in **1** with a trans-olefin resulted in improved compound permeability and solubility, and provided much better pharmacokinetic properties in preclinical species. Replacement of the dihydrouracil in **1** with an *N*-linked uracil provided better potency in the genotype 1 replicon assay. Results from Phase 1 clinical studies supported once-daily oral dosing with ABT-072 in HCV infected patients. A Phase 2 clinical study that combined ABT-072 with the HCV protease inhibitor ABT-450 provided a sustained virologic response at 24 weeks post dosing (SVR_{24}) in 10 of 11 patients who received treatment.

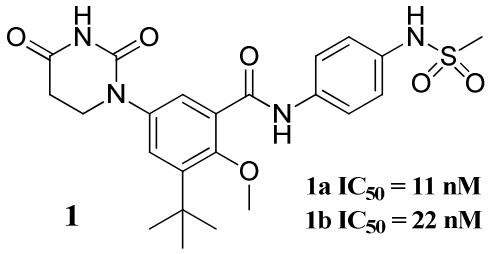
Introduction

Hepatitis C virus (HCV) infection is a global health problem, with over 185 million infected individuals at risk of serious and potentially life-threatening liver disease.[1] The majority of those currently infected are asymptomatic and thus likely unaware of their dilemma. Yet, 75-85% will eventually develop a chronic infection, and 10-20% will develop cirrhosis, a very serious condition that greatly increases the odds of developing hepatocellular carcinoma. HCV-related deaths are on the rise in the US, with close to 20,000 US death certificates listing HCV as the cause of death in 2014, according to recent CDC mortality statistics. In the US, the total number of combined deaths from 60 other infectious diseases, including HIV, tuberculosis, and pneumococcal disease is surpassed by the number of HCV deaths alone, and this is reported to be only a fraction of total deaths that can be attributed to complications from liver disease caused by HCV.[2]

Recent advances in HCV therapy have been realized through the use of drug combinations with orally administered direct-acting antivirals (DAAs).[3] Highly effective HCV combination therapy utilizes two or more DAAs targeting multiple HCV drug target proteins that play different roles in the viral life cycle.[4] DAAs that inhibit HCV NS5B polymerase are among the most clinically useful agents for HCV treatment.[5] These antiviral agents interfere with replication of the viral RNA genome, and are divided into two inhibitor classes. Nucleoside inhibitors bind to the catalytic site of NS5B as their triphosphate metabolites and function by interrupting the genetic message as RNA chain terminators. Non-nucleoside inhibitors (NNIs) bind to one of four different allosteric sites in NS5B, commonly referred to as NNI sites I, II, III, and IV. NNI site binders inhibit NS5B by affecting the conformation of the enzyme, thereby interfering with its catalytic function.[6]

Research at AbbVie targeting HCV polymerase was first to discover a series of *N*-aryl uracil analogs as a novel structural class of NS5B NNI.[7] Our original report described dihydrouracil analog **1**, which is a potent inhibitor of genotypes 1a and 1b polymerase and is active in genotype 1 (GT1) replicon assays (1a EC_{50} = 51 nM and 1b EC_{50} = 19 nM). Replicon activity is maintained when the assay is conducted in the presence of 40% human plasma (1a EC_{50} = 61 nM and 1b EC_{50} = 22 nM), indicating a negligible effect of plasma protein binding on cell potency. However, compound **1** has poor pharmacokinetic properties in rat, with high plasma clearance and poor oral bioavailability (F = 1.4%), providing low plasma concentrations of drug following oral dosing. Physical properties of this compound that can be associated with poor oral exposures include low aqueous solubility (< 1.5 μM at pH 7.2) and poor membrane permeability. The solubility problem is likely related to the compound's high melting point (>300 ^{o}C). Based on an excellent antiviral activity profile, compound **1** became the primary lead for our medicinal chemistry team, which launched an effort to design inhibitors with an improved pharmacokinetic profile.

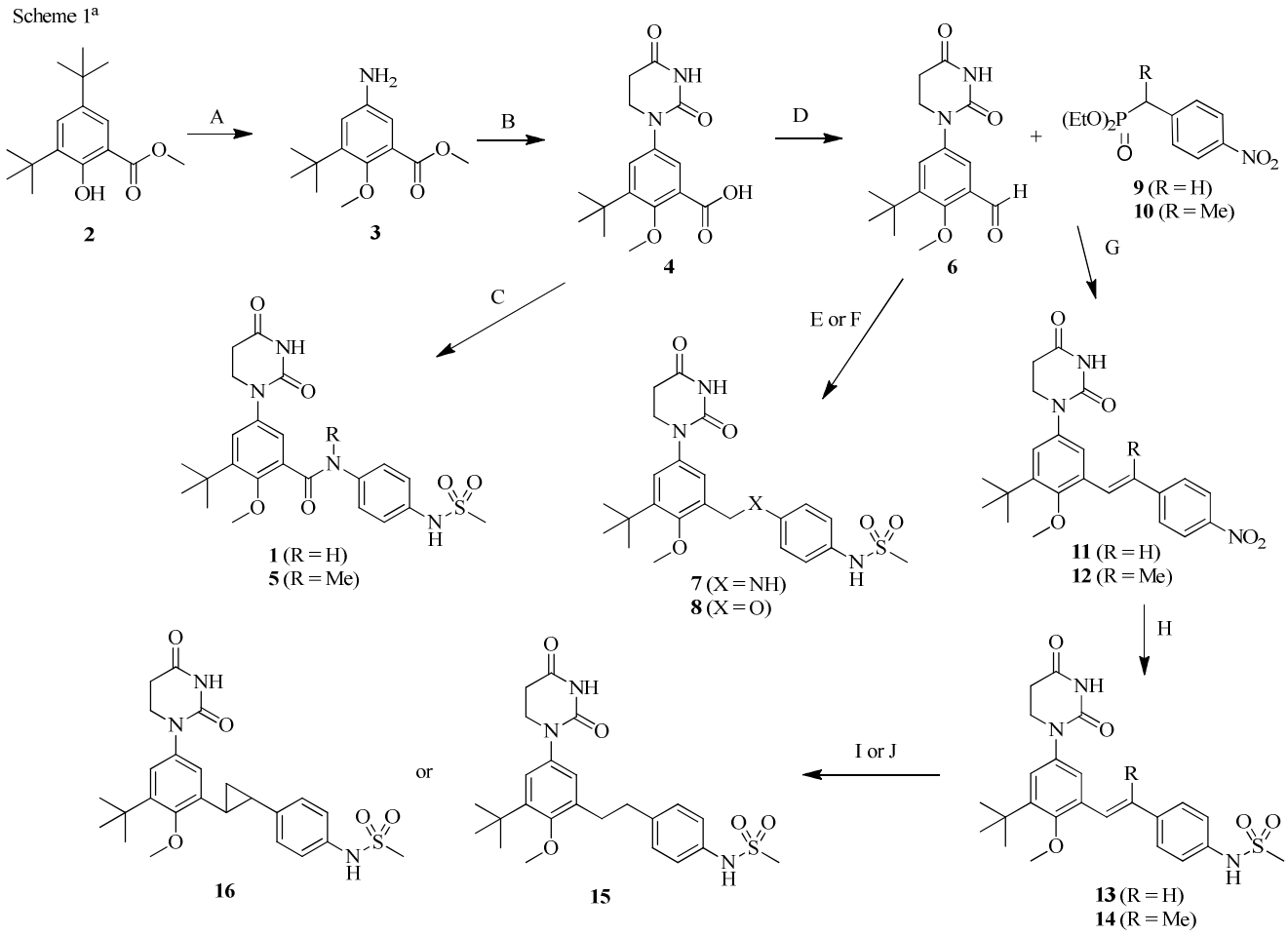
Figure 1. A novel inhibitor of HCV NS5B.[7]



Chemistry

Synthesis of dihydrouracil analogs was accomplished as shown in Schemes 1 and 2. Ipso-nitration of di-*tert*-butyl salicylate **2** resulted in replacement of the alkyl group para to the hydroxyl substituent. Methylation followed by nitro reduction provided compound **3**, which was N-alkylated with acrylic acid in refluxing toluene to form the propionic acid derivative. Heating this compound at 120 ^{o}C with urea in acetic acid formed the dihydrouracil, which was subjected to ester hydrolysis to provide **4**. Synthesis of amide analogs, such as **1** and **5**, proceeded via the acid chloride, formed by heating **4** in thionyl chloride. Alternatively, the acid chloride intermediate formed from **4** could be reduced to aldehyde **6** by the action of lithium tri-*tert*-butoxyaluminum hydride. Aldehyde **6** proved to be a versatile intermediate for making multiple analogs, including benzylamine **7**, formed by reductive amination with *N*-(4-aminophenyl)methanesulfonamide, and benzylether **8**, accessed through the benzyl bromide intermediate derived from the corresponding benzyl alcohol. Olefination of **6** using phosphonates **9** and **10** provided stilbene derivatives **11** and **12**, which were converted to **13** and **14** by nitro reduction and sulfonylation. Compound **13** was subjected to hydrogenation to provide **15**, or cyclopropanation to give **16**.

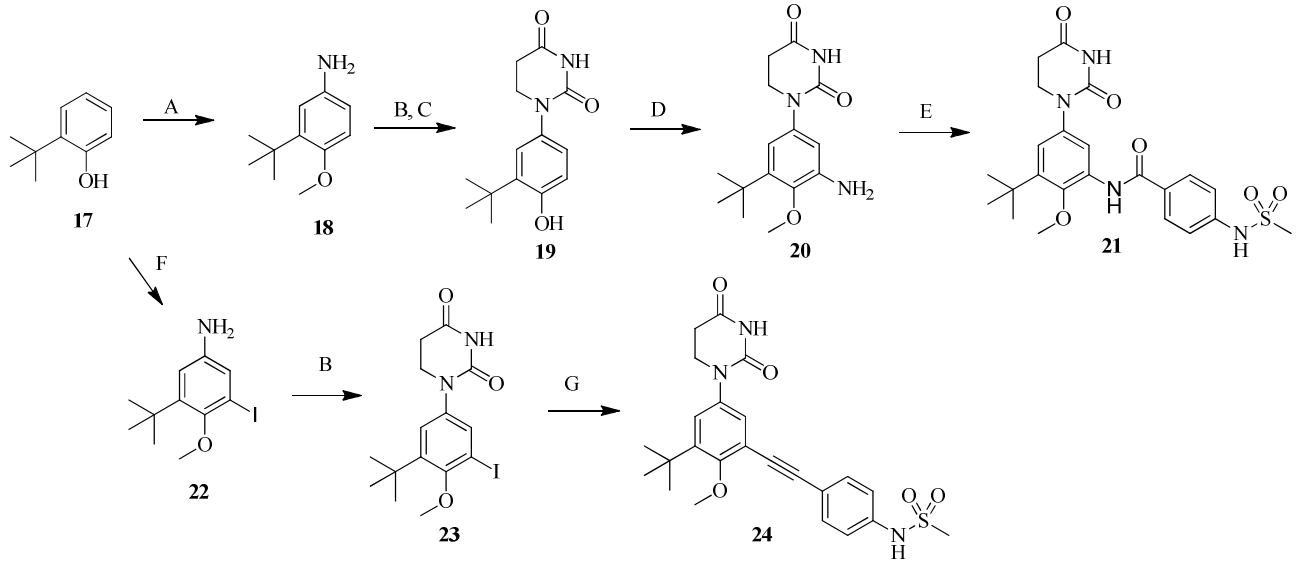
Scheme 1[a]



[a]Key: (A) i. HNO_3, AcOH, rt; ii. Me_2SO_4, K_2CO_3, acetone, reflux; iii. Fe, NH_4Cl, H_2O, MeOH, reflux. (B) i. acrylic acid, toluene, reflux; ii. urea, HOAc, 120 °C; iii. NaOH, H_2O, MeOH, THF, rt. (C) i. $SOCl_2$, reflux; ii. *N*-(4-aminophenyl)methanesulfonamide, pyridine, CH_2Cl_2, rt or *N*-(4-(methylamino)phenyl)methanesulfonamide, DMA, pyridine, 80 °C. (D) i. $SOCl_2$, reflux; ii. $LiHAl(OtBu)_3$, THF, -78 °C; (E) *N*-(4-aminophenyl)-methanesulfonamide, $NaCNBH_3$, AcOH, MeOH, rt. (F) i. $NaBH_4$, MeOH; ii. PPh_3, NBS, CH_2Cl_2, rt; iii. TBAI, Cs_2CO_3, *tert*-butyl (4-hydroxyphenyl)carbamate, DMF, rt; iv. HCl, dioxane, rt; v. CH_3SO_2Cl, pyridine, CH_2Cl_2, rt. (G) KOtBu, CH_2Cl_2, rt. (H) i. Fe, NH_4Cl, H_2O, EtOH, THF, reflux; ii. CH_3SO_2Cl, pyridine, CH_2Cl_2, rt; (I) H_2, Pd/C, MeOH, rt. (J) $Pd(OAc)_2$, trimethylsilyldiazomethane, THF, rt.

Other dihydrouracil derivatives were synthesized as shown in Scheme 2. Selective nitration para to the hydroxyl group in phenol **17**, followed by methylation and nitro reduction, provided aniline intermediate **18**. The dihydrouracil group was assembled by the 2-step process of heating with acrylic acid in toluene to affect *N*-alkylation to the propionic acid derivative, followed by heating with urea in acetic acid. Demethylation with boron tribromide gave **19**, a step which proved necessary in order to achieve satisfactory yields in the subsequent nitration. Methylation with TMS-diazomethane, followed by hydrogenolysis to reduce the nitro group, provided aniline **20**. Acylation with 4-nitrobenzoyl chloride, and 2-step conversion of the nitro group to methanesulfonamide, gave **21**. The nitration product of **17** was iodinated with iodochloride, followed by methylation and nitro reduction to give **22**. This compound was converted to dihydrouracil **23** and subjected to Sonogashira coupling with *N*-(4-ethynylphenyl)methanesulfonamide to provide acetylene analog **24**.

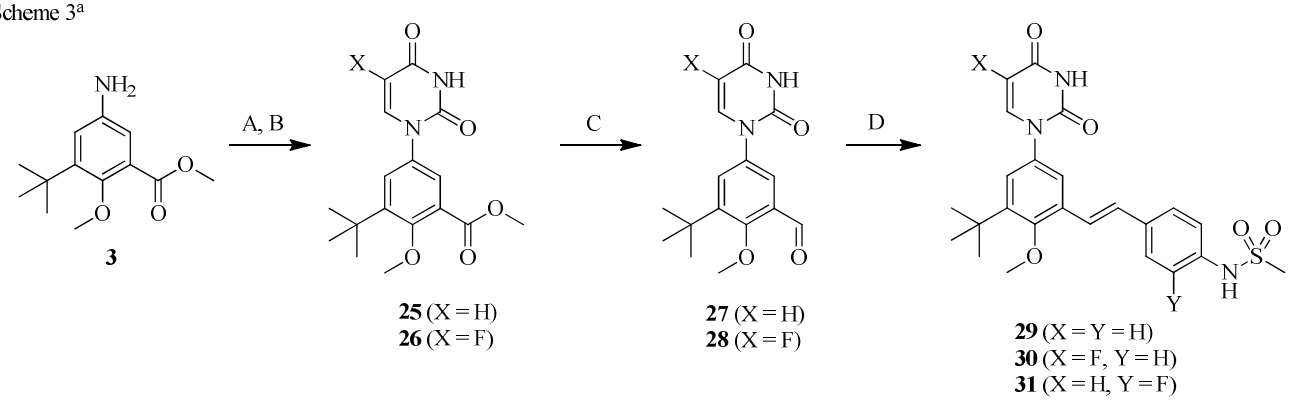
Scheme 2[a]



[a]Key: (A) i. HNO_3, AcOH, 0 °C; ii. Me_2SO_4, K_2CO_3, rt; iii. H_2, 10% Pd/C, EtOAc, rt. (B) i. acrylic acid, toluene, 100 °C; ii. urea, HOAc, 120 °C. (C) BBr_3, CH_2Cl_2, reflux. (D) i. HNO_3, AcOH, rt; ii. trimethylsilyldiazomethane, THF, rt; iii. H_2, 10% Pd/C, EtOAc, rt. (E) i. 4-nitrobenzoyl chloride, pyridine, CH_2Cl_2, rt; ii. H_2, 10% Pd/C, MeOH, CH_2Cl_2, rt; iii. CH_3SO_2Cl, pyridine, CH_2Cl_2, rt. (F) i. HNO_3, heptane, H_2O, rt; ii. ICl, MeOH, H_2O, rt; iii. trimethylsilyldiazomethane, MTBE, MeOH, 0 °C to rt; iv. Fe, NH_4Cl, EtOH, THF, H_2O, reflux. (G) *N*-(4-ethynylphenyl)methanesulfonamide, CuI, Et_3N, $PdCl_2(PPh_3)_2$, CH_3CN, 80 °C.

Uracil derivatives were synthesized as shown in Scheme 3. The uracil group was installed on compound **3** via a 2-step procedure that involved reaction with (*E*)-3-methoxyacryloyl isocyanate to give the corresponding *N*-(phenylcarbamoyl)acrylamide derivative, followed by heating with sulfuric acid in aqueous ethanol. Uracil intermediate **25** was converted to 5-fluoro derivative **26**. Ester hydrolysis, followed by conversion to the acid chloride and reduction with lithium tri-*tert*-butoxyaluminum hydride gave aldehyde intermediates **27** and **28**. Olefination with phosphonate **9**, followed by nitro reduction and methanesulfonylation provided stilbene analogs **29** and **30**. Compound **31** was prepared from **27** using the same sequence of steps, but replacing **9** with diethyl (3-fluoro-4-nitrobenzyl)phosphonate.

Scheme 3[a]



[a]Key: (A) i. (*E*)-3-methoxyacryloyl isocyanate, DMA, -25 °C to rt; ii. H_2SO_4, EtOH, H_2O, 100 °C. (B) i. 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate), CH_3CN, MeOH, 90 °C; ii. TEA, MeOH, rt. (C) i. NaOH, H_2O, EtOH, rt; ii. $SOCl_2$, 80 °C; iii. $LiHAl(OtBu)_3$, THF, -78 °C. (D) i. KOtBu, **9**, CH_2Cl_2, rt; ii. Fe, NH_4Cl, H_2O, EtOH, 75 °C; iii. CH_3SO_2Cl, pyridine, CH_2Cl_2, rt.

Results and Discussion

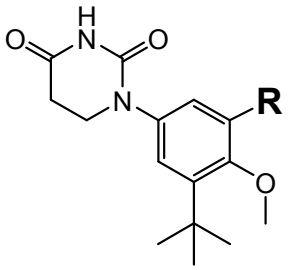
Our medicinal chemistry team investigated replacement of the amide linker between the two phenyl groups in **1**. Suitable alternative linkers that eliminated one or both of the two hydrogen-bonding groups were expected to provide compounds with better membrane permeability. Improvements in aqueous solubility might also be provided, if the resulting compounds were less crystalline than **1**.

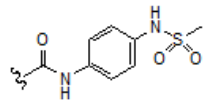
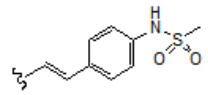
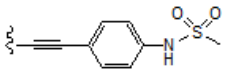
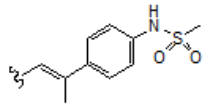
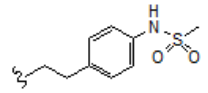
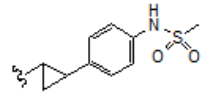
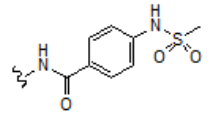
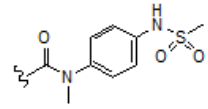
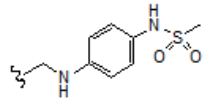
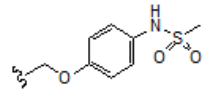
All-carbon linkers were found to be particularly well suited for our needs, providing good passive membrane permeability as demonstrated in a PAMPA assay, as well as better aqueous solubility (Table 1). In addition to these improvements, the stilbene (**13)** and acetylene (**24**) analogs were found to be more potent in GT1 enzyme and replicon inhibitory assays. Compound **13** is a sub-nanomolar inhibitor of both 1a and 1b polymerase as the result of a > 30-fold improvement in enzyme activity relative to amide-linked **1**. It is also a potent inhibitor in the GT1b replicon (EC_{50} = 1.1 nM) due to a nearly 20-fold improvement in cell-culture activity. Activity improvements for acetylene **24** relative to **1** were 10-fold in the enzyme assays and 5-fold in the replicon. The tri-substituted olefin **14** was also more active than **1**, although it was substantially less active and less soluble than stilbene analog **13**.

Both **13** and **24** have linker groups that impart constraints on compound conformation, as does the amide-linked analog **1**. The more flexible ethyl-linked compound **15** was considerably less active, illustrating that the degree of conformational control provided by **1**, **13**, and **24** is important to the activity of these inhibitors. The poor activity of cyclopropyl-linked analog **16** is likely the result of this particular linking group stabilizing a conformation that does not bind well to the enzyme.

Other alternative linking strategies investigated were unproductive. Reversing the amide group gave compound **21**, which had improved aqueous solubility, but similar activity and PAMPA permeability relative to **1**. Unfortunately, the increased solubility of **21** resulted in no significant improvement in oral PK properties in rat (F = 4.3%). Alkylation of the amide nitrogen was detrimental to activity. Tertiary amide analog **5** suffered a > 10-fold loss in potency in the enzyme assays, as well as a nearly 100-fold loss in replicon potency. Benzylamine-linked **7** had the best aqueous solubility of compounds made in this series, although it had poor PAMPA permeability and was substantially less active than **1**. The loss in activity for **7**, as well as benzyl ether **8**, is likely due in part to greater linker flexibility in these analogs relative to more active compounds, such as **1**, **13**, and **24**.

Table 1. In vitro properties of dihydrouracil HCV polymerase inhibitors.



Compound	R	NS5B IC_{50} (nM) 1a	NS5B IC_{50} (nM) 1b	1b Replicon EC_{50} (nM) 0% HP[a]	1b Replicon EC_{50} (nM) 40% HP[a]	PAMPA 10^{-6} cm/sec	aq sol. (μM) pH 7.2	HLM[b] % rem.	RLM[c] % rem.
1		10.8	21.7	19	22	0.25	< 1.5	>85	>85
13		0.3	0.7	1.1	3.1	0.92	10.3	48.1	33.5
24		1.1	3.0	3.9	29.8	0.83	2.3	35.8	50
12		4.9	16.7	12.4	82.8	0.58	< 2	18.5	36.5
15		26.5	40.3	39.1	91.3	4.06	18.8	2.5	1.6
16		267	514	56	431	2.8	18.8	26.5	32.3
21		8.8	46.0	29	21	0.22	32.8	>85	>85
5		140	273	1550	--	0.25	> 50	64	83
7		80	270	522	--	0.41	90.8	67	58
8		100	167	53	321	7.47	3.0	>85	80

[a]Human plasma; [b]Percent remaining after 30 min incubation in human liver microsomes; [c]Percent remaining after 30 min incubation in rat liver microsomes

Stilbene analog **13** and acetylene analog **24** have similar metabolic stability *in vitro*, with 34-50% of parent remaining following 30 minute incubation in human and rat liver microsomes. Substantial improvements in both PAMPA permeability and aqueous solubility for these two compounds in comparison to **1** made them good candidates for pharmacokinetic study in rat. Both compounds gave higher plasma exposures and better oral bioavailability following a 5 mg/kg oral dose (Figure 2 and Table 2). Stilbene **13** provided the best overall results, with relatively low IV clearance (0.64 L/hr/kg) and higher plasma levels with oral dosing (30-fold and 7-fold higher AUC relative to **1** and **24**, respectively). Plasma concentrations of **13** were maintained above 100 ng/mL (> 200 nM) out to 8 hours post dosing, exceeding the plasma-adjusted 1b replicon EC_{50} value for **13** (3.1 nM) by over 60-fold. By contrast, acetylene **24** achieved a maximum concentration of 60 ng/mL (128 nM), an exposure level that is only a few fold above its plasma-adjusted 1b replicon EC_{50} of 30 nM.

Figure 2. Plasma concentrations for **13** (a) and **24** (b) following a 5 mg/kg dose in rat.

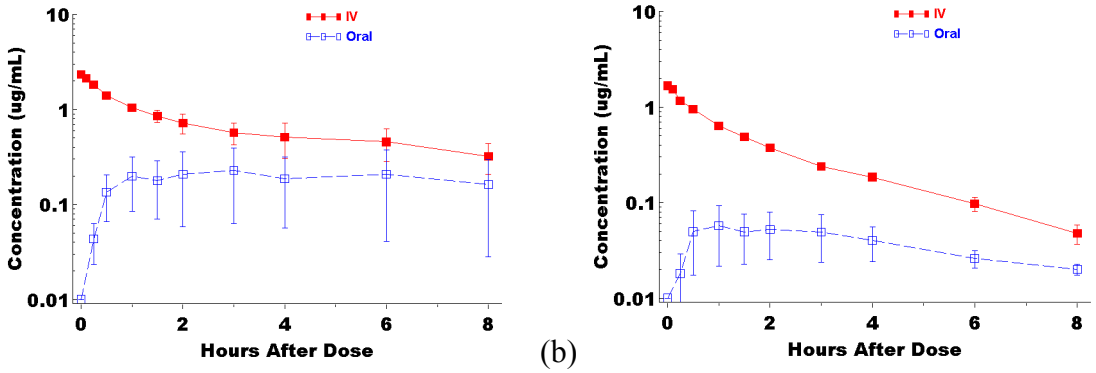
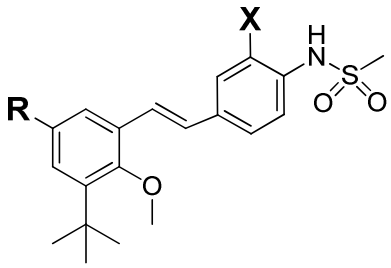


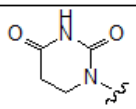
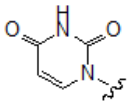
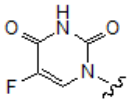
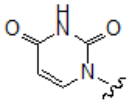
Table 2. Pharmacokinetic properties for dihydrouracil analogs.

Compound	IV CL_p (L/hr/kg)	PO $t_{1/2}$ (hr)	PO C_{max} (μg/mL)	PO AUC (μg·hr/mL)	F%
1	1.5	1.0	0.03	0.05	1.4
13	0.64	2.3	0.26	1.52	18
24	1.9	3.0	0.06	0.35	14

Uracil analogs of stilbene inhibitors have improved properties in comparison to dihydrouracil analogs (Table 3). Uracil **29** is 2-fold to 3-fold more active than **13** in both 1a and 1b replicon assays, both with and without the addition of 40% human plasma. In addition, **29** provided a longer plasma half-life and better plasma levels following a 5 mg/kg oral dose in rat, as well as a substantial increase in oral bioavailability from 18% to 44%. Further improvement in pharmacokinetic properties was provided with fluorinated analogs. 5-Fluorouracil analog **30** provided a higher plasma concentration and better oral bioavailability. However, the replicon activity for this compound was relatively poor. Fluorophenyl analog **31** provided even greater plasma exposures and relatively low clearance. While the replicon activity for **31** was better than fluorouracil **30**, it was still weak in comparison to the non-fluorinated analog **29**.

Table 3. Comparison of uracil and dihydrouracil analogs.



Compound	R	X	1a replicon EC_{50} (nM) 0% HP	1a replicon EC_{50} (nM) 40% HP	1b replicon EC_{50} (nM) 0% HP	1b replicon EC_{50} (nM) 40% HP	aq sol (μM) (pH 7.2)	Rat PK (5 mg/kg IV or PO) IV CL_p (L/hr/kg)	PO $t_{1/2}$ (hr)	PO AUC (μg·hr/mL)	F%
13		H	3.1	50	1.1	3.1	10.3	0.64	2.3	1.52	18
29		H	1.1	19	0.3	2.4	20.7	0.9	3.1	2.52	44
30		H	89.0	--	9.6	59.4	15.5	0.85	3.7	3.12	52
31		F	13.8	--	2.1	5.3	15.5	0.38	3.7	10.4	76

Due to a superior replicon activity profile and good PK properties in rat, compound **29** was selected for further *in vivo* characterization (Figure 3 and Table 4). Pharmacokinetic studies in dog revealed low plasma clearance and high oral bioavailability. Plasma exposures increased in a dose proportional manner following oral dosing in both rat and dog. Concentrations in liver tissue were high relative to plasma levels in both rat and dog. A 2.5 mg/kg oral dose in dog provided a 24-hour liver concentration of over 1 μg/g (liver to plasma ratio = 10).

Figure 3. Plasma and liver concentrations for **29** in rat (a) and dog (b).

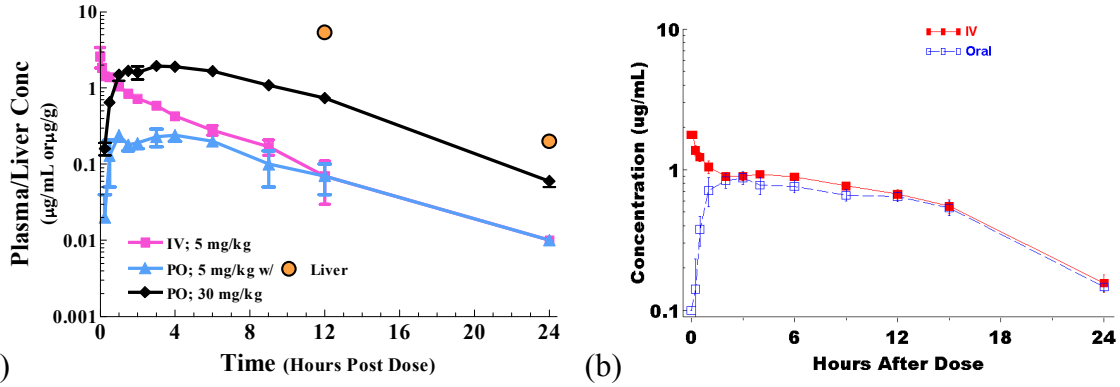
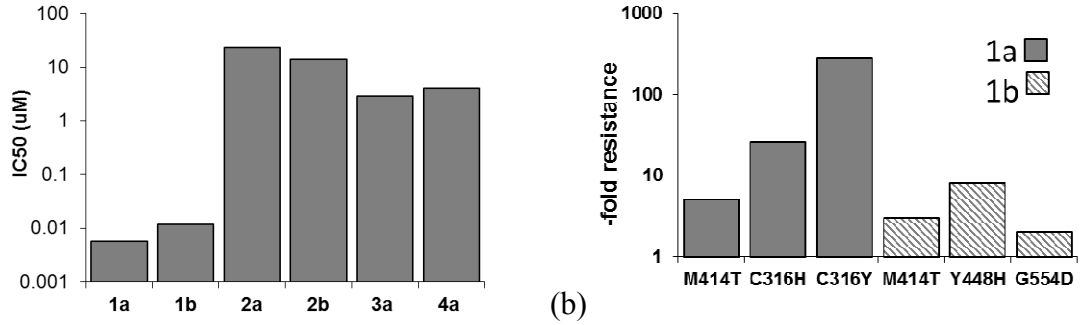


Table 4. Pharmacokinetic properties of **29** in rat and dog.

Species	Dose (route)	$t_{1/2}$ (hr)	V_{ss} (L/kg)	CL_p (L/hr/kg)	C_{max} (µg/mL)	AUC (µg·hr/kg)	F%	Liver:Plasma ratio 12 hr	Liver:Plasma ratio 24 hr
Rat	5 mg/kg (IV)	2.9	3.7	0.9		5.73			
	5 mg/kg (PO)	3.1			0.28	2.52	44	71	46
	30 mg/kg (PO)	3.5			2.00	21.64	63		
Dog	2.5 mg/kg (IV)	5.5	1.5	0.15		16.81			
	2.5 mg/kg (PO)	5.4			0.88	14.41	86	11	10
	30 mg/kg (PO)	9.6			7.56	123.8	61		

The NS5B inhibitory potency of **29** is far greater against GT1 proteins than against other genotypes (Figure 4). IC_{50} values obtained in polymerase assays for other HCV genotypes ranged from 3 μM for GT3a to 23 μM for GT2a. For the GT1a polymerase, the activity of **29** is highly sensitive to single-point amino acid variations. The greatest loss of inhibitory activity is seen with cysteine variants at position 316. Loss of inhibitory activity to the M414T variant is observed in both GT1a and GT1b. In GT1b, the Y448H variant imparts the greatest loss of inhibitory activity. Replicons containing these amino acid variants were selected during *in vitro* resistance selection studies (data not shown) and have clinical relevance as they were observed in human HCV infected subjects treated with dasabuvir, a compound that is structurally similar to **29**.[8,9] The overall enzyme inhibitory activity profile for **29**, shown in Figure 4, is consistent with binding to the NNI III (Palm 1) binding site of NS5B[10] based on analogies to two structurally similar compounds[11-13] that are highly resistant to the M414T variant, and whose Palm 1 binding sites were confirmed via X-ray crystallography.

Figure 4. Inhibitory activity of compound **29** in polymerase enzyme assays. (a) Activity against different HCV genotypes. (b) Activity against GT1 polymerase with single-point amino acid variations.



Human Pharmacokinetics

Based on these and other preclinical results, compound **29** was selected for clinical study and designated ABT-072. Human pharmacokinetic results are shown in Figure 5 and Table 5.[14] Single-dose administration of ABT-072 at 80, 160, and 320 mg in healthy subjects resulted in dose-proportional increases in plasma exposure. Maximum concentrations were achieved within 5-9 hours of dosing with a mean terminal phase half-life of 7-9 hours. Following dosing with ABT-072 at 160 mg once daily for 2 days in HCV GT1-infected patients (N=4), the HCV RNA least squares mean maximum decrease from baseline was 1.18 $\log_{10}$ IU/mL. The mean C_{max}, AUC, and $t_{1/2}$ values in HCV infected patients were similar to those obtained in healthy subjects. These results supported further development of ABT-072 as a once-daily non-nucleoside NS5B inhibitor for the treatment of HCV infection.

Figure 5. Mean (+SD) ABT-072 (**29**) plasma concentration-time profiles in healthy subjects.

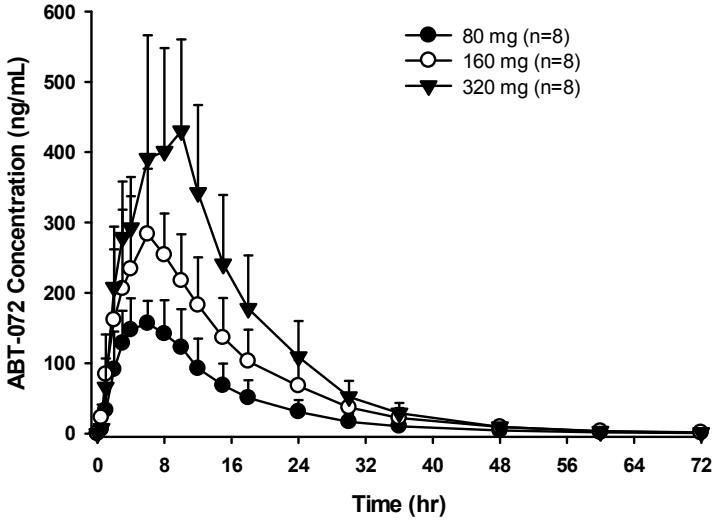


Table 5. Mean ± SD pharmacokinetics parameters of ABT-072 in healthy subjects and HCV GT1-infected patients.

	Healthy Subjects			HCV GT1-Infected Patients
Pharmacokinetic Parameter	**ABT-072 80 mg (N = 8)**	**ABT-072 160 mg (N = 8)**	**ABT-072 320 mg (N = 8)**	**ABT-072 160 mg (N = 4)**
C_{max} (ng/mL)	180 ± 37.6	303 ± 89.1	486 ± 154	415 ± 142
T_{max} (h)	5.1 ± 1.6	6.8 ± 1.8	8.8 ± 2.6	3.6 ± 0.5
$t_{½}$ [†] (h)	8.17 ± 1.34	7.63 ± 2.32	7.01 ± 0.87	8.56 ± 1.21
AUC_{∞} (ng•h/mL) [$]	2442 ± 885	4639 ± 1446	7197 ± 2328	4175 ± 1669

† Presented as harmonic mean and pseudo SD.

$ AUC_{24} presented for HCV-infected patients.

Conclusions

Research on a novel series of *N*-aryl-dihydrouracil HCV NS5B inhibitors has identified stilbene analogs with better antiviral potency and improved ADME properties in comparison to early lead molecule **1**. Replacement of the amide linker between the two aryl groups in **1** provided several analogs with much better permeability, and improved aqueous solubility. A trans-substituted olefin provided the most potent inhibitor, stilbene **13**, which gave > 30-fold improvement in IC_{50} against both GT1a and GT1b polymerase, and 20-fold improvement in GT1b replicon EC_{50} compared to **1**. Pharmacokinetic properties for **13** were also improved, with reduced clearance and increased plasma compound levels upon oral dosing in rat. The pharmacokinetic properties of stilbene analog **13** were further improved by replacing the dihydrouracil group with uracil to give **29**. Compound **29** is a potent inhibitor of HCV GT1 replicons, with an EC_{50} of 1 nM against GT1a and 0.3 nM against GT1b. Replicon potency is attenuated 8-fold to 17-fold when activity is measured in the presence of 40% human plasma. The resistance profile for **29** is consistent with binding to the NNI III (Palm 1) binding site of NS5B. Compound **29** was selected for clinical evaluation and designated ABT-072. Pharmacokinetic studies in human support development of ABT-072 as a once-daily non-nucleoside NS5B inhibitor for the treatment of HCV infection.[14,15] Phase 2 studies have demonstrated the effectiveness of ABT-072 for treating genotype 1 infected HCV patients, when used in combination with HCV protease inhibitor ABT-450, co-administered with a low dose of ritonavir (ABT-450/r), and ribavirin.[16-18] ABT-072, administered once daily as a 400 mg dose in combination with ABT-450/r and ribavirin, demonstrated a sustained virologic response 24 weeks post-treatment (SVR_{24}) of 91% (N=10/11) in HCV genotype 1 infected adults.[16]

Experimental Section

Biological Evaluation. Biochemical activity of compounds was determined by measuring their ability to inhibit the formation of RNA products in a standard polymerase inhibition assay.[9] Inhibition of genotypes 1a (H77) and 1b (BK) HCV NS5B was determined by measuring the amount of ^{3}H-UTP incorporated into RNA by scintillation counting. IC_{50} values for inhibitors were calculated using a standard IC_{50} equation.

Cell-culture activity of compounds was determined using subgenomic replicons transfected into Huh-7 cells.[9] The ability of compounds to inhibit replication of genotype 1a (H77) and 1b (Con1) replicons expressing the firefly luciferase gene was determined by measuring the level of luciferase in the cell lysate using a luminometer. Replicon inhibition activity, reported as EC_{50}, was measured both in the absence of human plasma (HP) and in the presence of 40% HP in order to determine the extent to which inhibitor-protein interactions affect compound potency.

Pharmacokinetic properties of compounds were determined using Sprague-Dawley rats (n = 3 for each experiment) via both intravenous and oral administration of a 5 mg/kg dose. Compounds were formulated using DMSO: PEG400 (1:9, v/v) and were dosed as a solution.[19]

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. 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}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.

Methyl 5-amino-3-*tert*-butyl-2-methoxybenzoate (3). Methyl 3,5-di-*tert*-butyl-2-hydroxybenzoate (**2**) (28.66 g, 108.4 mmol) was dissolved with stirring in 430 mL glacial acetic acid and the resulting mixture was treated drop-wise with fuming nitric acid (90%,179.26 mL). When the addition was complete, the resulting mixture was stirred for 2.5 h. The reaction mixture was poured onto 2.0 L of crushed ice and allowed to stand for 0.5 h.

Afterwards, 1.0 L of water was added and the ice water mixture was allowed to melt. The mixture was then filtered, and the collected solid product was washed with water and dried to provide methyl 3-*tert*-butyl-2-hydroxy-5-nitrobenzoate (24.57 g, 89%). This compound (11.41 g, 45.0 mmol), potassium carbonate (9.34 g, 67.6 mmol), acetone (200 mL), and dimethyl sulfate (6.46 g, 67.6 mmol) were added together, and the resultant mixture was heated to reflux for 16 h. After cooling, the mixture was filtered, the solid was washed with ethyl acetate, and the filtrate was concentrated under vacuum. The oily product was dissolved in ethyl acetate (600 mL), washed with water, dried over sodium sulfate, and filtered. The filtrate was concentrated under vacuum to give a crude product that was purified by column chromatography on silica gel, eluting with a solvent gradient of 5-40% ethyl acetate in hexanes to yield methyl 3-*tert*-butyl-2-methoxy-5-nitrobenzoate (10.42 g, 87%). This compound was combined with iron powder (10.89 g, 195 mmol), ammonium chloride (3.13 g, 58.5 mmol), water (30 mL), and methanol (150 mL), and the resulting mixture was refluxed for 1 h. The mixture was cooled to rt, filtered through Celite, and the Celite was washed with methanol. The filtrate was concentrated under vacuum, and the residue was dissolved in ethyl acetate (600 mL) and washed with water and

brine. The organic extract was dried over sodium sulfate, filtered and concentrated under vacuum to provide **3** as an oil (9.25g, 100%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.29 (s, 9H), 3.56 (s, 3H), 3.80 (s, 3H), 4.98 (s, 2H), 6.68-6.74 (m, 2H); MS (ESI) *m/z* 238 $(M+H)^+$.

3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-methoxybenzoic acid (4). A solution of **3** (16.44g, 69.3 mmol) in toluene (200 mL) was heated to reflux and acrylic acid was added slowly (1 mL of acrylic acid added every 3 h, 5.23 mL total, 76.2 mmol). The mixture was then refluxed for 24 h. The resulting mixture was cooled to rt and concentrated under vacuum to give 3-(3-*tert*-butyl-4-methoxy-5-(methoxycarbonyl)phenylamino)propanoic acid as an oil (21.4 g, 100%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.30 (s, 9H), 2.47 (t, J = 7.17 Hz, 2H), 3.19 (t, J = 6.80 Hz, 2H), 3.57 (s, 3H), 3.82 (s, 3H), 6.61 (d, J = 2.94 Hz, 1H), 6.72 (d, J = 2.94 Hz, 1H), 12.26 (s, 1H); MS (ESI) *m/z* 310 $(M+H)^+$.

A solution of 3-(3-*tert*-butyl-4-methoxy-5-(methoxycarbonyl)phenylamino)propanoic acid (21.4 g, 69.3 mmol) and urea (10.4 g, 173 mmol) in glacial acetic acid (200 mL) was heated at 120^{o}C for 18.5 h, and the resulting mixture was concentrated under vacuum to give an oil. Methanol (13 mL) and ethyl acetate (350 mL) were added, and the resulting mixture was allowed to stand for 24-48 h, during which time a precipitate formed. The resulting solid was collected by filtration, washed with methanol (10 mL), and air dried to yield methyl 3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-methoxybenzoate as a solid (15.26 g, 66%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.35 (s, 9H), 2.70 (t, J = 6.62 Hz, 2H), 3.72 (s, 3H), 3.76 (t, J = 6.80 Hz, 2H), 3.87 (s, 3H), 7.40 (d, J = 2.57 Hz, 1H), 7.46 (d, J = 2.57 Hz, 1H), 10.36 (s, 1H); MS (ESI) *m/z* 335 $(M+H)^+$.

To a solution of methyl 3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-methoxybenzoate (4.52g, 13.52 mmol) in methanol (70 mL) and tetrahydrofuran (70 mL) was

added a solution of aqueous sodium hydroxide (1.0 M, 68 mL). The mixture was stirred for 12 h and concentrated under vacuum to remove the organic solvents. Aqueous hydrochloric acid (1.0 M, 80 mL) was added, resulting in the formation of a solid, and the mixture was concentrated under vacuum. Concentrated hydrochloric acid (100 mL) was added, and the mixture was heated to 100°C for 1.5 h. The mixture was cooled to rt, water was added (100 mL), and the resulting solid was collected by filtration, washed with water, and dried to provide **4** as a solid (3.55 g, 82%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.35 (s, 9H), 2.70 (t, J = 6.62 Hz, 2H), 3.77 (m, 5H), 7.36 (d, J = 2.94 Hz, 1H), 7.44 (d, J = 2.94 Hz, 1H), 10.35 (s, 1H), 13.13 (s, 1H); MS (ESI) *m/z* 321 $(M+H)^+$.

3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-methoxybenzaldehyde (6). A solution of **4** (4.07g, 12.71 mmol) in thionyl chloride (40.82 mL, 559 mmol) was refluxed for 2 h and was concentrated under vacuum to provide a light yellow solid. The solid was dissolved in tetrahydrofuran (125 mL), and the solution was cooled to –78°C with stirring. To the cooled mixture was added lithium tri-*tert*-butoxyaluminum hydride (1.0 M in THF, 14 mL, 14.0 mmol) slowly over 10 min. The mixture was stirred at –78°C for 2 h, and was then quenched with aqueous hydrochloric acid (1.0 M, 25 mL) and allowed to warm to rt. Ethyl acetate (100 mL) was added, the organic layer was separated, and the aqueous layer was washed with ethyl acetate. The organic extracts were combined and washed with saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to provide **6** as a solid (3.73 g, 96%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38 (s, 9H), 2.71 (t, J = 6.62 Hz, 2H), 3.80 (t, J = 6.80, 2H), 3.93 (s, 3H), 7.56 (q, J = 2.70 Hz, 2H), 10.26 (s, 1H), 10.40 (s, 1H); MS (ESI) *m/z* 305 $(M+H)^+$.

1-(3-*tert*-butyl-4-methoxy-5-(4-nitrostyryl)phenyl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (11). To a solution of **6** (1.00 g, 3.29 mmol) and diethyl 4-nitrobenzylphosphonate (0.853 g, 3.12 mmol) in dichloromethane (50 mL) was added solid potassium *tert*-butoxide (0.737 g, 6.57 mmol) portionwise at rt. The resultant dark red solution was stirred at rt for 1.5 h, followed by the addition of aqueous hydrochloric acid solution (1.0 M, 50 mL). The mixture was stirred for 30 min, followed by the addition of dichloromethane (50 mL). The organic layer was separated and dried over sodium sulfate, filtered and concentrated to give a crude product that was purified by column chromatography on silica gel using 1% methanol in dichloromethane as eluent to provide **11** as a solid (1.12g, 80%). Note: the material obtained is a mixture of approx. 9/1 trans/cis olefin isomers. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.39 (s, 9H), 2.72 (t, J = 6.60 Hz, 2H), 3.78 (s, 3H), 3.81 (m, 2H), 7.24 (d, J = 2.5 Hz, 1H), 7.38 (d, J = 16.20 Hz, 1H), 7.57 (m, 1H), 7.61 (d, J = 2.2 Hz, 1H), 7.94 (d, J = 8.8 Hz, 2H), 8.24 (d, J = 8.8 Hz, 2H), 10.38 (s, 1H); MS (ESI) *m/z* 424 $(M+H)^+$.

(*E*)-*N*-(4-(3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-methoxystyryl)phenyl)methanesulfonamide (13). A mixture of **11** (1.1 g, 2.60 mmol), iron powder (0.725 g, 12.99 mmol), and ammonium chloride (0.208 g , 3.90 mmol) in tetrahydrofuran (40 mL), ethanol (40 mL), and water (12 mL) was heated to 90^{o}C for 45 min. The mixture was cooled to rt, filtered through Celite and washed with ethanol (20 mL), and the filtrate was concentrated under vacuum to give a solid. The solid was dissolved in ethyl acetate (100 mL), washed with water, dried over sodium sulfate, filtered and concentrated under vacuum. The resultant yellow solid (830 mg, 2.11 mmol) was dissolved in dichloromethane (50 mL) and pyridine (0.512 mL, 6.33 mmol) was added, followed by methanesulfonyl chloride (0.181 mL, 2.32 mmol). The resultant solution was stirred at rt for 16 h. Dichloromethane (100

mL) was added, and the solution was extracted with a 1N aqueous hydrochloric acid solution (2 x 50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using 2% methanol in dichloromethane to provide **13** as a solid (480 mg, 39%): ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.37 (s, 9H), 2.71 (t, J = 6.7 Hz, 2H), 3.01 (s, 3H), 3.75 (s, 3H), 3.79 (t, J = 6.6 Hz, 2H), 7.13 (d, J = 16.5 Hz, 1H), 7.15 (d, J = 2.4 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 7.25 (d, J = 16.5 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.61 (d, J =8.6 Hz, 2H), 9.80 (bs, 1H), 10.30 (s, 1H); MS (ESI) *m/z* 472 $(M+H)^+$.

3-*tert*-butyl-5-iodo-4-methoxyaniline (22). To a vigorously stirred solution of 2-*tert*-butylphenol (10.0 g, 66.6 mmol) in heptane (67 mL) was added at a fast drip a solution of 70% nitric acid (4.25 mL, 66.6 mmol) diluted with water (4.25 mL). The resulting dark red/brown mixture was stirred vigorously for 2 h. The suspended solid was collected by filtration then washed with hexane (300 mL), water (200 mL) and once again with hexane (200 mL) to provide 2-*tert*-butyl-4-nitrophenol as a solid (4.65 g, 36%): ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.44 (s, 9H), 5.85 (s, 1H), 6.76 (d, J = 8.82 Hz, 1H), 8.01 (dd, J = 8.64, 2.76 Hz, 1H), 8.22 (d, J = 2.94 Hz, 1H).

To a solution of 2-*tert*-butyl-4-nitrophenol (4.5 g, 23.05 mmol) in methanol (120 mL) and water (30 mL) was added iodine monochloride (1.16 mL, 23.05 mmol) drop-wise over a period of 10 min. The mixture was stirred for 2 h, and was poured into water (1.0 L) and allowed to stand overnight. The resulting solid was collected by filtration, washed with water (3 x 50 mL), and dried under vacuum to provide 2-(*tert*-butyl)-6-iodo-4-nitrophenol as a tan solid (7.14 g, 96%): ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.42 (s, 9H), 6.17 (s, 1H), 8.20 (d, J = 2.3 Hz, 1H), 8.47 (d, J = 2.7 Hz, 1H).

A solution of 2-*tert*-butyl-6-iodo-4-nitrophenol (5.5 g, 17.13 mmol) in methyl *tert*-butyl ether (15 mL) was placed in a 50 mL pressure vessel and cooled in an ice bath. A solution of trimethylsilyldiazomethane (2.0 M in diethyl ether, 12.85 mL, 25.7 mmol) was added, followed by drop-wise addition of methanol (1.0 mL). The vessel was sealed and stirred at rt for 16 h, and the vessel was cooled in an ice bath before being opened. The resulting solution was partitioned between ethyl acetate and water, and the organic layer was washed with 1.0M aqueous hydrochloric acid, saturated aqueous potassium carbonate solution, and brine. The organic layer was dried over sodium sulfate, filtered and concentrated to provide 1-*tert*-butyl-3-iodo-2-methoxy-5-nitrobenzene as a red oil (5.4 g, 84%): ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.42 (s, 9H), 3.98 (s, 3H), 8.24 (d, J = 2.7 Hz, 1H), 8.56 (d, J = 2.7 Hz, 1H).

A mixture of 1-*tert*-butyl-3-iodo-2-methoxy-5-nitrobenzene (5.80 g, 17.31 mmol), ammonium chloride (1.39 g, 26.0 mmol), and iron powder (4.83 g, 87 mmol) in a 2/2/1 mixture of tetrahydrofuran/ethanol/water (200 mL) was refluxed for 2 h, cooled and filtered through Celite. The filtrate was concentrated under vacuum, and the residue was partitioned between water and ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated to provide **22** as a brown oil (5.28 g, 100% yield): ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 1.42 (s, 9H), 3.63 (s, 3H), 4.89 (s, 2H), 6.51 (d, J = 2.7 Hz, 1H), 6.86 (d, J = 2.7 Hz, 1H); MS (ESI) *m/z* 306 $(M+H)^+$.

1-(3-*tert*-butyl-5-iodo-4-methoxyphenyl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (23). A solution of **22** (8.2 g, 26.9 mmol) was treated with acrylic acid (5.53 mL, 81 mmol) and stirred at rt overnight to give a thick, viscous mixture. To the mixture was added acetic acid (60 mL) and urea (7.3 g, 120 mmol), and the mixture was heated at 120°C for 24 h. The mixture was cooled to rt and concentrated under vacuum, and the residue was dissolved in toluene and

concentrated under vacuum to give a brownish solid. The solid was suspended in a mixture of ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate (100 mL) and stirred for 0.5 h to neutralize any remaining acetic acid. The solid was collected by filtration and washed repeatedly with 50 mL portions of water and finally with 3/1 hexane/ethyl acetate (50 mL) to provide **23** as an off-white solid (7.1 g, 66%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.37 (s, 9H), 2.69 (q, J = 6.74 Hz, 2H), 3.74 (t, J = 6.62 Hz, 2H), 3.82 (s, 3H), 7.26 (d, J = 2.57 Hz, 1H), 7.68 (d, J = 2.21 Hz, 1H), 10.34 (s, 1H); MS (ESI) *m/z* 403 $(M+H)^+$.

***N*-(4-((3-(*tert*-butyl)-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-methoxyphenyl)ethynyl)phenyl)methanesulfonamide (24).** To a solution of 4-ethynylaniline (3.64 g, 31.1 mmol) in dichloromethane (62 mL) was added pyridine (9.83 g, 124 mmol) followed by drop-wise addition of methanesulfonyl chloride (4.27 mL, 37.3 mmol). The mixture was stirred at 25°C for 2 h, and concentrated under vacuum. The residue was dissolved in ethyl acetate (350 mL) and washed with 1N aqueous hydrochloric acid, water, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum to give a crude product that was purified by column chromatography on silica gel, eluting with 0-1% methanol in dichloromethane to provide *N*-(4-ethynylphenyl)methanesulfonamide as a colorless solid (5.09 g, 84%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.03 (s, 3H), 4.11 (s, 1H), 7.19 (d, J = 8.82 Hz, 2H), 7.44 (d, J = 8.46 Hz, 2H), 10.03 (s, 1H); MS (ESI) *m/z* 196 $(M+H)^+$.

A mixture of **23** (1.77 g, 4.40 mmol), *N*-(4-ethynylphenyl)methanesulfonamide (0.945 g, 4.84 mmol), copper(I) iodide (0.029 g, 0.154 mmol), triethylamine (3.07 mL, 22.0 mmol), and dichlorobis(triphenylphosphine)palladium(II) (0.154 g, 0.22 mmol) in acetonitrile (60 mL) was purged by bubbling with nitrogen for 5 min, and then heated at 80°C for 30 min. The mixture

was cooled to rt and diluted with ethyl acetate (400 mL). The mixture was extracted with 1N aqueous hydrochloric acid, water, and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum to give a crude product that was purified by column chromatography on silica gel, eluting with 0-5% methanol in dichloromethane to provide **24** as a colorless solid (0.98 g, 47%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.35 (s, 9 H) 2.70 (t, J=6.62 Hz, 2 H) 3.06 (s, 3 H) 3.77 (t, *J* = 6.62 Hz, 2 H) 4.05 (s, 3 H) 7.25 (dd, *J* = 5.52, 2.94 Hz, 3 H) 7.35 (d, *J* = 2.57 Hz, 1 H) 7.55 (d, *J* = 8.46 Hz, 2 H) 10.09 (s, 1 H) 10.37 (s, 1 H); MS (ESI) *m/z* 470 $(M+H)^+$.

Methyl 3-(*tert*-butyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methoxybenzoate (25). A solution of **3** (2.0 g, 8.43 mmol) in *N*,*N*-dimethylacetamide (30 mL) was cooled to -25°C. A 0.5 M solution of *E*-3-methoxyacryloyl isocyanate in benzene (21.9 mL, 10.96 mmol) was added drop-wise, and the resulting solution was stirred at rt for 4 h, and then poured into water. The product was extracted into dichloromethane, washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum to provide a solid. This material (3.1 g, 8.51 mmol) was dissolved in ethanol (60 mL). To this solution was added a mixture of concentrated sulfuric acid (6 mL) and water (60 mL), and the heterogeneous mixture was heated at 100°C for 3 h. The ethanol solvent was removed under vacuum, and the aqueous mixture was extracted with dichloromethane. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum to give a crude product that was purified by column chromatography on silica gel, eluting with 1% methanol in dichloromethane to provide **25** as a solid (1.23 g, 44%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.32 (s, 9H), 3.76 (s, 3H), 3.88 (s, 3H), 5.64 (d, *J* = 8.09 Hz, 1H), 7.46 (d, *J* = 2.94 Hz, 1H), 7.56 (d, *J* = 2.57 Hz, 1H), 7.72 (d, *J* = 7.72 Hz, 1H), 11.41 (s, 1H); MS (ESI) *m/z* 333 $(M+H)^+$.

3-*tert*-butyl-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methoxybenzaldehyde (27). A solution of **25** (1.23 g, 3.7 mmol) in ethanol (5 mL) and 1M sodium hydroxide solution (10 mL) was stirred at rt for 18 h. The solution was acidified with 1M aqueous hydrochloric acid and the resulting solid was collected by filtration and dried (0.945 g, 80%). This material (0.945 g, 2.97 mmol) was mixed with thionyl chloride (4.5 mL), and the mixture was heated at 80°C for 40 min. After evaporating to dryness, the resulting acid chloride intermediate was dissolved in dry tetrahydrofuran (8 mL) and cooled to -78°C. A 1.0 M solution of lithium tri-*tert*-butoxyaluminum hydride in tetrahydrofuran (3.0 mL, 3.0 mmol) was added drop-wise. The mixture was stirred at -78°C for 45 min, and was quenched with 1M aqueous hydrochloric acid (5 mL). The mixture was extracted into ethyl acetate, and the organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 0-1% methanol in dichloromethane to provide **27** (0.635 g, 71%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.39 (s, 9H), 3.97 (s, 3H), 5.67 (dd, J = 7.9, 2.0 Hz, 1H), 7.61 (d, J = 2.8 Hz, 1H), 7.67 (d, J = 2.8 Hz, 1H), 7.75 (d, J = 7.9 Hz, 1H), 10.27 (s, 1H), 11.44 (s, 1H); MS (ESI) *m/z* 303 $(M+H)^+$.

(*E*)-*N*-(4-(3-*tert*-butyl-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methoxystyryl)phenyl)methanesulfonamide (29). To a solution of **27** (0.634 g, 2.1 mmol) and diethyl 4-nitrobenzylphosphonate (0.573 g, 2.1 mmol) in anhydrous dichloromethane (25 mL) was added potassium *tert*-butoxide (0.494 g, 4.4 mmol) portion-wise, and the resulting red/brown heterogeneous mixture was stirred for 1.5 h. The reaction was quenched with 1M aqueous hydrochloric acid (15 mL) and was partitioned between water and ethyl acetate. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to give a crude product that was purified by column chromatography on silica gel, eluting with 1%

methanol in dichloromethane to provide a solid (0.735 g, 83%). This solid (0.735 g, 1.74 mmol) was mixed with ammonium chloride (0.14 g, 2.62 mmol) and iron powder (0.487 g, 8.72 mmol) in ethanol (10 mL), water (5 mL), and tetrahydrofuran (10 mL). The mixture was stirred and heated at 75° C for 1 h. The cooled mixture was filtered through Celite, and the filtrate was concentrated under vacuum. The resulting crude aniline intermediate (0.683 g, 1.75 mmol) was dissolved in pyridine (0.564 mL, 6.98 mmol) and dichloromethane (15 mL). Methanesulfonyl chloride (0.163 mL, 2.1 mmol) was added drop-wise and the solution was stirred at rt for 18 h. The mixture was partitioned between 1M aqueous hydrochloric acid and dichloromethane, and the organic layer was dried over sodium sulfate, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using a solvent gradient of 1-2% methanol in dichloromethane to provide **29** as a colorless solid (0.465 g, 57%): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38 (s, 9H), 3.01 (s, 3H), 3.79 (s, 3H) 5.65 (d, J = 7.7 Hz, 1H), 7.17-7.28 (m, 5H), 7.58-7.70 (m, 3H), 7.75 (d, J = 7.7 Hz, 1H), 9.86 (s, 1H), 11.42 (s, 1H); MS (ESI) *m/z* 470 $(M+H)^+$.

ASSOCIATED CONTENT

Supporting Information

Synthetic methods and analytical data for compounds **1**, **5**, **7**, **8**, **10**, **12**, **14-16**, **18-21**, **26**, **28**, **30**, **31**; molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations used. GT, genotype; DAA, direct-acting antiviral; SVR_{24}, sustained virologic response at 24 weeks post dosing; NNI, non-nucleoside inhibitor; HP, human plasma; CL, drug clearance; C_{max}, maximum concentration of drug measured in the plasma; T_{max}, time after drug administration to reach C_{max}; N, number of test subjects; SD, standard deviation.

Disclosure - JTR, ACK, PLD, JKP, DL, RW, HBL, JMB, NSP, LC, WMK, LEH, KLL, and EOD are employees of AbbVie. CEM, TWR, MDT, RM, YL, GK, DWAB, KDS, AM, and CJM were employees of Abbott at the time of the study. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

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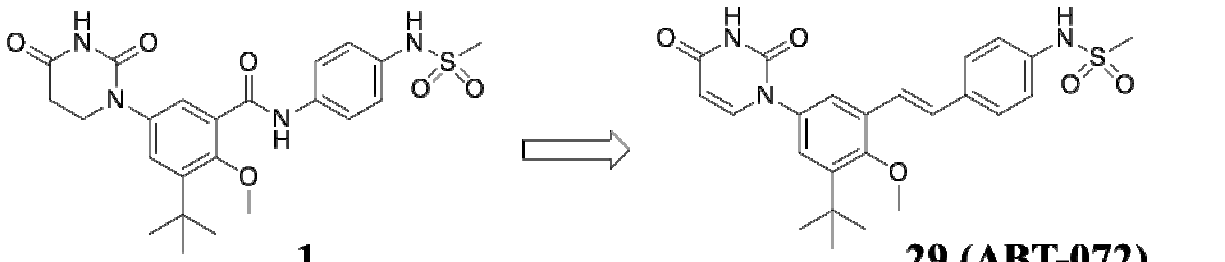
19. All animal studies were approved by the AbbVie Institutional Animal Care and Use Committee (IACUC) and conducted in an AAALAC accredited facility to ensure high standards of animal care and use.

Table of contents graphic



1

HCV GT1b EC_{50} = 19 nM
aq sol. (pH 7.2) < 1.5 µg/mL
Rat PK (5 mg/kg) F = 1.4%

29 (ABT-072)

HCV GT1b EC_{50} = 0.3 nM
aq sol. (pH 7.2) = 20.7 µg/mL
Rat PK (5 mg/kg) F = 44%
Dog PK (2.5 mg/kg) F = 86%