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Discovery of Novel Urea-Based Hepatitis C Protease Inhibitors with High Potency against Protease-Inhibitor-Resistant Mutants

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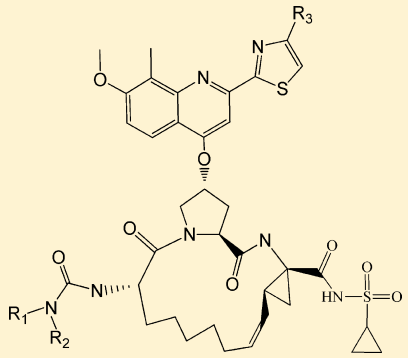
Supporting Information

ABSTRACT: The macrocyclic urea **2**, a byproduct in the synthesis of benzoxaborole **1**, was identified to be a novel and potent HCV protease inhibitor. We further explored this motif by synthesizing additional urea-based inhibitors and by characterizing them in replicase HCV protease-resistant mutants assay. Several compounds, exemplified by **12**, were found to be more potent in HCV replicon assays than leading second generation inhibitors such as danoprevir and TMC-435350. Additionally, following oral administration, inhibitor **12** was found in rat liver in significantly higher concentrations than those reported for both danoprevir and TMC-435350, suggesting that inhibitor **12** has the combination of anti-HCV and pharmacokinetic properties that warrants further development of this series.



INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease that can lead to cirrhosis, carcinoma, and liver failure. WHO estimates that 130−170 million people are chronically infected with HCV, a leading cause of liver transplants.[1] As a result of the very recent approval of first HCV protease inhibitors (PIs) boceprevir[2] and telaprevir,[3] the current golden standard for HCV infection involves a combination therapy of protease inhibitor, injectable pegylated interferon-α (PEG IFN-α), and ribavirin. While more effective in patients with genotype (gt) 1 than the previous interferon + ribavirin only regimen, it is nonetheless less than ideal therapy because of the well-known interferon side effects.[4]

Other HCV PIs, ITMN-191 (danoprevir),[5] TMC-435350,[6] MK-7009 (vaniprevir),[7] and BI201335, are in advanced clinical trials (Figure 1).[8] However, new generation inhibitors with improved potency against HCV-PI mutants could be very valuable to manage a rapid emergence of HCV resistance observed for HCV PIs in the clinic.[9] Such compounds would also be important in the future direct acting antivirals (DAA) based combination therapies, which aim to eliminate the use of PEG IFN-α and thus have the potential to improve the side effect profile of anti-HCV therapy, in turn increasing patients' eligibility and compliance. In addition, owing to their pharmacokinetic properties, both boceprevir and telaprevir require significant, multiple daily doses. New PIs supporting low once-a-day dose would add significant benefit by decreasing dosing frequency and improving compliance. Despite the advances of current therapy, there remains a strong need to develop additional HCV PIs. In this report we describe our own effort in this area.

RESULTS AND DISCUSSION

We recently published several studies describing design, synthesis, and biological properties of boron-based novel HCV protease inhibitors.[10−13] These compounds incorporated cyclic boronates and benzoxaboroles as putative warheads on position P1 targeting HCV protease catalytic triad Ser[139] and on positions P1′ and P4 to identify opportunistic covalent and noncovalent interactions of these boron-bearing moieties with the HCV protease. This effort led us to the discovery of very potent boron-containing analogues, such as **1**[11] (Figure 2).

Investigation of unpurified product **1** revealed the presence of dihydroxy **2**, a type of product that has been described to form under oxidative deboronation conditions.[14] While **2** was less potent than **1** in the HCV gt1a replicon assay, it was equipotent in the HCV gt1b replicon assay and more potent in the gt1a enzyme assay[10−13] (Table 1). The potency of **2** compared well with other advanced HCV protease inhibitors (Table 1). Moreover, macrocyclic P4-urea HCV PIs offered

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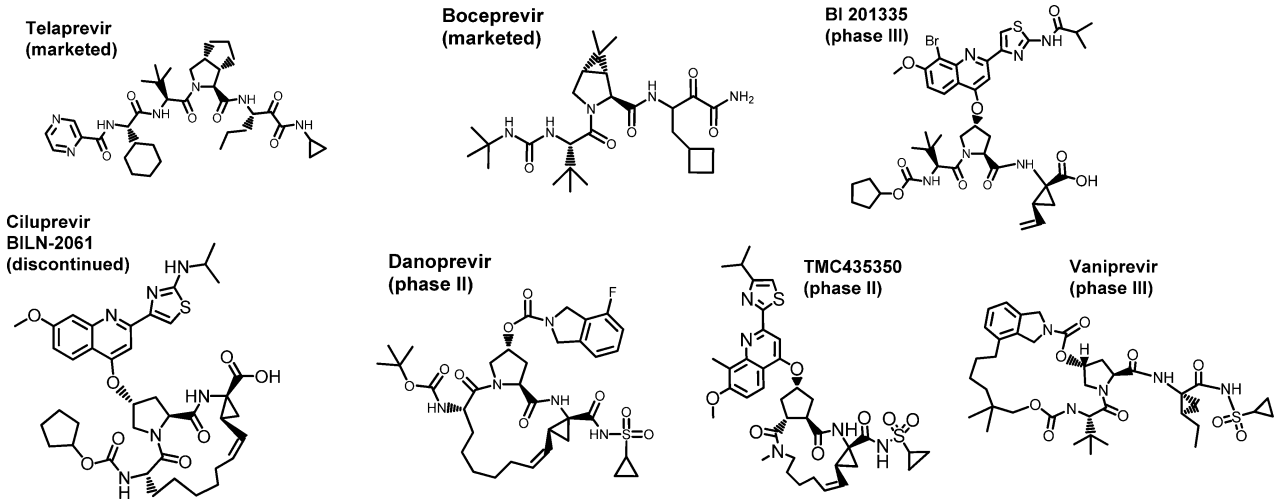

Figure 1. Selected advanced HCV protease inhibitors (PIs).

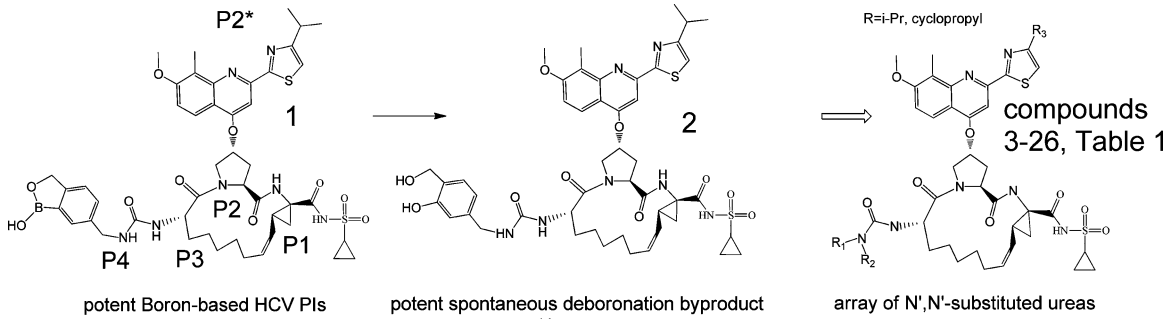

Figure 2. Evolution of potent P4-benzoxaborole **1**[11] into the current series of P4-urea derivatives.

substantial opportunity for lead optimization and appeared novel and unexplored in the literature.

We set out to further explore this motif by synthesizing a focused set of urea analogues **3**–**26** (Table 1). The goal was to discover novel compounds with superior potency or potency profile on PI-resistant HCV mutants. To this end, final compounds were evaluated in the panel of laboratory and clinical HCV protease mutants A156S, A156T, A156V, D168A, D168V, and R155K (Table 1).

Structure–Activity Relationships. All final compounds were tested in a gt1a protease assay, gt1a and gt1b stable replicon assays, and a panel of transient replicon assays. The transient replicon allows establishment of a panel of key protease resistance mutation sequences. In our experience, the transient replicon assay can be somewhat more sensitive to inhibitors than the stable replicon but provides important information about mutation sensitivity to the inhibitor when compared to the wt gt1b EC_{50} (Table 1).

In general, urea substitutions resulted in lowering of the stable replicon EC_{50} values and improving (decreasing) EC_{50}(mutant)/EC_{50}(wt) ratios. For example the EC_{50}(D168V)/EC_{50}(wt) is ∼5600 for unsubstituted urea **3** and ∼1400 for dimethylurea **4**. Many (but not all, notably **19**) diverse urea substituents especially shift the stable 1a replicon potency EC_{50} values into the subnanomolar range. This suggests that the NS3/4A enzyme tolerates many substituents, which therefore can be used as "handles" to modulate PK properties or to install prodrug moiety. For example, the piperidine-based **12** had much lower in vivo rat clearance (vide infra) than the more hydrophilic morpholine-based **9** (PK data not shown). Compounds in Table 1 were generally very potent in gt1a and gt1b stable replicon assays, with an EC_{50} (highest to lowest) spread of ∼60-fold in gt1a values (**2** vs **23**) and ∼15-fold spread in gt1b values (**15** and **4**). The SAR trends were generally less pronounced for the gt1b, consistent with the narrower range in EC_{50} values. Compounds with certain hydrophobic substituents on the urea moiety were very potent in particular in the gt1a assay, e.g., dimethyl **4**, morpholino **9**, piperidine **12**, bicyclic **17**, and Me-*i*-Pr **23**. Compounds with more polar substituents, such as carboxamide **15**, carboxylate **16**, and sulfone **20**, were about 3× to 5× less potent than unsubstituted **3** in gt1a assay but were essentially equipotent to the unsubstituted **3** in the gt1b assay. In particular, **4**, **10**–**13**, **22**–**24** were more potent in both stable replicon assays than the advanced HCV PIs (Table 1).

Compounds were also profiled in the transient replicon gt1b protease mutant assay (Table 1). Most PIs were more potent in the transient wt than stable wt assay. Interesting SAR was observed in the mutant assays. The A156S mutant did not affect susceptibility to urea analogues, which generally maintained their subnanomolar potency. Mutants A156T and A156V somewhat affected susceptibility to urea analogues, with compound potency decreasing up to 200-fold vs wt. On the other hand, EC_{50} values of some analogues were highly shifted against R155K, D168A, and D168V mutants. Transient EC_{50}(mutant)/EC_{50}(wt) ratios for compounds **5**, **7**, **8**, **23**–**26** were similar to ratios observed for clinical/marketed HCV PIs (<1000-fold across the mutant panel). However, stable replicon EC_{50} values for **5**, **7**, **8**, **23**–**26** were generally superior to the advanced reference PIs in Table 1.

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Table 1. Potencies of Compounds 1−26 and Advanced HCV PIs in gt1a Enzyme Assay and Stable and Transient Replicon Assay[a]

			Enzyme IC_{50} [nM]	STABLE replicon EC_{50} [nM]		gt1b Transient replicon, EC_{50} [nM]						
			1a domain	1a	1b	wt	A156S	A156T	A156V	D168A	D168V	R155K
1			2	3.4	0.8	0.9	0.2	7	9	249	144	49
2			0.5	15.5	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	N(R1,R2)	R3										
3		i-Pr	2	1.8	0.5	0.4	0.4	6	16	873	2261	66
4		i-Pr	2	0.3	0.1	0.04	0.1	1	5	36	57	10
5		i-Pr	6	0.7	0.4	0.2	0.5	3	11	60	147	25
6		i-Pr	1	0.9	0.7	0.1	0.5	2	8	56	131	15
7		i-Pr	4	0.8	0.4	0.1	0.2	2	8	60	97	20
8		i-Pr	2	0.8	0.5	0.1	0.1	1	6	15	58	14
9		i-Pr	2	0.3	0.5	0.01	0.03	0.2	2	17	33	4
10		i-Pr	4	0.4	0.4	0.1	0.2	2	9	86	200	15
11		i-Pr	2	0.6	0.5	0.1	0.1	2	11	28	138	17
12		i-Pr	5	0.4	0.5	0.04	0.1	2	7	32	79	6
13		i-Pr	2	0.6	0.3	0.1	0.1	2	9	42	154	7
14		i-Pr	1	0.7	0.3	0.1	0.1	2	9	47	141	14
15		i-Pr	2	5.6	1.5	0.2	0.1	3	10	431	n.d.	65
16		i-Pr	0.9	2.2	0.8	0.1	0.2	1	3	7	161	21
17		i-Pr	5	0.3	0.3	0.01	0.1	1	15	23	83	11
18		i-Pr	7	1.6	0.9	0.2	0.2	5	30	182	386	69
19		i-Pr	2	10.6	1.2	0.7	1.0	23	69	423	2088	233
20		i-Pr	2	2.3	0.5	0.4	0.3	5	38	372	1416	115
21		i-Pr	2	0.6	0.5	0.5	1.9	12	98	1622	n.d.	102
22		i-Pr	2	0.5	0.3	0.1	0.3	5	32	169	477	60
23		cpy	0.8	0.3	0.4	0.04	0.05	2	5	17	37	5
24		cpy	1	0.4	0.4	0.2	0.7	8	24	50	151	16
25		cpy	n.d.	1.0	1.0	0.2	0.4	7	9	71	84	28
26		cpy	2	0.7	0.9	0.2	0.6	11	43	64	105	46
	ciluprevir		2	2.2	0.5	1	3	604	627	205	438	241
	telaprevir		500	540	372	34	883	1136	1965	638	207	1778
	TMC-435350		10	2	1	1	0.3	86	344	149	140	56
	danoprevir		0.7	2	1.5	0.2	2	10	8	39	26	82

[a] n.d.: not determined.

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Table 2. Pharmacokinetics of Compounds 7, 8, and 12 in the in Vivo Rat Model

	compound		
	7	8	12
PK (Rat, iv, 1 mg/kg)			
CL (mL·h^{-1}·kg^{-1})	9618	5462	5004
AUC (h·μg/mL)	0.107	0.203	0.268
PK (Rat, po, 5 mg/kg)			
AUC (h·μg/mL)	0.0405	0.069	0.266
terminal $t_{1/2}$ (h)	2.26	0.806	1.16
% max absorption	7.6	6.8	20
% bioavailability (JV)	4.2	4	9.4
Liver Concentration (Rat, po Solution, 5 mg/kg)			
AUC (h·μg/mL)			308
terminal $t_{1/2}$ (h)			8.1

Among the clinical compounds listed in Table 1, telaprevir has the least variable EC_{50} profile EC_{50}(mutant)/EC_{50}(wt), with the range 6−57 (lowest to highest ratio) for these mutants. This range is significantly wider for both TMC-435350 (0.3−370) and danoprevir (10−466).

Inhibitors **4**, **7**, **8**, **9**, **12**, **17**, **23**, and **24** were substantially more potent than telaprevir, TMC-435350, boceprevir, vaniprevir,[15] and danoprevir in the mutant panel. This is demonstrated by the ratio of TMC-435350 EC_{50} to EC_{50} of a representative compound **12** for each virus: wt, 23×; A156S, 3.5×; A156T, 43×; A156V, 49×; D168A, 5×; D168V, 2×; R155K, 9×. For danoprevir and compound **12**, ratios were as follows: wt, 5×; A156S, 21×; A156T, 5×; A156V, 1×; D168A, 1×; D168V, 0.3×; R155K, 14×. Taken together, these results suggest that many compounds in Table 1, especially **4**, **9**, **12**, **17**, are some of the most potent HCV protease inhibitors reported to date. Many of these compounds are more potent on mutant R155K than danoprevir. Mutant R155K emerged in the 14-day danoprevir monotherapy study and a 12-week combination study of danoprevir with peginterferon alfa-2a/ribavirin.[16] In addition to changes on positions 80, 156, and 168, R155K was also detected in all subjects in the 5-day monotherapy with TMC-435350. Although these mutations spontaneously returned to the baseline over a period of time, they reappeared in two patients in the follow-up study with TMC435/PegIFNa-2a/ribavirin, underscoring the need for more effective agents.[17−19]

DMPK. Selected inhibitors were evaluated in the in vivo rat PK model.[20] Azetidine **7**, difluoroazetidine **8**, and piperidine **12** exhibited higher liver clearances (Table 2) in comparison with the ones reported for TMC-435350 (Cl of 505 and 2300

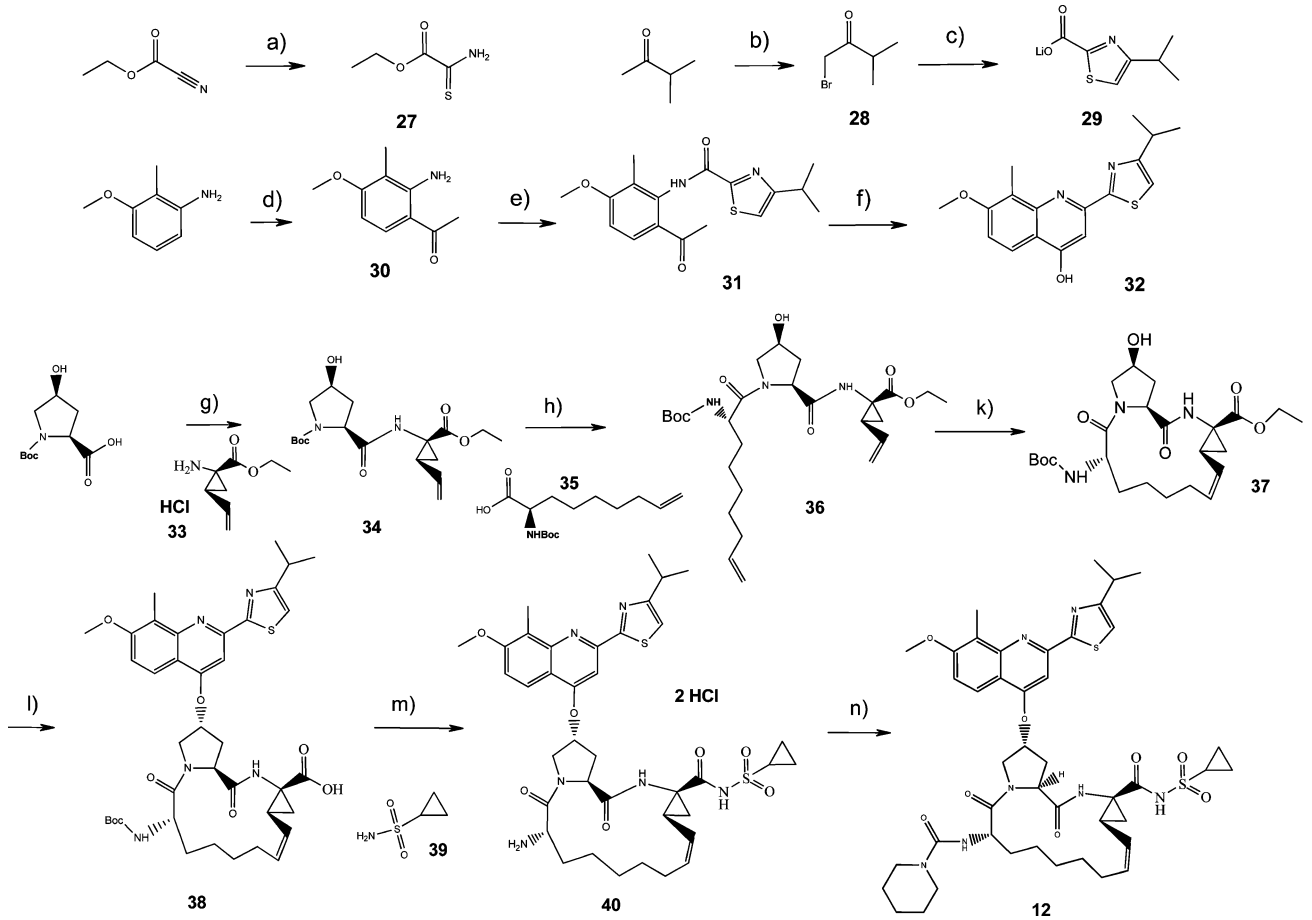

Figure 3. Reagents and conditions: (a) H_2S, ether, 95%; (b) Br_2, methanol, −30 °C, 54%; (c) (i) **27**, ethanol, reflux; (ii) LiOH, water/tetrahydrofuran/methanol, 50% two steps; (d) BCl_3, $AlCl_3$, acetonitrile, 71%; (e) **29**, HATU, DIEA, DMF, 40%; (f) ^{t}BuOH, KO^{t}Bu, 100 °C, 99%; (g) **33**, HATU, DIEA, dichloromethane, rt, 95%; (h) (i) HCl, dioxane, dichloromethane; (ii) **35**, HATU, DIEA, dichloromethane, 88% two steps; (k) Zhan 1B, DCE, c = 0.01 M, 75 °C, 62%; (l) (i) **32**, DIAD, Ph_3P, THF; (ii) LiOH, tetrahydrofuran, 61%, two steps; (m) (i) **39**, CDI, DMF, DBU; (ii) HCl, dioxane, 60% two steps; (n) cyclohexylamine, triphosgene, dichloroethane, yield 51%.

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mL·h^{-1}·kg^{-1} at 2 and 4 mg/kg iv doses, respectively).[6,18] Compound **24** was less stable in vitro than **12** and was not advanced to further in vivo studies. When administered at 5 mg/kg po, the dose-normalized (DN) po AUC values for **7**, **8**, and **12** (0.008, 0.014, and 0.053 h·μg·kg·mL^{-1}·mg^{-1}, respectively) were lower than ones for TMC-435350 (calculated po DNAUC = 0.194 h·μg·kg·mL^{-1}·mg^{-1} for reported AUC = 7.740 h·μg/mL at 40 mg/kg dose) and danoprevir (po DNAUC = 0.317 h·μg·kg·mL^{-1}·mg^{-1} for AUC = 9.500 h·μg/mL at 30 mg/kg dose). The bioavailability of TMC-435350 was 44% at 40 mg/kg dose.[5,18] We determined the concentration of **12** in rat liver at the 5 mg/kg po dose. Remarkably, **12** exhibited a high $t_{1/2}$ of 8.1 h in the liver, with corresponding liver AUC_{∞} of 308 h·μg/mL and liver/plasma ratio of 1158. For TMC-435350 the reported liver/plasma ratio was 32−65 at 40 mg/kg po dose.[18] For danoprevir, liver AUC_{∞} was 90.8 h·μg/mL at 30 mg/kg po dose, corresponding to liver/plasma ratio of ∼10.[5] While different doses and conditions used in these PK studies complicate comparisons, the PK data strongly suggest that although **12** is not as bioavailable as danoprevir or TMC-435350, it is present at much higher concentration in the liver, the main HCV reservoir.

Synthesis. Compounds **3**−**26** were synthesized as described in Figure 3. Several improvements made to the literature syntheses of the P2* phenol **32**,[6,20−22] such as the use of HATU in step e instead of $POCl_3$ and performing the thiazole cyclization at higher temperature, resulted in high yield and purity of **32**.

Macrocycle **37** was synthesized in high yield by RCM of bis-olefin **36** at high dilution in the presence of Zhan 1B catalyst. Ether **38** was secured from **37** and **32** employing Mitsunobu conditions. Key intermediate **40** was then obtained from **38** after coupling and deprotection steps and was then used to produce compounds **3**−**22**. The cyclopropyl-substituted (instead of isopropyl) analogues **23**−**26** were synthesized in a similar manner.

CONCLUSIONS

We designed, synthesized, and evaluated a number of novel, urea-based HCV protease inhibitors. Compounds with hydrophobic urea substituents, such as **4**, **9**, **12**, **17**, **23**, were found to be more potent in both stable and transient replicon assays than the leading second generation inhibitors danoprevir and TMC-435350. While plasma-based PK data suggest that compounds in this class have low to moderate bioavailability, more detailed investigation of the abundance of **12** in the rat liver following oral administration revealed liver concentrations that were substantially higher than these reported for danoprevir and TMC-435350. We believe that the combined data for key compounds in this report support further optimization and/or development of compounds in this class. These results will be communicated in due time.

ASSOCIATED CONTENT

Supporting Information

Details of PK methods, assays, and synthesis procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

DAA, directly acting antiviral; SAR, structure−activity relationship; IFN, interferon; RCM, ring-closing metathesis; HCV, hepatitis C virus; PI, protease inhibitor; AUC, Area Under Curve

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