Potent Inhibitors of Hepatitis C Virus NS3 Protease: Employment of a Difluoromethyl Group as a Hydrogen-Bond Donor

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Supporting Information

ABSTRACT: The design and synthesis of potent, tripeptidic acylsulfonamide inhibitors of HCV NS3 protease that contain a difluoromethyl cyclopropyl amino acid at P1 are described. A cocrystal structure of **18** with a NS3/4A protease complex suggests the presence of a H-bond between the polarized C–H of the CHF₂ moiety and the backbone carbonyl of Leu135 of the enzyme. Structure–activity relationship studies indicate that this H-bond enhances enzyme inhibitory potency by 13- and 17-fold compared to the CH₃ and CF₃ analogues, respectively, providing insight into the deployment of this unique amino acid.

KEYWORDS: Hepatitis C virus, NS3 protease, enzyme and replicon inhibitor, difluoromethylcyclopropyl amino acid, difluoromethyl, tripeptide acylsulfonamide, hydrogen-bond donor

Treatment for hepatitis C virus (HCV) has evolved significantly in recent years with the approval of direct-acting antiviral agents (DAAs) that provide improved cure rates and reduced duration of treatment when compared to earlier interferon-based regimens.¹ Proof-of-concept for curative therapy with DAA treatment was established in clinical trials using the combination of the NS3 protease inhibitor asunaprevir (**1**) and the NS5A inhibitor daclatasvir (**2**) (Figure 1), the first interferon-free combination to be approved worldwide.^{2,3}

The discovery of **1** has been described in detail, and this compound was a replacement for BMS-605339 (**3**), which exhibited a cardiovascular (CV) signal in the clinic at unexpectedly low plasma concentrations based on profiling in preclinical studies.^{4,5} The structural changes that differentiate **1** from **3** are subtle and limited to the P2* region of these molecules, with repositioning of the CH₃O at C4 and the introduction of a Cl substituent at C7 of the isoquinoline ring, providing a compound free of a CV signal in both preclinical and clinical studies. As **1** was progressing through clinical trials, BMS-890068 (**4**) was identified as a back-up compound that, based on preclinical PK data, offered the potential to provide once-daily dosing while maintaining the potency and favorable preclinical CV profile found in **1**.⁶ Among the structural changes that differentiated **4** from **1**, modifications to the P1 position proved

Figure 1. Structures of asunaprevir (**1**), daclatasvir (**2**), BMS-605339 (**3**), BMS-890068 (**4**), and P1 cyclopropyl prototype **5**, which form the basis of this study.

pivotal, with the bis-cyclopropyl group at the P1 position of **4** leading to enhanced absorption that translated into a significant

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improvement in the pharmacokinetic (PK) profile in rats. This observation underscored the potential value in exploring additional structural modifications at P1, as represented generically by **5** (Figure 1).

The S1 subsite of HCV NS3 protease is a shallow, lipophilic pocket defined by Leu135, Phe154, and Ala157, and the P1 moieties of potent inhibitors are lipophilic in nature.⁷ The carbonyl moiety of Leu135, which lies at the base of the pocket, has been recognized as a potential H-bond acceptor in interactions with bound ligands. The hexameric peptide **6**, which incorporates a CHF₂-substituted amino acid at P1, was reported as a moderately potent inhibitor of NS3 protease (Figure 2).⁸ The CHF₂-based P1 functionality in **6** was elegantly

Figure 2. Hexameric peptide-based NS3 inhibitors.

designed as a bioisosteric replacement of the P1 cysteine that is present in endogenous substrates and inhibitor **7**.⁹ A cocrystal structure of an analogue of **6** with a NS3/4A protease complex indicated the presence of a H-bond between the terminal CHF₂ moiety at P1 and the carbonyl oxygen atom of Leu135.⁹ The role of a CHF₂ as a H-bond donor had previously been recognized, but its application in the context of **6** demonstrated its potential in drug design.^{10–12} In this Letter, efforts to employ a P1 CHF₂-bearing amino acid moiety in the context of tripeptide-based acyl sulfonamides as inhibitors of the NS3 protease are described.

Incorporation of a P1 CHF₂CH₂ moiety in a tripeptidic acylsulfonamide series provided diastereoisomers **12** and **13**, both of which were poorly active NS3 inhibitors. The ethyl homologues **14** and **15** offered similar enzyme inhibitory activity, suggesting minimal potency contribution from the terminal CHF₂ group in **12** and **13**. A P1 CHF₂CH₂ group was also examined in the context of **16**, which, while of similar activity to **12** and **13**, was cocrystallized with a NS3/4A protease complex (Figure 3b), allowing comparison with **1** (Figure 3a).¹³ Note that while **16** was synthesized and tested as a mixture of diastereoisomers epimeric at P1, only the (*S*)-stereoisomer was observed in the cocrystal structure.

In the cocrystal structures, a significant point of differentiation between **1** and **16** was the distinct binding mode of the acyl sulfonamide moieties. One of the diastereotopic sulfone oxygens of **1** established a H-bond with the backbone N–H of Gly137 in the oxyanion hole, while the other engaged both the backbone N–H and the side chain hydroxyl of the catalytic Ser139.⁵ In addition, the terminal cyclopropyl ring of **1** effectively interfaced with the small, well-defined S1' pocket. However, these interactions were not recapitulated in the cocrystal structure of **16**, which presumably accounts, in part, for its poor inhibitory activity. The reduction in activity observed for **16** was not unexpected since structure–activity relationship (SAR) studies had established the importance of a cyclopropyl group at the P1 position, as exemplified by **9** and **10**, which were poor NS3/4A inhibitors with IC₅₀ values of 33 and 2.2 μM, respectively.¹⁴ An analysis of the binding mode of **1** (Figure 3a) indicated that the bite angle, defined by the N and C substituents attached to the α-carbon of the P1 amino acid, is optimal in the cyclopropyl series. This angle controls the relative positions of the H-bond donor

Figure 3. (a) Cocrystal structure of **1** bound to the NS3/4A protease complex illustrating interactions between the P1 and P1' elements of the inhibitor and the enzyme subpockets. (b) Similar perspective from the cocrystal structure of **16** bound to the NS3/4A protease complex. (c) CADD model of **11** bound to the NS3/4A protease complex. The arc represents the bite angle, while the red wedge depicts the dihedral angle.

(N–H) and H-bond acceptor (C=O) that flank the cyclopropyl ring (Figure 3a). The spatial complementarity of these elements to the H-bond acceptor (Arg255) and donors (Ser139 and Gly137) within the enzyme is essential to the efficiency of H-bonding and hence activity. The measured bite angle for the bound structure of **1** is ~118° (Figure 3a), which is, interestingly, the same as that found (118°) in the single crystal X-ray structure of **1**, suggestive of an effective preorganization for optimal binding with the protein.⁴ The bite angle for **16** when bound to the enzyme is also 118° (Figure 3b),¹³ however, the average bite angle for a prototypical acyclic amino acid is ~108°, which suggests that upon binding **16** requires significant reorganization to enable optimal H-bonding with the enzyme.¹⁵

The cyclopropyl ring system at P1 also provides an optimal dihedral angle between the backbone N and carbonyl O moieties that flank the P1 cyclopropyl. In the bound state, the P1 dihedral angle in **1** is ~176°, rendering it near planar (Figure 3a), which compares with the ~160° dihedral angle measured in the single crystal X-ray structure of **1**.⁴ Coupled with the bite angle analysis, this observation reinforces the critical role of the cyclopropyl group in preorganizing the P1 moiety for optimal binding with the enzyme. Not surprisingly, small structural modifications can result in a significant loss in activity, exemplified by the P1 spirocyclobutyl derivative **17**, which is 6-fold less potent than its matched pair **11** (Figure 3c).

Given the critical nature of the cyclopropyl group at P1, the potential of a CHF₂ substituent was given consideration. A model of the proposed compound **5** (R₁ = CHF₂) bound to the NS3/4A protease complex suggested the potential for a H-bonding interaction between the C–H of the CHF₂ group and the carbonyl oxygen atom of Leu135. This model relied upon the (1*R*, 2*R*) stereochemistry of the CHF₂-substituted cyclopropyl amino acid moiety analogous to that found in **1** and **3**.

The synthesis of the desired P1 difluoromethylcyclopropyl amino acid is presented in Scheme 1.^{16,17} The commercially available CHF₂CHO derivative **25** was subjected to a Horner–Wadsworth–Emmons homologation with **24** to provide the unsaturated amino acid derivative **26**,¹⁸ with the *Z*-olefin

Scheme 1. Synthesis of the *N*-Boc Difluoromethylcyclopropyl Amino Acid **29**^a

^aReagents and conditions: (a) KOtBu, THF, -78°C to rt, 44%; (b) separation by a SFC column; (c) (Boc)₂O, DMAP, rt, 2 h, THF, 85%; (d) (CH₃)₃SO⁺I⁻, NaH, DMSO, 80°C , 2 h, 48%; (e) NaOH, MeOH, rt, 18 h, 87%.

geometry assigned by ¹H NMR.¹⁹ The carbamate N atom of **26** was protected with a Boc moiety to provide intermediate **27**, which underwent cyclopropanation upon exposure to (CH₃)₃SO⁺I⁻ and NaH, providing **28** as a mixture of stereoisomers (*syn/anti* = 3.5:1). De-esterification of **28** using NaOH/MeOH afforded **29**, with the relative stereochemistry of the cyclopropane ring substituents confirmed by a single crystal X-ray structure determination (Figure 4). Amino acid **29** was

Figure 4. Single crystal X-ray structures of **18** and **29** (racemate).

incorporated into the tripeptide acylsulfonamide series using the previously described chemistry outlined in Scheme 2, with the final compound isolated as a mixture of stereoisomers **18** and **19**.

Scheme 2. Synthesis of the Tripeptide Acylsulfonamide Derivatives **18** and **19**^a

^aReagents and conditions: (a) CDI, DBU, THF, reflux, 84%; (b) HCl/dioxane, rt, 100%; (c) HATU, 4-methylmorpholine, DCM, rt, 78%.

A single crystal X-ray structure of **18** confirmed the absolute stereochemistry of the P1 moiety as (1*R*, 2*R*), consistent with that found at the P1 position of **1**.

Compound **18** was found to be a potent inhibitor of HCV NS3/4A protease, IC₅₀ = 1 nM, with low nanomolar antiviral activity observed in the cell-based replicon assay (EC₅₀ = 8 nM). A cocrystal structure of **18** with the NS3/4A protease complex indicated that **18** bound to the enzyme analogously to **1** (Figure 5).¹³ The P1 bite angle for **18** was measured as 115.6°, while the

Figure 5. (a) Cocrystal structures of **18** bound to HCV NS3/4A. (b) Details of the P1 subsite of **18** bound to the NS3/4A protease complex.

dihedral angle between the backbone N and carbonyl O that flanks the P1 cyclopropyl was measured as -174.7° . Moreover, the CHF₂ group projected toward Leu135, with the C–H of the CHF₂ group positioned 2.7 Å from the carbonyl O atom, a distance consistent with a H-bond. In the single crystal X-ray structure of **18**, the P1 bite angle (115.06°) and the dihedral angle (178.7°) were similar to that found in the cocrystal structure, once again illustrating the role of the cyclopropyl moiety in preorganizing the P1 site.

Compound **18** was 13-fold more active than the CH₃ analogue **20** and 17-fold more active than the corresponding CF₃ homologue **21**. Illustrating the importance of the correct positioning of the CF₂H moiety, the one carbon homologue **22** was 14-fold less active than **18**, but similar in potency to the ethyl analogue **23**, while the CHF₂ moiety in **19**, a diastereoisomer of **18**, projects in a manner that does not enable H-bonding within the S1 pocket. These SAR points indicate the importance of the C–H H-bond between the P1 CHF₂ group in **18** and the C=O of Leu135. The H-bonding potential of the P1 amino acid deployed in **18** bears similarity to serine and cysteine but is distinct by virtue of its conformational rigidity as well as a more lipophilic disposition. For example, the cLogP value of **29** is 1.4, while that for the *N*-Boc serine is -0.12 and that for the *N*-Boc cysteine is 1.0, suggesting potential as a replacement for serine or cysteine in cases where enhanced rigidity and lipophilicity may provide an advantage.

The $t_{1/2}$ of **18** in human liver microsomes (HLMs) was 120 min, while permeability across a confluent Caco-2 layer was low, <15 nm/s (Pc A-B); 47 nm/s (Pc B-A) (Table 1). Following IV administration, **18** demonstrated a short $t_{1/2}$, which was driven by moderate clearance, while the oral bioavailability was poor ($F < 1\%$), consistent with the low Caco-2 cell permeability. These PK parameters were inferior compared to those recorded for the P1 bis-cyclopropyl analogue **8**, which had an oral bioavailability of 34% and a prolonged plasma $t_{1/2}$ of 6 h, further underscoring the significance of small structural changes on the PK profile of analogues within this series. The reduced exposure observed with **18** compared to **8** suggests that the P1 moiety in **8** is important

Table 1. Virology and PK Parameters for a Series of P1-Modified Tripeptidic HCV NS3 Protease Inhibitors

CPd#	R	Structure AA	Activity		HLM $t_{1/2}$ (min)	Rat PK ^c
			GT-1a ^a enzyme IC_{50} (nM)	GT-1b ^b replicon EC_{50} (nM)		
1			1	6	40	$F^d = 12\%$; $AUC^e = 1.0 \mu M \cdot h$, $Cl^f = 38 \text{ mL/min/kg}$; $t_{1/2}^g = 4.2 \text{ h}$, PO 24h liver conc. = $15.2 \mu M$
8			6	18.3	83	$F = 44.2\%$; $AUC = 19.6 \mu M \cdot h$, $Cl = 5.2 \text{ mL/min/kg}$; $t_{1/2} = 10 \text{ h}$, PO 24h liver conc. = $35.7 \mu M$
9			33,000	23,000	53.5	
10			2,194	>1,000		
11			40	336	120	
12			976	509		
13			2,479	>1,000		
14			3,079	>1,000		
15			7,979	>1,000		
16			>1,000			
17			261	1,280		
18			1	7.6	120	$F = 0.9\%$; $AUC = 0.05 \mu M \cdot h$, $Cl = 58 \text{ mL/min/kg}$; $t_{1/2} = 2.6 \text{ h}$, PO 24h liver conc. = $0.27 \mu M$
19			12	58.9		
20			13	64	96	
21			17	125	47	
22			14	31	200	
23			5	16	34	$F = 43\%$; $AUC = 6.89 \mu M \cdot h$, $Cl = 18 \text{ mL/min/kg}$; $t_{1/2} = 12 \text{ h}$, PO 24h liver conc. = $40.3 \mu M$

^aGT-1a = genotype-1a; HCV NS3 enzyme inhibitory activity was assessed according to the conditions previously described.²⁰ ^bGT-1b = genotype-1b; HCV replicon inhibitory activity was assessed in the presence of 10% fetal bovine serum (FBS) according to the conditions previously described.²¹ ^cIV/PO dose: 5/15 mg/kg, $n = 3$. Vehicle: PEG-400/ethanol (90/10, v/v). ^dOral bioavailability. ^ePharmacokinetic area under curve. ^fPharmacokinetic clearance. ^gPlasma half-life.

for optimal absorption and that the lipophilicity intrinsic to the cyclopropyl group of **8** may more effectively balance the polarity associated with the adjacent acylsulfonamide moiety than does the CHF_2 in **18**. Consistent with this hypothesis, the $cLogP$ value for **18** was 3.2, while that for **8** was 0.3 Log unit greater at 3.5.

Likewise, the ethyl derivative **23**, $cLogP = 3.7$, demonstrated improved PK compared to **18**. Ultimately, the poor oral bioavailability of **18** limited the progression of the CHF_2 -based chemotype in this acyclic series, and efforts were diverted to the bis-cyclopropyl chemotype, which, as previously noted, yielded a

back-up candidate to **1**. However, the potential of the CHF₂-substituted cyclopropyl amino acid moiety has been further confirmed by its presence in the recently approved HCV NS3 inhibitors voxilaprevir and glecaprevir.^{22,23} While details of these research efforts have yet to be provided, the presence of the CHF₂-substituted cyclopropyl-based P1 moiety in these marketed drugs coupled with the design insights provided herein suggest the potential utility of this interesting and useful amino acid.

In summary, the incorporation of a difluoromethyl cyclopropyl amino acid in the tripeptide **18** conferred 13-fold higher potency than the corresponding des-fluoro analogue **20**. A cocrystal structure of **18** with an NS3/4A protease complex construct indicated the presence of a H-bond between the C–H of the CHF₂ group and the carbonyl oxygen atom of Leu135. While the PK profiles of this series were poor, the results demonstrate the potential of this amino acid moiety as a tool to drive activity through the use of the CHF₂ group as a H-bond donor.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.7b00503.

Synthetic methods and characterization data for compounds **18** and **26–31**, and methods for in vitro, in vivo, and pharmacokinetic assays (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AUC, area under the curve; Cl, clearance; CV, cardiovascular; DAA, direct-acting antiviral agent; HCV, hepatitis C virus; HLM, human liver microsomes; PK, pharmacokinetic; PO, per os; SAR, structure–activity relationship

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