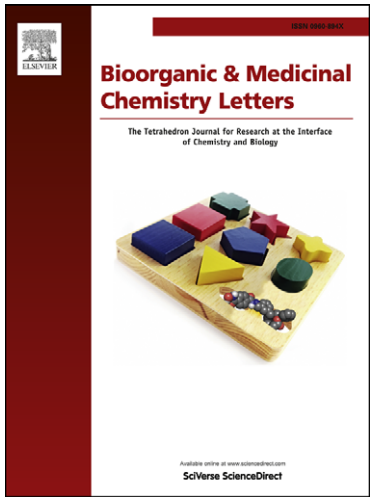


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# Discovery of pyrido[2,3-d]pyrimidine-based inhibitors of HCV NS5A.

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Efforts to improve the genotype 1a potency and pharmacokinetics of earlier naphthyridine-based HCV NS5A inhibitors resulted in the discovery of a novel series of pyrido[2,3-d]pyrimidine compounds, which displayed potent inhibition of HCV genotypes 1a and 1b in the replicon assay. SAR in this system revealed that the introduction of amides bearing an additional “E” ring provided compounds with improved potency and pharmacokinetics. Introduction of a chiral center on the amide portion resulted in the observation of a stereochemical dependence for replicon potency and provided a site for the attachment of functional groups useful for improving the solubility of the series. Compound **21** was selected for administration in an HCV-infected chimpanzee. Observation of a robust viral load decline provided positive proof of concept for inhibition of HCV replication in vivo for the compound series.

It’s estimated that more than 170 million people are chronically infected with the hepatitis C virus (HCV) worldwide. While the infection is often asymptomatic, chronic infection can lead to cirrhosis, hepatocellular carcinoma or liver failure. HCV is classified into six genotypes (1–6), with subgenotypes 1a and 1b most commonly found in the United States, Japan, and Western Europe. Treatment of genotype 1 (GT 1) patients with pegylated interferon alpha (pegIFN-α) and ribavirin is associated with significant tolerability issues and poor efficacy (40-50% cure rate). Combination of an NS3-4A protease inhibitor such as telaprevir or boceprevir with pegIFN-α and ribavirin has provided improved efficacy and is now the standard of care. However, there still remains a large unmet medical need for therapies to treat patients who are unable to tolerate or did not respond to previous therapy with pegIFN-α + ribavirin. The combination of

new direct-acting antivirals (DAAs) holds significant promise for improved cure rates and shortened treatment duration.[1]

The genome of HCV encodes several nonstructural (NS) proteins. While inhibition of the enzymatic activities of NS3/4A protease and NS5B polymerase has been the subject of intense drug discovery efforts, the NS5A protein has no known intrinsic enzymatic activity, although it is essential for viral RNA replication.[2] The discovery of compounds that select mutations in NS5A established it as a potential drug target.[3,4] NS5A inhibitors with dimeric structures have recently become the subject of intense research, due to their potent antiviral activity and clinical validation from human clinical trials.[5-8] Recent discovery efforts at Abbott to identify HCV inhibitors using the subgenomic replicon assay yielded potent naphthyridine inhibitors such as **1** that lacked activity against HCV protease and polymerase (Figure 1).[9] Studies to elucidate the mode of action concluded that the compounds were associated with NS5A, and analysis of resistant variants selected during in vitro passage revealed a number of mutations within the NS5A gene. Efforts to improve the GT 1a potency of **1** resulted in the discovery of compound **2**, which displayed potent inhibition of GT 1a and 1b.[10] While **2** displayed improved potency, it lacked sufficient metabolic stability and oral pharmacokinetics for further advancement as a lead series. Herein, we describe our efforts to identify additional SAR in this system resulting in the discovery of amides bearing an additional "E" ring (Figure 1), which displayed both improved GT 1a potency and pharmacokinetics.

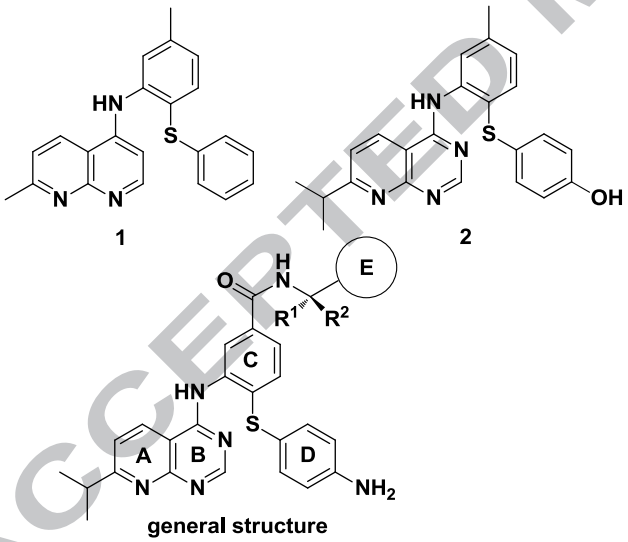
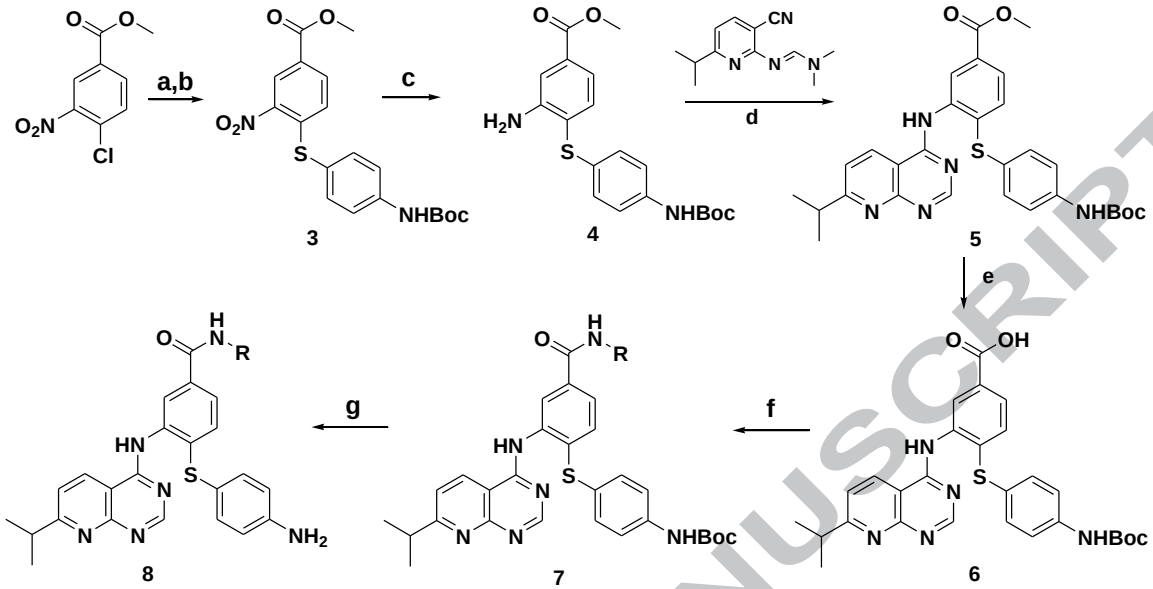



**Figure 1.** Design of HCV NS5A Inhibitors

The key acid intermediate **6** was prepared starting from methyl 4-chloro-3-nitrobenzoate according to Scheme 1. Nucleophilic aromatic substitution of methyl 4-chloro-3-nitrobenzoate with 4-aminothiophenol and subsequent protection of the amino group provided **3**. After reduction of the nitro group, the pyrido[2,3-d]pyrimidine **5** was formed by reaction with the appropriate pyrido dimethylamidine in hot acetic acid, as described previously.[10] Saponification of the ester gave the acid **6** which was allowed to react with a variety of amines to

give the protected amides **7**. Removal of the Boc group with trifluoroacetic acid provided the final products **8**.



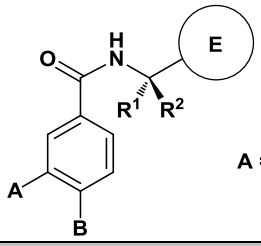

**Scheme 1**. Reagents and conditions: (a) 4-aminothiophenol, $K_2CO_3$, 90 °C; (b) $Boc_2O$, dioxane, 90 °C; (c) Fe powder, $NH_4Cl$, aqueous EtOH, reflux; (d) AcOH, 120 °C, 20 min.; (e) LiOH, THF, $H_2O$; (f) *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate [TBTU], *N*,*N*-diisopropylethylamine, DMSO, $RNH_2$, rt; (g) TFA.

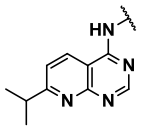
Amide compounds were tested in the GT 1a and GT1b subgenomic replicon assays[9] and the data are shown in Table 1. In an effort to improve the drug-like properties of polyaromatic lead molecules **1** and **2**, sp3 hybridized carbons were introduced to disrupt the planarity of the system. Introduction of a chiral center as in α-methyl benzyl amine analogs **10** and **11** resulted in observation of a distinct stereochemical dependence for their potency. The S-isomer **10** was several fold more potent than either the R-isomer **11** or the achiral analog **9**. The gem-disubstituted analogs **12** and **13** also displayed weaker potency than **10**. Similar stereochemical dependence was observed for the ethyl analogs **14** and **15**. The introduction of a hydroxyl group on these amide side chains (**16**-**19)** was poorly tolerated. When a quaternary center was introduced, however, the R-isomer **21** retained high potency, particularly against GT 1b.

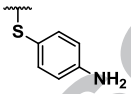
Further exploration of the substituents led to the discovery of additional analogs with potent activity against both subgenotypes, such as the $CF_3$ analog **22**, p-fluoro analog **25**, and p-bromo analog **37**. In general substitution at the 4-position of the phenyl ring yielded the most potent analogs, such as **25**, **28**, **34** and **37**, compared to the corresponding 2- or 3-substituted analogs. Cyclization of the R1 or R2 group onto the phenyl E ring resulted in the indane analogs **38** and **39**, which also displayed strong stereochemical dependence for potency. Interestingly, the more active enantiomer **39** (R isomer) had the opposite stereochemistry compared to the more active acyclic enantiomer **10** (S isomer).

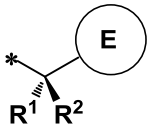
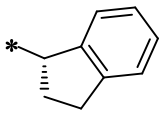
The introduction of heterocyclic E rings, such as pyridine in analogs **40**-**42** and an isosteric thiophene in analog **43**, was generally not well tolerated. The larger naphthalene analog **44** and a saturated cyclohexane analog **45** also proved to be less potent.

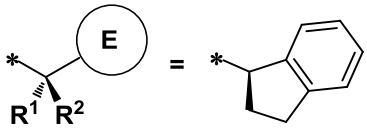
**Table 1.**
In vitro activity of amide analogs against HCV GT 1a and GT 1b in the replicon assay




A = 

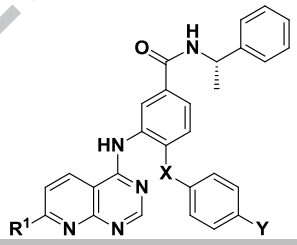

B = 


| | | | | | Replicon $EC_{50}$ (μM) | |
|---|---|---|---|---|---|---|
| no. | Stereochem. | $R^1$ | $R^2$ | E | GT 1a | GT 1b |
| **9** | - | H | H | Ph | 0.150 | 0.130 |
| **10** | *S* | Me | H | Ph | 0.045 | 0.007 |
| **11** | *R* | H | Me | Ph | 0.180 | 0.100 |
| **12** | - | Me | Me | Ph | 0.284 | 0.181 |
| **13** | - |  | | Ph | 1.07 | 0.700 |
| **14** | *S* | Et | H | Ph | 0.035 | 0.033 |
| **15** | *R* | H | Et | Ph | 0.266 | 0.127 |
| **16** | *R* | $-CH_2OH$ | H | Ph | 4.57 | 0.314 |
| **17** | *S* | H | $-CH_2OH$ | Ph | 3.03 | 0.237 |
| **18** | *S* | $-CH_2CH_2OH$ | H | Ph | 1.47 | 0.242 |
| **19** | *R* | H | $-CH_2CH_2OH$ | Ph | 3.27 | 0.347 |
| **20** | *S* | Me | $-CH_2OH$ | Ph | 1.20 | 0.060 |
| **21** | *R* | $-CH_2OH$ | Me | Ph | 0.188 | 0.002 |
| **22** | *R* | $CF_3$ | H | Ph | 0.013 | 0.002 |
| **23** | *RS* | Me | H | 2-F-Ph | 0.280 | 0.020 |
| **24** | *RS* | Me | H | 3-F-Ph | 0.092 | 0.015 |
| **25** | *S* | Me | H | 4-F-Ph | 0.010 | 0.002 |
| **26** | *RS* | Me | H | 2-Me-Ph | 0.300 | 0.030 |
| **27** | *RS* | Me | H | 3-Me-Ph | 0.100 | 0.010 |
| **28** | *S* | Me | H | 4-Me-Ph | 0.022 | 0.005 |
| **29** | *RS* | Me | H | 2-OMe-Ph | 0.610 | 0.040 |
| **30** | *S* | Me | H | 3-OMe-Ph | 0.024 | 0.004 |
| **31** | *S* | Me | H | 4-OMe-Ph | 0.089 | 0.052 |
| **32** | *S* | Me | H | 2-$CF_3$-Ph | 0.370 | 0.020 |
| **33** | *S* | Me | H | 3-$CF_3$-Ph | 0.210 | 0.021 |
| **34** | *RS* | Me | H | 4-$CF_3$-Ph | 0.066 | 0.041 |
| **35** | *S* | Me | H | 2-Br-Ph | 0.169 | 0.028 |
| **36** | *RS* | Me | H | 3-Br-Ph | 0.033 | 0.009 |
| **37** | *S* | Me | H | 4-Br-Ph | 0.016 | 0.004 |
| **38** | *S* |  | | =  | 0.451 | 0.054 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **39** | *R* |  | | | 0.016 | 0.013 |
| **40** | *RS* | Me | H | 2-pyridine | 0.919 | 0.164 |
| **41** | *RS* | Me | H | 3-pyridine | 1.424 | 0.377 |
| **42** | *RS* | Me | H | 4-pyridine | 1.172 | 0.331 |
| **43** | *RS* | Me | H | 2-thiophene | 0.378 | 0.159 |
| **44** | *S* | Me | H | 2-naphthalene | 0.340 | 0.030 |
| **45** | *S* | Me | H | cyclohexane | 0.240 | 0.045 |

Compound **10** was chosen as the starting point for additional SAR studies on other regions of the molecule. Data from these studies are shown in Table 2. Replacement of the A/B ring isopropyl group with H, methyl or trifluoromethyl (**46**, **47** and **48**, respectively) resulted in less potent compounds, while the t-butyl analog **49** retained similar potency to **10**. Replacement of the sulfur linkage between the C and D rings with oxygen (**50**) resulted in a significant loss of activity (50-fold for GT1a and 100-fold for GT1b). The sulfur atom's larger size, greater polarizability, longer bond length, and lower bond angle (C-S-C) compared to oxygen may result in significantly different topologies for **10** and **50**, and may account for the potency difference.[11] Replacement of the D-ring amino group with a hydroxyl group (**51**) resulted in a >10-fold loss of potency. While methyl carbamate analog **52** retained good potency, both the amide and sulfonamide analogs (**53** and **54**, respectively) were less potent.

**Table 2.**
In vitro activity of amide analogs against HCV GT 1a and GT 1b in the replicon assay



| | | | | Replicon $EC_{50}$ (μM) | |
|---|---|---|---|---|---|
| no. | $R^1$ | X | Y | GT 1a | GT 1b |
| **46** | H | S | $NH_2$ | 7.89 | 6.25 |
| **47** | Me | S | $NH_2$ | 1.20 | 0.60 |
| **48** | $CF_3$ | S | $NH_2$ | 0.20 | 0.25 |
| **49** | *t*-Bu | S | $NH_2$ | 0.046 | 0.003 |
| **50** | iPr | O | $NH_2$ | 2.31 | 0.79 |
| **51** | iPr | S | OH | 0.69 | 0.12 |
| **52** | iPr | S | $NHCO_2Me$ | 0.042 | 0.004 |
| **53** | iPr | S | NHCOMe | 5.03 | 0.149 |
| **54** | iPr | S | $NHSO_2Me$ | 0.349 | 0.051 |

On the basis of their HCV subgenomic replicon potencies, compounds were chosen for pharmacokinetic evaluation in male Sprague-Dawley rats (Table 3). Compound **10** demonstrated low clearance and moderate oral bioavailability.

Compound **21** demonstrated low clearance, although the oral bioavailability was lower than that observed for **10**. Compounds **22** and **37**, which displayed uniform 1a and 1b potency, demonstrated similar pharmacokinetics to **10**. Potent analogs **28** and **45** displayed higher clearance than compound **10**. Methyl carbamate analog **52** demonstrated moderate clearance, although it was not orally bioavailable. Because of its superior rat pharmacokinetics, compound **10** was advanced to dog pharmacokinetic studies. The results were similar to rat, with the observation of low clearance, a good half life and similar oral bioavailability.

**Table 3.**
Pharmacokinetic parameters of selected compounds[a]

| | | IV | | PO | | | |
|---|---|---|---|---|---|---|---|
| no. | Species | $t_{1/2}$ | Cl | $t_{1/2}$ | $C_{max}$ | AUC | *F* |
| **10** | rat | 1.6 | 0.33 | 2.7 | 1.48 | 6.49 | 42.4 |
| **21** | rat | 1.1 | 0.75 | 2.7 | 0.32 | 0.90 | 13.3 |
| **22** | rat | 2.9 | 0.24 | 1.8 | 0.93 | 3.89 | 18.7 |
| **28** | rat | 0.7 | 1.10 | 2.6 | 0.16 | 0.60 | 12.2 |
| **37** | rat | 1.9 | 0.34 | 2.0 | 0.33 | 3.84 | 24.9 |
| **45** | rat | 0.5 | 1.29 | 0.8 | 0.20 | 0.25 | 5.0 |
| **52** | rat | 0.4 | 0.77 | - | 0.0 | 0.0 | 0.0 |
| **10** | dog | 3.2 | 0.26 | 3.0 | 0.49 | 2.35 | 18.8 |

[a] Units: $t_{1/2}$ (h); Cl (L/h/kg); Cmax (μg/mL); AUC (μg*h/mL); *F* (%). Doses: 5 mg/kg in rat; 2.5 mg/kg in dog.

Because adaptive mutations in NS5A that increase replication occur during development of the replicon cell culture assay,[2] it remained to be demonstrated that compounds in this series would exert an antiviral effect in vivo against the native virus. Two compounds, **10** and **21**, were selected for further characterization for potential administration in an HCV infected chimpanzee proof of concept study. Compound **21** retained high potency in the presence of plasma proteins (0.034 μM $EC_{50}$ in the GT 1b subgenomic replicon assay in the presence of with 40% human plasma), while compound **10** was considerably weaker (1.2 μM). In addition, **21** demonstrated higher solubility(19.7 μM for compound **21** and 5.0 μM for compound **10**) in aqueous buffer and was more suitable for IV administration in a chimpanzee. Both **10** and **21** had short half-lives in monkey (ca. 1 h), while **21** displayed a good half-life in a chimpanzee (4.1 h). As a result, **21** was chosen for evaluation in an HCV GT1b-infected chimpanzee. Intravenous administration of compound **21** (2.5 mg/kg, 5 single doses, q8h) resulted in a significant viral load decline (1.65 log10 over the first 32 hours of dosing) as well as the development of resistance-associated variants in NS5A (K26R, Q24R + K26R, L28M, Y93C + K26R).[9] Mutations at tyrosine 93 have been found to confer resistance to multiple classes of NS5A inhibitors,[3,4] and the Y93C mutant demonstrated 900-fold reduced susceptibility to compound **21**. These observations provided positive proof of concept that compounds in this series exert an antiviral effect in vivo.

In conclusion, we discovered new pyrido[2,3-d]pyrimidine-based HCV NS5A inhibitors such as compound **10** that displayed potent inhibition of GT 1a and 1b as well as oral bioavailability in rats and dogs. The introduction of a chiral

center resulted in the observation of a stereochemical dependence for HCV replicon potency and provided a site for the introduction of functional groups useful for improving the solubility of the series. On the basis of its high potency against GT 1b replicon in the presence of 40% human plasma and good aqueous solubility, compound **21** (A-972338) was selected for administration in an HCV infected chimpanzee study. Observation of a robust viral load decline provided positive proof of concept for inhibition of HCV replication in vivo for the compound series.

Acknowledgement
The authors gratefully acknowledge the work of Dr. Robert Lanford for studies conducted in the HCV-infected chimpanzee.

Disclosure Statement
This research was sponsored by AbbVie. AbbVie contributed to the research, and interpretation of data, writing, reviewing, and approving the publication. DD, DG, DL, JP, PK, TP-M, KM, AM, DK and CM are employees of AbbVie, and own AbbVie stock. DB, MT, and WH own AbbVie stock.

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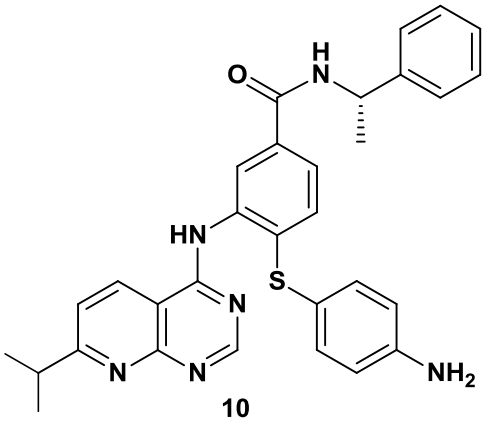
Graphical Abstract




10

**GT 1a $EC_{50}$ = 45 nM**
**GT 1b $EC_{50}$ = 7 nM**
**Oral Bioavailability (rat) = 42%**