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Discovery of BMS-961955, an Allosteric Inhibitor of the Hepatitis C Virus NS5B Polymerase

Barbara Zhizhen Zheng,* [a] Stanley V. D'Andrea,[a] Umesh Hanumegowda,[bδ] Jay O. Knipe,[b] Kathy Mosure,[b] Xiaoliang Zhuo,[b] Julie A. Lemm,[c] Mengping Liu, [c] Karen L. Rigat,[c] Ying-Kai Wang,[d] Hua Fang,[d] Chris Poronsky,[b] Jingfang Cutrone,[b] Dauh-Rurng Wu,[e] Pirama Nayagam Arunachalam,[f] T. J. Balapragalathan,[f] Arunachalam Arumugam,[f] Arvind Mathur,[e] Nicholas A. Meanwell,[a] Min Gao,[c] Susan B. Roberts,[c] and John F. Kadow[aδ]

[a]*Department of Discovery Chemistry and Molecular Technologies, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, Connecticut 06492, United States*

[b]*Department of Preclinical Candidate Optimization, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, Connecticut 06492, United States*

[c]*Department of Virology, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, Connecticut 06492, United States*

[d]*Department of Lead Evaluation, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, Connecticut 06492, United States*

[e]*Department of Discovery Synthesis, Bristol-Myers Squibb Research and Development, PO Box 4000, Princeton, NJ 08543, United States*

[f]*Biocon Bristol-Myers Squibb R&D Center, Biocon Park, Bommasandra IV phase, Jigani Link Road, Bengaluru – 560099, India.*

Abstract: The synthesis, structure-activity relationship (SAR) data, and further optimization of the metabolic stability and pharmacokinetic (PK) properties for a previously disclosed class of cyclopropyl-fused indolobenzazepine HCV NS5B polymerase inhibitors are described. These efforts led to the discovery of BMS-961955 as a viable contingency backup to beclabuvir which was recently approved in Japan for the treatment of HCV as part of a three drug, single pill combination marketed as Ximency™.

Keywords: HCV NS5B, Polymerase, Cyclopropyl-fused indolobenzazepine, Metabolic stability, Direct-acting antiviral agent, Antiviral agent

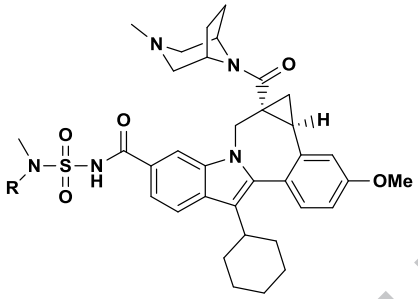
Recent estimates suggest that hepatitis C virus (HCV) infects approximately 130-170 million people or 2.8% of the world's population.[1, 2] While primary infection with HCV is often asymptomatic, most HCV infections progress to a chronic state that can slowly progress for decades, eventually causing liver cirrhosis, liver failure or hepatocellular carcinoma.[3, 4] There are more than 700,000 deaths per year from HCV-related diseases.[5]

The development of direct acting antiviral agents (DAAs) against HCV is made more complicated by both its heterogeneity, as evidenced by the recent classification of seven major genotypes (GTs) with several subtypes within each group, and its rapid rate of replication by a highly error-prone NS5B polymerase.[6] While GT 1 HCV predominates in North America and Europe, infections by other GTs such as 2 and 3 are also prevalent in significant numbers and may predominate in other geographic areas.[7] Historically, GT 1 HCV has been considered the most difficult to cure. For a considerable span of time, the standard of care for treatment of HCV infection was a combination of pegylated α-interferon (PEG-IFN) and ribavirin (RBV). However, the sustained viral response (SVR) to this therapy was limited to between 40% and 60% in patients, depending on the infecting genotype, with some of the poorer response rates associated with GT 1 HCV infections.[8] Moreover, interferon-based therapies were poorly tolerated, associated with side effects such as fatigue and flu-like symptoms which often significantly reduced adherence or even discouraged the initiation of therapy.[9] The first DAAs against HCV that were approved for clinical use, telaprevir and boceprevir, targeted the HCV NS3 protease.[10, 11] Combination of one of these two protease inhibitors with PEG-IFN and RBV improved efficacy as measured by SVRs after the end of treatment of 68-75% in GT 1 patients; unfortunately, these new therapies required frequent dosing and more significantly were burdened with significant additional side effects, a profile that eventually led to their withdrawal from the market.[12, 13] The heterogeneity and rapid mutation rate of HCV strongly suggested that the development of PEG-IFN-free DAA-only regimens would require combinations of compounds acting by diverse mechanisms.[14]

The NS5B polymerase, a viral RNA-dependent RNA polymerase, is essential for HCV replication.[15] We have pursued NS5B inhibitors as potential partners to be used in combination therapy with mechanistically distinct inhibitors. Beclabuvir (BMS-791325, **1)** in Figure 1, which binds to the polymerase at the thumb 1 domain[16, 17] is an allosteric inhibitor of NS5B that was recently approved in Japan as a part of fixed-dose combination with two mechanistically distinct

DAAs, the NS3 protease inhibitor asunaprevir and the NS5A inhibitor daclatasvir.[18] After the discovery of **1**, additional contingency backup molecules were targeted to ensure the ultimate success of the program in the event of any unforeseen issues that might arise during its clinical development.

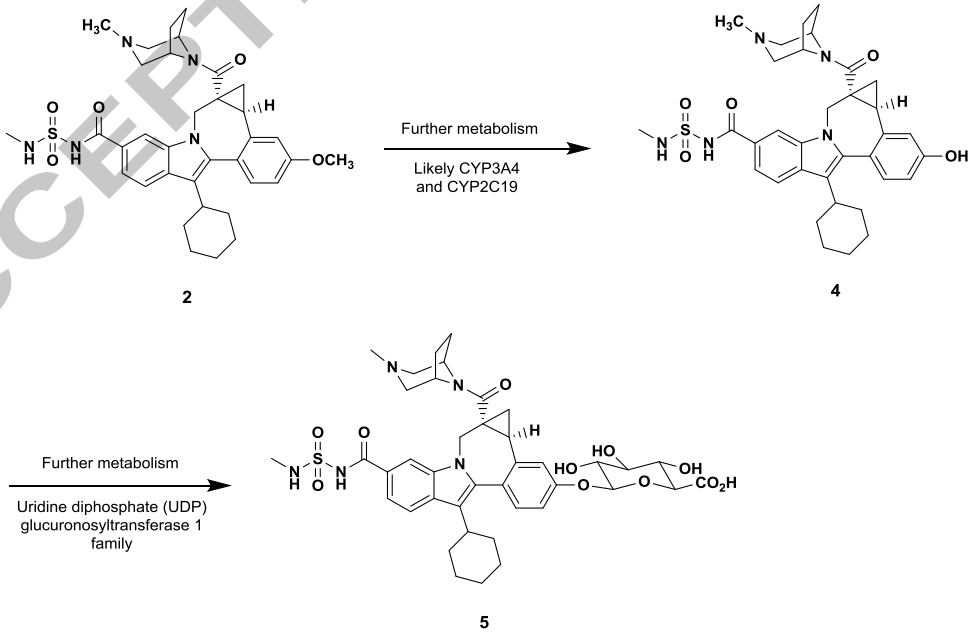
Figure 1. The structures of compound **1** and **2.**



1, R=Me, BMS-791325
2, R=H

Biotransformation studies with **1** (see scheme 1) had indicated that CYP 3A4-mediated oxidative demethylation of the *N, N*-dimethylsulfamide moiety was the primary path of metabolism and produced the equipotent mono-desmethyl derivative **2** as the major metabolite.[16] Sulfonamide **2** was further oxidized by CYP 3A4 or CYP 2C19 to form the O-demethylated product **4** which was shown to produce metabolite **5**, the product of subsequent glucuronidation.

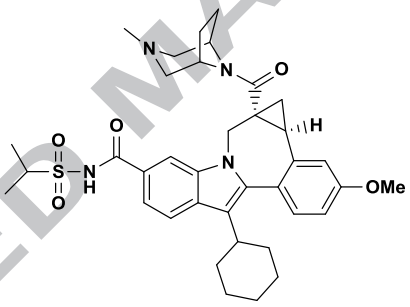
Scheme 1. Biotransformation studies with compound **1.**



2 **4** **5**

The initial campaign to generate a contingency backup to **1** centered on preventing the generation of the primary metabolite **2** which proceeded at a reasonable rate as reflected by the $t_{1/2}$ values in *in vitro* microsomal stability studies in human liver microsomes (HLM) of 46.1 minutes and cynomolgous monkey liver microsomes (CLM) of 20.8 minutes. Compounds in which this major pathway of metabolism was blocked were more stable in liver microsomes. Specifically, as reported previously, when the left-hand *N*,*N*-dimethylsulfamide was replaced with the metabolically more stable isopropyl acylsulfonamide, the resulting molecule **3** in figure 2**,** demonstrated a much enhanced $t_{1/2}$ in both HLM (>200 minutes) and CLM (87.5 min.).[17] However, this initially promising compound was advanced directly into pre-IND toxicity studies because of its structural similarity to **1** but ultimately was not a suitable backup due to unexpected findings of an unknown mechanism.[17]

Figure 2. The structure of compound **3**.



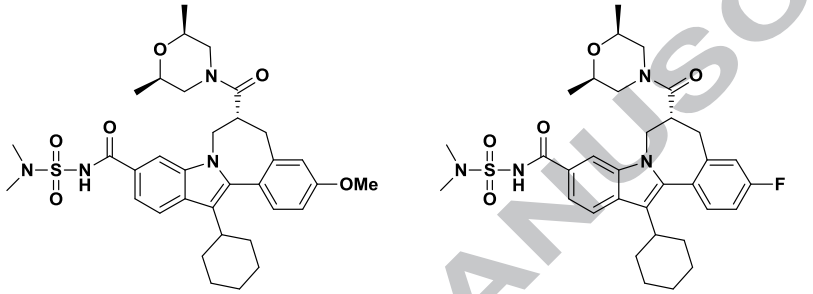
3, BMS-821095

Therefore our objective was to identify a metabolically more stable analog of **1** which was structurally differentiated from **3** so that it might avoid the toxicological issues seen with that compound. It was felt that two structural changes would be preferential in order to avoid the toxicity issues associated with **3**. Addressing the metabolism of the aryl methoxy would provide additional structural and metabolic differentiation, thereby serving a dual purpose. Thus, changes in this region of the molecule were pursued in combination with efforts to avoid the oxidative *N*-demethylation of the sulfamide moiety of **1**. In order to block *O*-demethylative metabolism, replacement of the methoxy group of **1** with a fluoro atom was examined in conjunction with an exploration of the SAR associated with acylsulfamide and acylsulfonamide replacements.

The success of this approach was not assured since earlier studies of fluoro-substituted molecules had revealed an erosion of potency toward GT 1a and 1b viruses in both the enzyme

inhibition assay and in cellular replicons when compared to the corresponding methoxy analogs. As shown by the comparison of **6** and **7** in Figure 3, the EC_{50} values for **7**, with and without 40% human serum, against GT 1b were between 2- and 4.5-fold higher than that of the corresponding methoxy analogue **6**. The origin and SAR of the morpholine amides in the context of the methoxy series that includes **6** has been previously disclosed.[16] Against the backdrop of this SAR point, optimization of the fluorophenyl series in the more potent cyclopropyl indolobenzazepine core was pursued.

Figure 3. The structures of compound **6** and **7**.

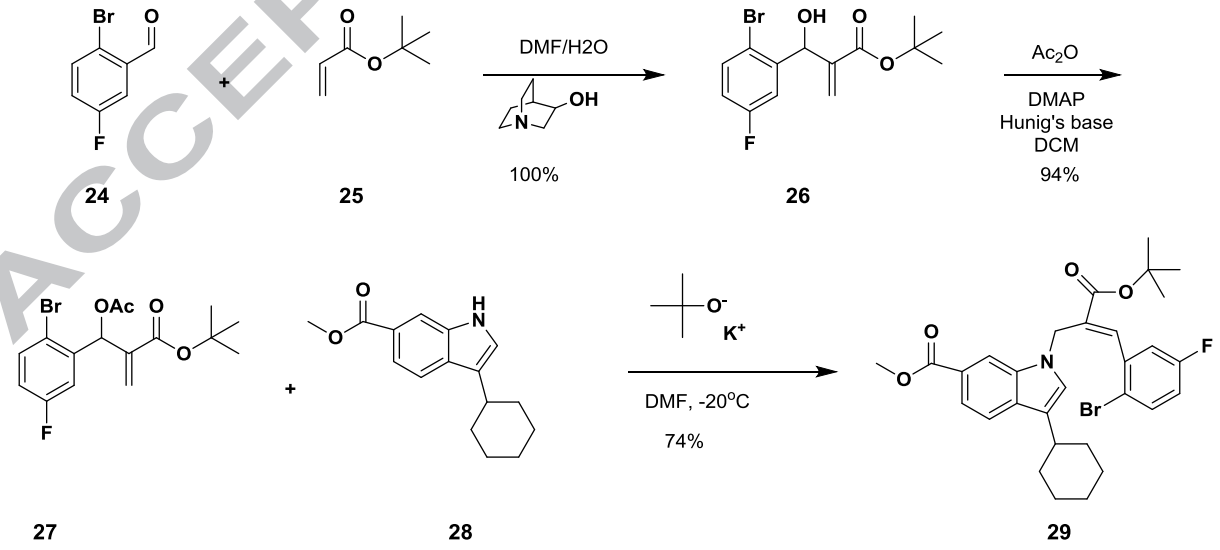


6
GT 1b IC_{50} = 11 nM
Neo Fret FBS GT 1b EC_{50} = 36.2 nM
Neo Fret GT 1b HS EC_{50} = 119 nM

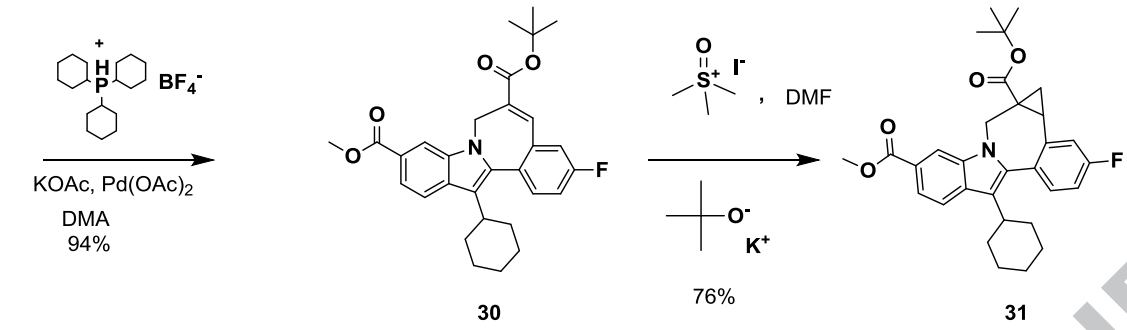
7
GT 1b IC_{50} = 17 nM
Neo Fret FBS GT 1b EC_{50} = 62.8 nM
Neo Fret HS GT 1b EC_{50} = 541 nM

The synthesis of the fluoroaryl acylsulfonamides and sulfamides are shown in Scheme 2.

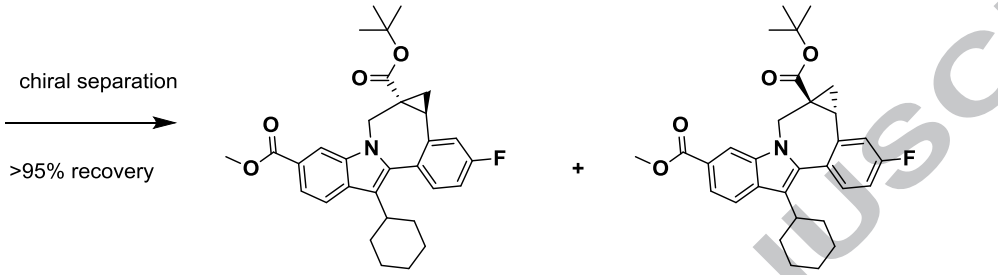
Scheme 2. The synthesis of the fluoroaryl acylsulfonamides and sulfamides.



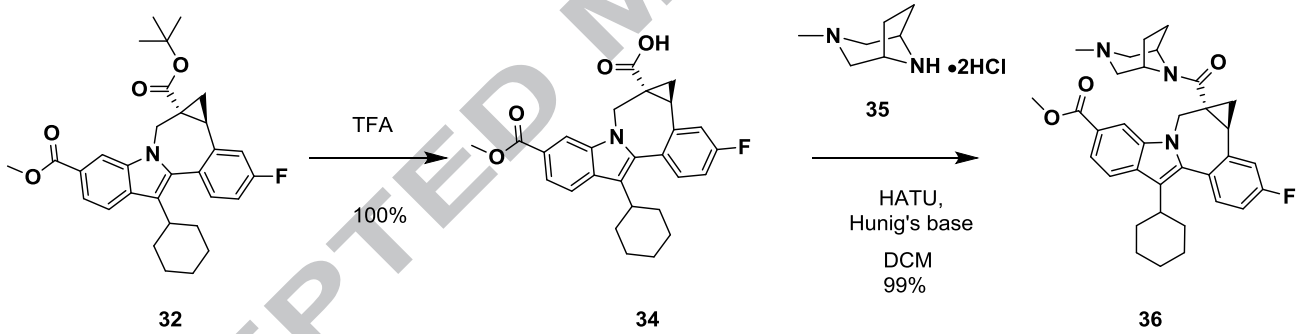
24 **25** **26** **27** **28** **29**



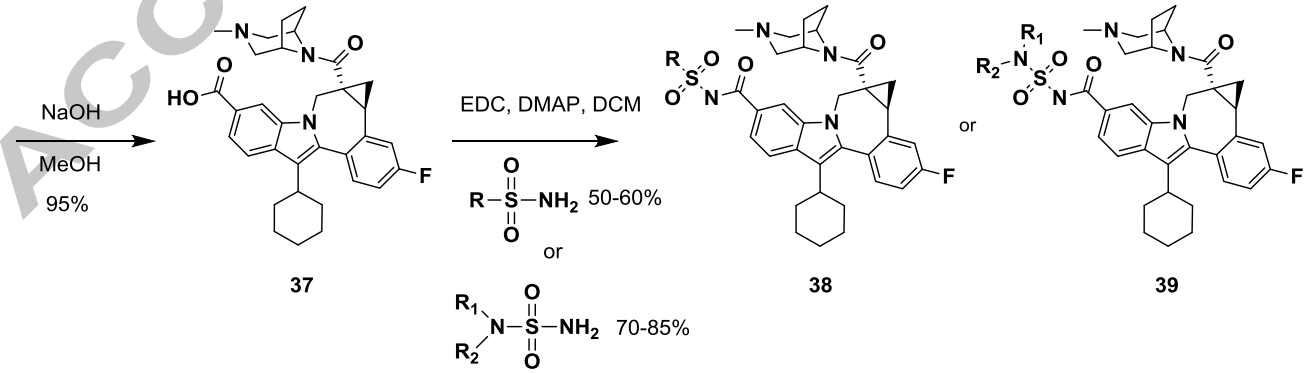
30 31



32 33



32 34 36

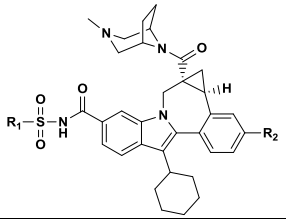


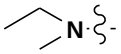
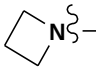
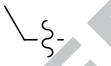
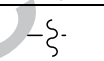
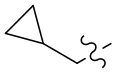
37 38 39

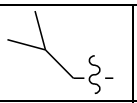
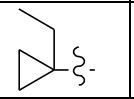
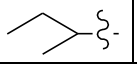
Baylis-Hillman reaction of 2-bromo-5-fluorobenzaldehyde (**24**) with *tert*-butyl acrylate (**25**) yielded **26**, which was acetylated and subsequently coupled with 3-cyclohexyl-1*H*-indole-6-carboxylate (**28**) to generate bromide **29**. An intramolecular Heck reaction of **29** produced the diester **30** which was subjected to a Corey-Chaykovsky cyclopropanation reaction to afford **31** as a racemic mixture, which was resolved using SFC chromatography to isolate the desired (*R*, *S*) enantiomer[16] **32** which was isolated as the first eluent. The *tert*-butyl ester protecting group of **32** was removed using TFA and the resulting acid **34** coupled with bicyclic amine **35** to give amide **36**. Saponification of the methyl ester of **36** provided the corresponding acid **37** which was activated with CDI and imidazole and then coupled with a variety of sulfonamides to yield the acylsulfonamide series **38**. Sulfamide analogues **39** were prepared in a similar manner *via* the coupling of acid **37** with a variety of sulfamides.

To gauge the potential for realizing the target profile and the impact of the fluoro substituent in the context of the more optimized background, the analog of **1** in which the methoxy group was replaced with a fluoro atom was prepared along with several close sulfamide analogs. The potency of this series of compounds was well maintained when compared to **1** with analogues **8**-**11** showing similar potency to the prototype (Table 1). However, the metabolic stability of **8** in CLM was similar to **1** while **9** and **10** were poorer. The metabolic stabilities of these compounds in HLM were comparable or only modestly better than **1** leading to the conclusion that the sulfamides in the fluoro series were not significantly differentiated with respect to metabolic stability. The azetidinyl sulfamide **11** demonstrated improved metabolic stability in HLM (>120 minutes) and CLM (43 minutes) but this compound displayed poor absorption after oral dosing to rats (see table 2), a result that was consistent with what has been observed previously for the sulfamide analogue in the methoxy series (Table 2).[17]

Table 1: Potency and metabolic stability of fluorinated analogues and their methoxy comparators.



Compound	R_1	R_2	GT-1b replicon EC_{50} (nM)	GT-1a replicon EC_{50} (nM)	GT-1b enzyme IC_{50} (nM)	CLM $t_{1/2}$ (min)	HLM $t_{1/2}$ (min)
1		OMe	6	4	20.4	20.8	46.1
3		OMe	10	4	5.0	87.5	> 200
8		F	5	5		27	75
9		F	7.1	4.5		9.9	51.5
10		F	14.9	6.2		11.7	61
11		F	5.3	3.0		43	> 120
12		F	17	11		59	> 120
13		F	6	5		50	> 120
14		F	6	6.5		46	> 120
15		F	23	10		47	> 120
16		F	7.9	6.5		38	> 120
17		F	7.9	4.3	4.6	65.5	> 120
18		F	22	9.7		35	>120
19		F	10.5	6.4		48	>120
20		F	7.0	4.0		>120	>120

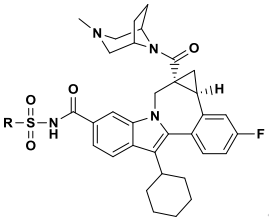
21		F	9.2	3.0		36	>120
22		F	5.2	2.9		42.5	>120
23		F	25	10			

Having established that a fluorophenyl ring was capable of providing targeted antiviral potency in the sulfamide series, the acylsulfonamide analogues were explored in a similar structural context. Previous SAR studies in the methoxy series had shown that small groups were tolerated on the acylsulfonamide moiety[19] and the series of compounds **12-23** was prepared and evaluated. Overall, the compounds displayed excellent potency in antiviral replicon assays with the majority demonstrating EC_{50} values toward GT 1a/1b HCV replicons of <10 nM. The metabolic stability of these acylsulfonamides were evaluated in HLM and CLM with the result that they were generally quite stable in HLM, with compounds **11-22** in Table 1 displaying $t_{1/2}$ values of >120 minutes. The majority of the acylsulfonamides also displayed enhanced metabolic stability in CLM, with $t_{1/2}$ values ranging from 35 to >120 minutes. Compounds **12**, **15**, **18**, **19** and **23** were deprioritized due to double digit nM potency in one of the initial replicon assays assessing GT 1b inhibitory potency. The *tert*-butyl acylsulfonamide **20** exhibited the best metabolic stability in both HLM ($t_{1/2}$ >120 minutes) and CLM ($t_{1/2}$ >120 minutes). The potency of **20** was very similar to compounds **1** and **3** and this compound was selected for PK evaluation in a snapshot rat screening study conducted over 6 hours. However, **20** showed low exposure after oral dosing (AUC =1.3 μM.h; F = 8%, 6 h) which was attributed to poor absorption (PAMPA permeability = 135 nm/s at pH 5.5 and 238 nm/s at pH 7.4).

The acylsulfonamides with replicon EC_{50} values toward GT 1a/1b of <10 nM were advanced into additional *in vitro* profiling and rat PK studies with the exception of **14** and **16** which were discarded due to a potential risk for CYP induction as predicted by a PXR transactivation signal in an *in vitro* screen (Table 2). Cyclopropyl acylsulfonamide **13** was a potent antiviral agent, displaying EC_{50} values of 6 nM and 5 nM against GT 1b and 1a replicons, respectively, and showed no potential for CYP induction in the PXR transactivation assay (EC_{50} >50 μM). However, this compound exhibited high clearance (41 mL/min/kg) and insufficient exposure following oral administration in a rat PK study. Compounds **21** and **22** showed targeted

potency and a clean PXR profile; however, these compounds did not offer an advantage in metabolic stability in CLM when compared to **17** so they were not profiled further in favor of compounds that did. From this survey, **17** (BMS-961955) emerged as the best overall candidate to move into PK studies in higher species.

Table 2: PK parameters and PXR trans-activation activity associated with acylsulfonamide- and *N, N*-dimethylsulfamide-based HCV NS5B inhibitors.



Compound	Structure	Antiviral activity		PXR EC_{50} (μM)	Rat PK*			
	R	GT 1b replicon EC_{50} (nM)	GT 1a replicon EC_{50} (nM)		Cl (mL/min /kg)	$AUC_{0\text{-}24h}$ (μM•h)	$t_{1/2}$ (h)	% F
1		6	4	> 50	2.4			43
3		10	4	> 50	14.5			65
11		5.3	3.0		12 (rat screen)	0.5 (0-6h)		19
13		6	5	50	41	1.508	4.2	23
14		6	6.5	2.78	42	0.857	5.4	13.6
16		7.9	6.5	8.33				
17		7.9	4.3	16.7	10	12.38	6.2	52
20		7.0	4.0	16.7	12 (rat screen)	1.3 (0-6h)		8

*IV dose: 5mg/kg, PO dose: 10 mg/kg

The methyl-substituted cyclopropyl acylsulfonamide **17** exhibited potency comparable to both **1** and **3** against GT 1a and 1b replicons with EC_{50} values of 4 nM and 7 nM, respectively. The EC_{50} value of **17** toward GT 1a increased by 8.2-fold in the presence of 40% human serum, indicative of a minimal effect.[20] Compound **17** potently and specifically inhibited the HCV NS5B polymerase derived from HCV GT 1, and GTs 3-6, with IC_{50} values of 4 nM (1a), 5 nM (1b), 2 nM (3a), 44 nM (4a), 15 nM (5a) and 124 nM (6a) while GT 2 NS5B was found to be less susceptible with IC_{50} values of 597 nM (2a) and 212 nM (2b).[21] Compound **17** displayed significantly enhanced metabolic stability in CLM and HLM when compared to **1** and it was not an inhibitor of recombinant CYP isoforms in HLM. In addition, no evidence of CYP induction was observed in human hepatocytes. Compound **17** exhibited high permeability in a PAMPA assay with Pc values of 523 nm/s at pH 5.5 and 590 nm/s at pH 7.4 and also displayed excellent PK properties in the rat, dog and cynomolgus monkey (Table 3). Clearance was low to moderate across the species while the volume of distribution and half-life values were moderate and bioavailability ranged from 48-84%. The predicted human dose based on the replicon potency and 3-species PK was 70 mg once a day (QD). As a result of its excellent potency, clean preclinical profile and excellent QD dosing potential, **17** was advanced into a two week toxicology study in rats.[22] No significant liabilities or issues were identified with only minimal findings at adequate exposure margins. A two week toxicology study in dogs was used to determine that **17** had a toxicology safety profile that supported advancement and clearly differentiated this compound from **3**. However, further development of **17** was not pursued based on the successful continued development of beclabuvir.[23, 24, 25, 26]

Table 3: Rat, dog and cynomolgus monkey PK data for **17**.

Species	Route	Dose (mg/kg)	AUC (0-24h) (μM•h)	$t_{½}$ (h)	Cl (mL/min/kg)	Vs (L/kg)	% F
Rat	IV	5	12.3 ± 3.3	6.2 ± 1.7	10.0 ± 2.5	3.9 ± 1.4	
	PO	10	12.4 ± 0.3				52
Dog	IV	1	25.7 ± 4.3	12.2 ± 1.1	0.8 ± 0.2	0.6 ± 0.1	
	PO	3	62.1 ±5.8				84
Monkey	IV	1	2.3 ± 0.2	8.7 ± 3.4	10.5 ± 0.5	3.1 ± 1.3	
	PO	3	3.1 ±0.3				48

The treatment landscape for patients with chronic hepatitis C infection has changed significantly over the last 5 years. The rapid evolution of clinical data has demonstrated that treatment with PEG-IFN-free combinations of DAAs[14] achieves high rates of SVR with shorter treatment durations. By 2016, the standard of care for HCV infection had evolved to consist of all oral therapies. The oral DAAs approved for use in the U.S. include: Daklinza™ (daclatasvir), Sovaldi® (sofosbuvir), Olysio® (simeprevir), Harvoni (a fixed-dose combination of ledipasvir and sofosbuvir), Technivie™ (ombitasvir/paritaprevir/ritonavir), Viekira Pak® (ombitasvir-paritaprevir-ritonavir and dasabuvir), Zepatier™ (the combination of elbasvir and grazoprevir) and Epclusa® (the combination of sofosbuvir and velpatasvir). Several other pan-genotype inhibiting regimens are being reviewed for possible approval including glecaprevir/pibrentasvir and a single tablet regimen that combines sofosbuvir, velpatasvir and voxilaprevir. Notably, both active-site[27, 28] and allosteric inhibitors[29, 30] of the HCV NS5B polymerase are currently present

in approved regimens. Since it is believed that the design and realization of polymerase inhibitors from this chemotype that act at thumb pocket 1 and are potent inhibitors of GT2 HCV would be challenging, compounds **1** and **17** represent the most optimal molecules realized in our efforts vs. this target. Further efforts aimed at identifying pan genotype inhibitors at an alternate allosteric site will be disclosed in the future.

In conclusion, after the discovery of compound **17**, preclinical profiling showed that the compound was a metabolically-stable HCV NS5B thumb site 1 inhibitor with a low projected human dose for the potential treatment of GT 1 HCV viruses and the compound was accepted as a candidate for development. Inhibitor **17** has two regions of structural differentiation from the beclabuvir (**1**), both located at sites of metabolic modification. However, the ultimately successful progression of **1** in clinical development eventually obviated further advancement of **17**.

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*Author for correspondence: Zhizhen Zheng@bms.com; Tel.: 203-677-5818

[δ]Current address: ViiV Healthcare, 5 Research Parkway, Wallingford, CT 06410.

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19. Unpublished results.
20. The human serum assay was performed essentially the same as described in the standard GT 1a replicon assay in the presence of 4% fetal bovine serum supplemented with an additional 40% human serum.
21. IC_{50} Human Pol. Enzyme assay α, β, γ = >25 μM; IC_{50} BVDV Pol. Enzyme assay >25 μM; EC_{50} BVDV replicon cell assay = 10 μM; EC_{50} Dengue replicon cell assay = 7 μM; EC_{50} HIV-2 = 2 μM; Cytotoxicity (Huh-7, Vero, MT2 cells) CC_{50} = 2.9-11.8 μM
22. The doses for the two week rat toxicity studies were 10, 30, 100 and 300 mg/kg/day.
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Graphical abstract

Discovery of BMS-961955, an Allosteric Inhibitor of the Hepatitis C Virus NS5B Polymerase

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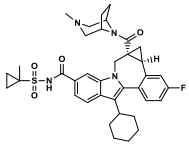
Barbara Zhizhen Zheng,*[a] Stanley V. D'Andrea,[a] Umesh Hanumegowda,[bδ] Jay O. Knipe,[b] Kathy Mosure,[b] Xiaoliang Zhuo,[b] Julie A. Lemm,[c] Mengping Liu,[c] Karen L. Rigat,[c] Ying-Kai Wang,[d] Hua Fang,[d] Chris Poronsky,[b] Jingfang Cutrone,[b] Dauh-Rurng Wu,[e] Pirama Nayagam Arunachalam,[f] T. J. Balapragalathan,[f] Arunachalam Arumugam,[f] Arvind Mathur,[e] Nicholas A. Meanwell,[a] Min Gao,[c] Susan B. Roberts,[c] and John F. Kadow[aδ]



BMS-961955
EC_{50} GT 1a/1b = 4.3/7.9 nM