Non-Nucleoside Benzimidazole-Based Allosteric Inhibitors of the Hepatitis C Virus NS5B Polymerase: Inhibition of Subgenomic Hepatitis C Virus RNA Replicons in Huh-7 Cells

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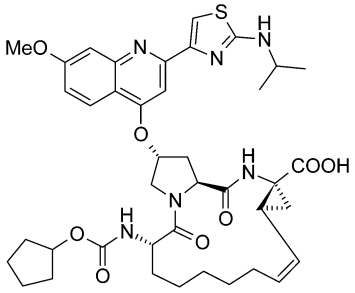
A previously disclosed series of non-nucleoside allosteric inhibitors of the NS5B polymerase of the hepatitis C virus (HCV) was optimized to yield novel compounds with improved physicochemical properties and activity in cell-based assays. Replacement of ionizable carboxylic acids with neutral substituents in lead compounds produced inhibitors with cellular permeability and antiviral activity in a cell-based assay of subgenomic HCV RNA replication (replicon EC_{50} as low as 1.7 μM). The improvement in potency in this ex vivo model of HCV RNA replication validates, in part, the mechanism by which this class of allosteric benzimidazole derivatives inhibits the polymerase and represents a significant step forward in the discovery of novel HCV therapeutics.

Introduction

Recently identified as the etiological agent responsible for non-A and non-B hepatitis,[1] in 2002 it was estimated that the hepatitis C virus (HCV) had spread to at least 170 million people worldwide.[2] While the disease can be resolved by some, for many (>50%), the infection becomes chronic and may progress to end-stage diseases such as liver cirrhosis and hepatocellular carcinomas (HCC). Current treatments for chronic HCV infection rely on the use of modified (pegylated) interferons (IFN-α) in combination with a broad spectrum antiviral agent, ribavirin.[3] This therapy achieves a sustained viral response in approximately 50% of people infected with HCV genotype 1, the most prevalent form of the virus in North America and Europe, and suffers from significant side effects.[4]

Though the virus still cannot be robustly propagated in cell culture, which has hampered efforts to better understand its life cycle, several important advances in recent years have resulted in the identification of key enzymatic functions that are essential for viral replication.[5] These have become prime targets in the search for specific chemical compounds capable of interfering with their role in the replication of the virus and the development of novel HCV therapies. The HCV genome encodes a unique ~3000 amino acid polyprotein, the C-terminal two-thirds of which are nonstructural factors that catalyze and regulate vital viral functions.[6] The NS3 serine protease is an essential maturation enzyme that has been the focus of intensive drug discovery activities.[7] BILN 2061 (Figure 1), a specific HCV NS3 protease inhibitor, recently demonstrated clinical activity in a two-day proof-of-concept trial in infected patients.[8] This unprecedented result has provided validation for many of the tools that are currently being used



BILN 2061

Figure 1.

in HCV drug research, such as the cell-based replicon system, which is the most robust method for evaluating the potency of compounds in arresting cellular HCV RNA replication.[9] Another essential virally encoded enzyme is the RNA-dependent RNA polymerase (NS5B), which is encoded by the C-terminus of the HCV polyprotein.[5,6b,10] This enzyme, which has no mammalian counterpart, plays a central role in the virus' replicative machinery and has attracted the attention of medicinal chemists as reflected by the intense activity in the patent and open literature over the last years. Screening of our corporate sample collection with a modified NS5B polymerase construct from a 1b genotype, that had lower affinity for a primer/template (oligoU/polyA) substrate, identified benzimidazole carboxylic acid derivatives (e.g. **1**, Figure 2) as specific inhibitors of HCV polymerase activity.[11] Initial "hit-to-lead" activities were reported recently and identified compound **2** (Figure 2) as the minimum core for biological activity (IC_{50} values in the low μM range).[12] Subsequent optimization of the right-hand side of the molecule exposed potentially novel ligand−protein interactions, which led to the discovery of compounds that inhibited the polymerase in vitro at low nanomolar concentrations (e.g. compound **3**, IC_{50} = 0.207 μM, Figure 2), a 450-fold increase in intrinsic potency relative to the starting

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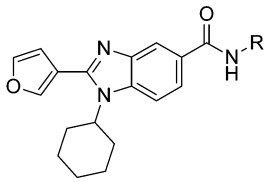
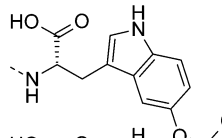
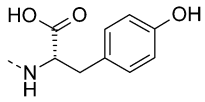
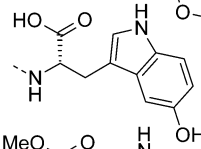
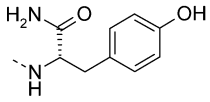
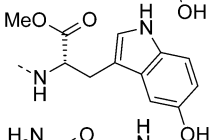
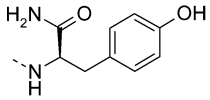
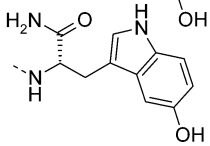
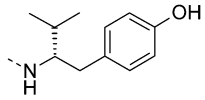
Table 1.



entry	HNR	IC_{50} (μM)	EC_{50} (μM)	entry	HNR	IC_{50} (μM)[a]	EC_{50} (μM)[a]
3		0.027 ± 0.006	64 ± 26	**7**		0.5 ± 0.1	~ 48[b]
4		0.05 ± 0.01	> 74	**8**		0.8 ± 0.1	13 ± 3
5		0.38 ± 0.01	cytotoxic	**9**		3.2 ± 0.8	~ 31[b]
6		0.22 ± 0.06	8 ± 1	**10**		4.3 ± 0.3	3.5 ± 1

[a] Values are the average of at least two determinations performed on two seperate weighings ($n = 4$ or more) unless standard deviations are not provided. [b] Slight cytotoxicity was observed at the hightest concentrations tested (250 μM and 100 mM for compounds **7** and **9**, respectively).

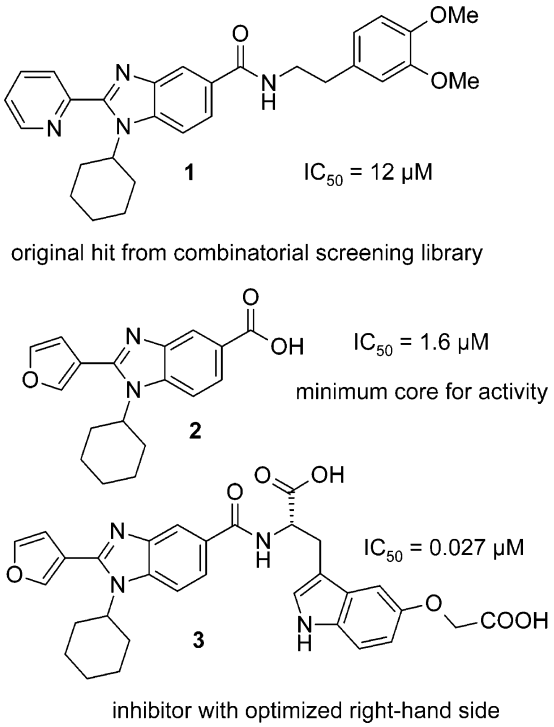


Figure 2.

lead, **1**.[13] However, the most potent compounds of this series did not show significant inhibition of HCV RNA replication in a cell-based replicon assay,[9] presumably as a consequence of their overall charge in a physiological environment and poor cellular permeability. In this paper, we describe specific modifications of the inhibitor structures by replacement of ionizable acidic functions, which led to the discovery of inhibitors of HCV subgenomic RNA replication ex vivo and confirmation of the effect[11,14] of this class of allosteric, non-nucleoside NS5B polymerase inhibitors.

Results and Discussion

5-Carboxybenzimidazole derivatives such as those described in Figure 2 specifically inhibit HCV polymerase activity by preventing productive binding of the enzyme with its RNA substrate.[11,14] The lack of activity in the cell-based replicon assay, which has thus far characterized this class of inhibitors, may be the result of several factors including (i) suboptimal physicochemical properties exhibited by anionic compounds such as **3**[13] that are poorly permeable across cell membranes due to their negatively charged carboxylate functions, (ii) relevance of the observed in vitro polymerase inhibition in our biochemical assay to cellular RNA replication (IC_{50} versus EC_{50}), and (iii) insufficient intrinsic potency as measured in our biochemical assay. In an effort to distinguish between these possibilities, our primary objective was to modify the physicochemical properties of the compounds to promote cellular permeation.

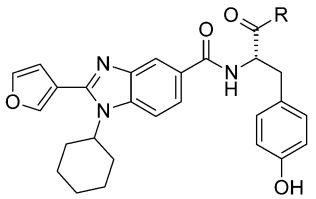
As described in a previous report,[13] removal of the acetic acid from the 5-hydroxyl group on the indole ring of **3** (Figure 2) reduced potency merely 2-fold for a compound with only a single negative charge at physiological pH (compound **4**, Table 1). The increase in lipophilicity resulting from this modification was reflected by the respective distribution coefficient (log *D*) values at pH 7.2 (−1.6 and 0.45, solubility in pH 7.2

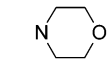
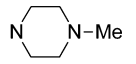
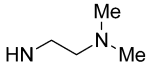
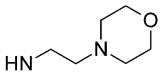
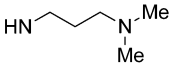
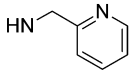
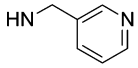
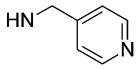
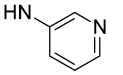
phosphate buffer >850 μg/mL for both compounds)[15] of compounds **3** and **4**. Since the potency in the replicon assay of the latter derivative did not improve significantly (37% inhibition at 74 μM), we focused on neutralizing the remaining negative charge by exploring replacements for the carboxyl group. Previous studies[13] had shown that the α-carboxyl group contributes significantly to the potency of the inhibitor (removal results in a 30-fold loss in potency). When the α-carboxylic acid charge was neutralized by conversion to the methyl ester, the compound was 7.6-fold less potent (compound **5**, $IC_{50} = 0.38$ μM, Table 1). Having generated a neutral inhibitor of the polymerase that retained reasonable potency in the biochemical assay, we tested compound **5** in the cell-based replicon. The further increase in lipophilicity of this compound (log $D = 3.1$, solubility = 0.6 μg/mL at pH 7.2) would presumably promote cellular permeation and inhibition of the target viral enzyme. However, only 30% inhibition in the replicon assay was observed at 3.4 μM, and beyond this concentration, the compound elicited a cytotoxic effect.[16] Carboxamide derivative **6** (Table 1), however, was only 4-fold less potent than acid **4** ($IC_{50} = 0.22$ μM), suggesting that a predominant role for the α-carboxyl group may be to orient the inhibitor backbone toward a favorable conformation for polymerase binding, rather than a direct "salt-bridge" or hydrogen-bonding interaction between the acid and a basic protein residue.[17] When tested in the cell-based HCV replicon assay, compound **6** specifically inhibited replication of subgenomic HCV RNA with an $EC_{50} = 8$ μM, without detectable cytotoxicity. Inhibition in the replicon assay validated the mechanism of action for this class of benzimidazole-based allosteric HCV polymerase inhibitors.[18] As shown in Table 1, a similar study conducted in parallel with somewhat less potent tyrosine analogues[13] provided similar conclusions: when tyrosine derivative **7** (which only showed weak activity in the cell-based assay, $EC_{50} \sim 48$ μM, with slight cytotoxicity at the highest concentration tested) was converted to its carboxamide analogue **8**, the compound had comparable potency to the former ($IC_{50} = 0.5$ and 0.8 μM respectively), but unlike **7**, amide **8** inhibited in the cellular assay with an $EC_{50} = 13$ μM and was devoid of cytotoxic effects (log $D = 3.1$ at pH 7.2). Furthermore, the D-tyrosine amide enantiomer **9** was 4 times less potent than the L-isomer in the enzymatic assay and the EC_{50} shifted by a similar factor of approximately 3-fold ($EC_{50} \sim 31$ μM), reflecting the structure-dependent inhibition and confirming that the measured antiviral activity was not a consequence of nonspecific cytotoxic effects.

Having established a biological proof-of-concept for this class of inhibitors, the subsequent objective was to improve the cellular potency of these compounds. Replacement of the carboxamide group in **8** by an isosteric sp^3-hybridized isopropyl group (compound **10**, Table 1) decreased potency 5-fold, which indicated the preference for an sp^2-hybridized center as an orienting group. The aliphatic modification, however, may have enhanced cellular uptake as reflected by the 3-fold improvement in cellular potency ($EC_{50} = 3.5$ μM, log $D = 3.4$ at pH 7.2). In an effort to further improve cellular activity by increasing the lipophilicity of compounds such as **8**, a series of substituted tyrosine amide derivatives was prepared. The results in Table 2 did not show an improvement in intrinsic potency of the compounds. Tertiary amides (compounds **11**−**13**) were significantly less potent (6−14-fold) than secondary amides (**14**−**20**), which in turn were slightly less potent (3−5-fold) than the original unsubstituted reference point (compound **8**, Table 1). Rationalized on steric grounds, the bulkier disubstituted amides may affect productive binding of the inhibitors to the protein by altering their backbone conformation or hindering interactions of the carbonyl with protein residues. The lack of SAR patterns among this series of secondary amide tyrosine derivatives, other than those mentioned above, reinforced the proposal that this substituent orients toward solvent.[17] None of the compounds in Table 2 showed significantly improved cell culture activity (compound **20** displayed a 2-fold increase: $EC_{50} = 6$ μM). Predictions were that a similar exercise in the tryptophan series would result in an equally unproductive outcome, and an alternative strategy for improving cell culture activity was proposed.

Functional groups that are capable of hydrogen-bonding to solvents (e.g. amide bonds such as those present in compounds **6** and **8**, Table 1) are generally

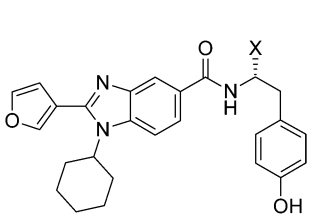
Table 2.

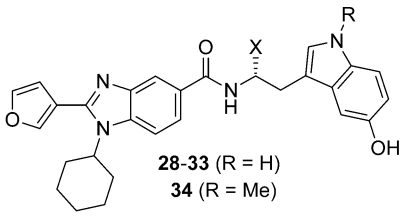


entry	R	IC_{50} (μM)[a]	EC_{50} (μM)[a]
8	NH_2	0.8 ± 0.1	13 ± 3
11	NMe_2	4.9 ± 0.6	
12		9.5 ± 1.3	
13		11.0 ± 1.0	> 9
14		2.1 ± 0.7	
15		3.2 ± 0.3	> 13
16		1.9 ± 0.5	
17		2.7 ± 0.9	10 ± 1
18		2.6 ± 0.6	16 ± 2[b]
19		3.7 ± 0.3	> 9
20		3.0 ± 1.2	6 ± 0.4[b]

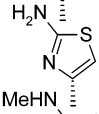
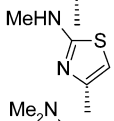
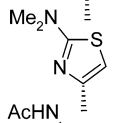
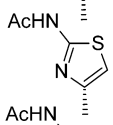
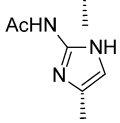
[a] Values are the average of at least two determinations performed on two seperate weighings ($n = 4$ or more). [b] Values are an average of two determinations.

Table 3.





28-**33** (R = H)
34 (R = Me)

X	entry	IC_{50} (μM)[a]	EC_{50} (μM)[a]	entry	IC_{50} (μM)[a]	EC_{50} (μM)[a]
	**21**	6.6 ± 1.6	4.3 ± 0.7	**28**	0.3 ± 0.1	1.7 ± 0.5
	**22**	3.3 ± 0.6	7.4 ± 0.4	**29**	0.3 ± 0.1	2.4 ± 0.4
	**23**	1.3 ± 0.2	not tested	**30**	0.19 ± 0.02	5.7 ± 1.3
	**24**	2.8 ± 0.4	7.1 ± 1.5	**31**	0.5 ± 0.1	3.0 ± 0.8
	**25**	5.1 ± 0.6	8.3[c]	**32**	0.9 ± 0.2	3.7 ± 0.6
	**26**	4.8 ± 1.6	2.4 ± 0.4	**33**	0.55 ± 0.15	5.8 ± 1.0
	**27**[b]	4.2 ± 1.6	not tested	**34**	0.9 ± 0.1	3.4 ± 0.6
				(X = 2-aminothiazolyl, R = Me)		

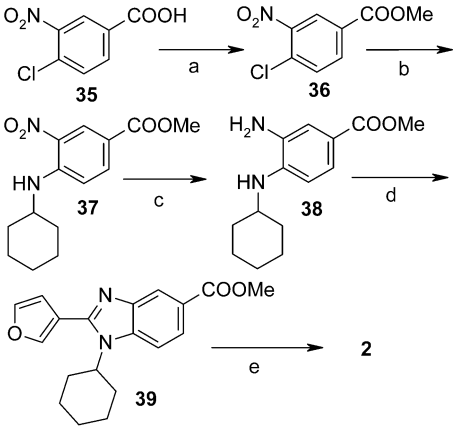
[a] Values are the average of at least two determinations performed on two seperate weighings ($n = 4$). [b] Compounds **27** was 80−85% homogeneous by reversed-phase HPLC. [c] Compound **25** was only tested once in the replicon assay ($n = 1$).

believed to be detrimental to cellular permeation.[19] Since conformational rigidity suggested a preference for sp^2-hybridized orienting groups (compounds **7**, **8** versus **10**), we rationalized that an sp^2 center within an aromatic heterocyclic system may provide an appropriate mimic of the orienting feature of a planar amide bond while minimizing solvation effects. Carboxylic acid functions are conveniently transformed into substituted thiazole heterocycles using the Hantzsch thiazole synthesis.[20] A series of tyrosine isosteres bearing a 4-thiazolyl replacement of the carboxyl function was prepared (compounds **21**−**26**, Table 3). All compounds showed low micromolar potency in the enzymatic assay, a 3−13-fold loss compared to the corresponding carboxylic acid **7** (Table 1), but only 1.6−8-fold less relative to amide **8**. The 2-aminothiazole analogue (**23**, Table 3) was the most potent ($IC_{50} = 1.3$ μM). Unlike the carboxylic acid derivatives and amides shown in Table 2, however, all thiazole compounds were active in the cell-based replicon assay with EC_{50} values ranging from 2 to 8 μM. Imidazole **27** had similar potency to thiazole **26** (Table 3). As shown previously,[13] expanding the size of the right-hand side from a tyrosine to a tryptophan moiety generally results in a ∼10-fold improvement in intrinsic potency. A similar observation was made with the isosteric thiazole derivatives, confirming that the two classes of compounds share the same mode of bonding. As seen in Table 3, tryptophan-derived analogues (**28**−**33**) were 6−22-fold more potent than their tyrosine counterparts. These potencies did not translate into improved cell culture activity, which remained comparable (1.7−5.8 μM) to that of the tyrosine series. The increase in size and molecular weight of the tryptophan analogues may negate the gain in intrinsic potency, resulting in net decreased cellular permeability. Typically, these derivatives exhibited physicochemical properties characteristic of highly lipophilic compounds [e.g. compound **29** in Table 3 had log $D = 4.6$ (pH 7.2) and solubility at pH 7.2 <0.1 μg/mL]. The best analogue in the series was unsubstituted thiazole **28** ($EC_{50} = 1.7$ μM, log $D = 3.8$, Table 3). *N*-Methylation of the indole nitrogen had no significant effect on cell culture activity (compare **30** and **34** in Table 3).

Compounds were also tested for specificity relative to another RNA-dependent RNA polymerase from the poliovirus and a mammalian DNA-dependent RNA polymerase II isolated from calf-thymus, as previously described.[11] In particular, for analogues in Table 3 that were active in cell culture, and as previously reported for this class of inhibitors,[12,13] the compounds were highly specific for HCV polymerase, showing <50% inhibition at 200−250 μM against the other enzymes.

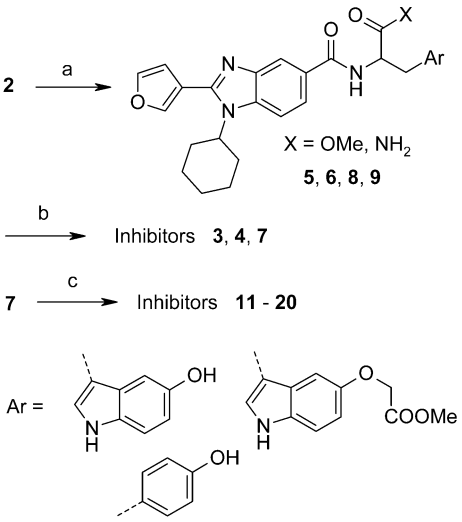
Synthesis of Inhibitors. Carboxylic acid derivative **2** (Figure 2) was prepared using a methodology devel-

Scheme 1[a]



[a] Reagents and conditions: (a) MeOH/$SOCl_2$/reflux. (b) cyclohexylamine/Et_3N/DMSO/70−80 °C. (c) H_2 gas (1 atm)/20%$Pd(OH)_2$/MeOH. (d) 3-furaldehyde/oxone/DMF−water. (e) NaOH/MeOH−water then AcOH.

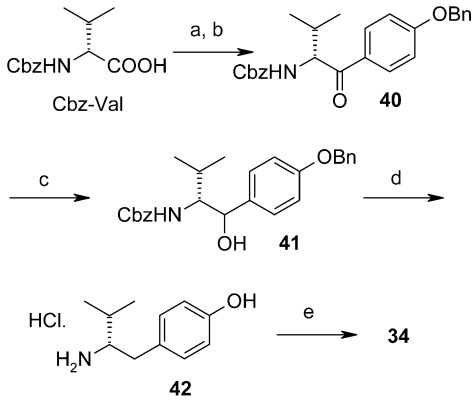
Scheme 2[a]



[a] Reagents and conditions: (a) amino acid derivative/TBTU/DIEA/DMF or DMSO. (b) NaOH then preparative reversed-phase HPLC. (c) Amine/EDC/DIEA/HOBt/DMSO.

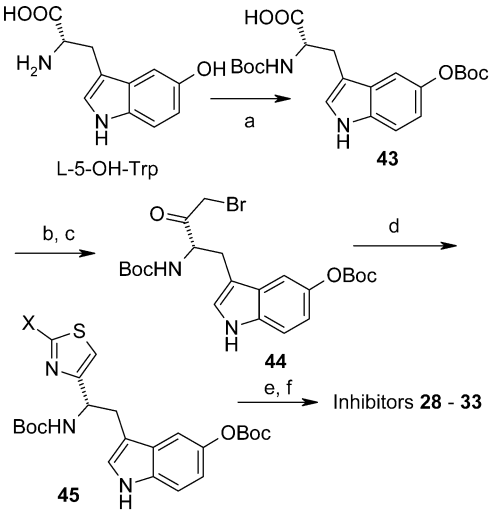
oped for the high-throughput synthesis of benzimidazole derivatives.[21] As shown in Scheme 1, commercially available 4-chloro-3-nitrobenzoic acid **35** was esterified (e.g. as the methyl ester **36** or the corresponding ethyl ester) and the cyclohexylamine substituent introduced via an S_NAr reaction to give nitroamine **37**. Hydrogenolysis of the nitro group gave the 1,2-phenylenediamine **38** required for an oxone-mediated benzimidazole ring-formation with 3-furaldehyde.[21] Saponification under standard conditions gave key intermediate **2**. Inhibitors were obtained through amide coupling of carboxylic acid derivative **2** with amines, followed by cleavage of protecting groups (generally esters), if necessary, as shown in Scheme 2. In some cases, inhibitors were further elaborated through additional amide bond formation (compounds **11**−**20**, Table 2). The chiral amine derivative required for the preparation of inhibitor **10** (Table 1) was prepared as described in Scheme 3 and coupled to carboxylic acid **2** in the usual manner. Thiazole isosteres of tyrosine (compounds **21**−**26**, Table 3) and 5-hydroxytryptophan (compounds **28**−**33**, Table 3) and compound **34** were prepared from the

Scheme 3[a]



[a] Reagents and conditions: (a) NHMeOMe·HCl/TBTU/DIEA/DMF. (b) (4-BnO)-C_6H_4-MgBr/THF. (c) $NaBH_4$/MeOH. (d) 10% Pd/C/AcOH−concd HCl/H_2 gas (50 psi). (e) **2**/TBTU/DIEA/DMSO.

Scheme 4[a]



[a] Reagents and conditions: (a) Boc_2O/NaOH/dioxane−water. (b) Isobutyl chloroformate/Et_3N/THF/−20 °C then excess etheral diazomethane. (c) 40% aq HBr. (d) Thioamides or thioureas/dioxane/reflux. (e) 4 N HCl in dioxane then free basing. (f) **2**/TBTU/DIEA/DMF or DMSO.

corresponding amino acids as shown in Scheme 4 for tryptophan analogues: L-5-hydroxytryptophan was protected as the bis-*N*,*O*-Boc derivative **43**[22] and the carboxylic acid converted to the corresponding bromomethyl ketone **44** by sequential activation with isobutylchloroformate, conversion to the diazomethyl ketone using diazomethane, and final treatment with hydrobromic acid. Bromomethyl ketone **44** was then converted to thiazole derivatives by heating **44** with thioamide and thiourea derivatives.[20] Amines **45** were obtained following removal of remaining protecting groups with 4 N HCl in dioxane. Amines **45** were then coupled to carboxylic acid **2** in the usual manner to provide the desired inhibitors. A similar sequence of steps starting from tyrosine provided analogous compounds.

Inhibitor **34** (Table 3) was obtained starting from 5-hydroxy-1-methyl-L-tryptophan, prepared using the method of Bennani et al.[23] Imidazole analogue **27** (Table 3) was prepared from the bromomethyl ketone derived from *N*,*O*-bis-Boc-tyrosine (prepared as shown from tryptophan analogues in Scheme 4) and *N*-acetylguanidine. All inhibitors were purified by preparative reversed-phase HPLC to greater than ∼95% homogeneity as

determined in two solvent systems and were isolated as TFA salts. Characterization data is provided in the Supporting Information.

Conclusion

Previously reported benzimidazole-based allosteric inhibitors of HCV NS5B polymerase were initially found to be inactive in a cell-based HCV replicon assay. Modification of the physicochemical properties of these charged molecules by replacement of ionizable functions with neutral entities reduced the intrinsic potency of the inhibitors but improved their cell culture activity. These neutral derivatives, at low micromolar concentrations, inhibited replication of subgenomic HCV RNA in Huh-7 cells. The best inhibitor, compound **28** (Table 3), had an $EC_{50} = 1.7$ μM and was not cytotoxic to the cells. The activity in the cellular replicon assay provides mechanistic relevance to inhibition of viral replication for this class of benzimidazole-based allosteric inhibitors of NS5B.

Experimental Section

General. NMR spectra were recorded at 400 MHz for ^{1}H NMR and 100 MHz for ^{13} C NMR in deuterated solvents and are referenced to TMS or the solvent signal (δ scale). Low-resolution mass spectra were obtained on a Micromass Platform LCZ model ZMD 4000 in electrospray mode. High-resolution mass spectra (HRMS) were obtained on a Micromass AutoSpec instrument in FAB+ ionization mode, using an NBA matrix. HPLC homogeneities were determined under reversed-phase conditions. System A: Waters (Milford, MA) Symmetry C18 column (5 μM, 2.1 × 150 mm), 5 → 100% CH_3CN (+0.1% TFA) in 0.1% TFA linear gradient in 25 min then 5 min isocratic, 0.5 mL/min flow rate, UV detection at 215 nM. System B: same column as system A, 5 → 80% CH_3CN in 50 mM NaH_2PO_4 (pH 3.0) linear gradient in 25 min then 5 min isocratic, 0.5 mL/min flow rate, UV detection at 215 nM.

Solvents and reagents were used as received from commercial sources.

1-Cyclohexyl-2-furan-3-yl-1*H*-benzimidazole-5-carboxylic Acid 2. Ethyl 3-amino-4-(cyclohexylamino)benzoate[21] (88.62 g, 338 mmol) was dissolved in DMF (350 mL), and oxone (145.5 g, 237 mmol, 0.7 equiv) was added followed by water (50 mL). The reaction mixture was immersed in a room-temperature water bath and stirred with a mechanical stirrer. 3-Furaldehyde (35.70 g, 372 mmol, 1.1 equiv) in DMF (50 mL) was added dropwise over 2 h. After completion, the reaction mixture was stirred for an additional 2 h, at which point HPLC analysis of the mixture showed disappearance of the starting diamine. The reaction mixture was diluted with a mixture of 5 N NaOH (340 mL) and MeOH to give a brown solution with a small amount of insoluble solids that were removed by filtration. Volatiles were removed under reduced pressure to give a thick residue that was dissolved in DMF (500 mL). NaOH (10 N, 170 mL) was added and the mixture stirred at 60 °C for 2 h, after which HPLC analysis showed complete hydrolysis of the ester. The reaction mixture was suspended in water (4 L) and the turbid solution filtered to remove insoluble particulates. The filtrate was then acidified to pH 3 and the precipitated product collected by filtration, washed with water, and dried in air. The crude product (86.2 g, ~80% homogeneity) was dissolved in THF (400 mL) at 60 °C and hexane (400 mL) was slowly added to the hot solution. After cooling the slurry to room temperature and then 5 °C overnight, the product was collected by filtration and washed with 1:1 THF−hexane and then hexane. Carboxylic acid **2** was obtained as a grayish solid (62.07 g, 59% yield): mp >280 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (br s, 1H), 8.28 (s, 1H), 8.20 (d, $J = 1.2$ Hz, 1H), 7.92 (s, 1H), 7.91 (d, $J = 8.4$ Hz, 1H), 7.83 (dd, $J = 8.4$, 1.4 Hz, 1H), 6.94 (d, $J = 1.0$ Hz, 1H), 4.44 (tt, $J = 12.3$, 3.9 Hz, 1H), 2.26 (m, 2H), 1.95−1.80 (m, 4H), 1.67 (m, 1H), 1.50−1.36 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.8, 148.3, 144.4, 143.4, 143.0, 136.8, 124.4, 123.2, 120.9, 116.0, 112.7, 111.1, 56.6, 30.5, 25.4, 24.4; ES-MS(+) *m/z* 311 (MH^+). Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.58; H, 5.76; N, 9.07.

[(*R*)-1-(4-Benzyloxybenzoyl)-2-methylpropyl]carbamic Acid Benzyl Ester 40: Cbz-L-valine (0.400 g, 1.59 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (0.186 g, 1.91 mmol, 1.2 equiv), and TBTU[24] (0.767 g, 2.38 mmol, 1.5 equiv) were dissolved in DMF (2 mL), and the solution was cooled in ice−water. DIEA (0.83 mL, 4.77 mmol, 3 equiv) was added and the mixture stirred for 1 h at room temperature. The reaction mixture was then poured into 1 M $KHSO_4$ (30 mL) and the product extracted with EtOAc (15 mL). The extract was washed consecutively with $KHSO_4$ (20 mL), aqueous $NaHCO_3$ (4 × 20 mL), and brine. After drying ($MgSO_4$), evaporation of the solvent under reduced pressure gave the desired Weinreb amide as a clear oil in quantitative yield: TLC R_f 0.8 (5% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (br d, $J = 8.9$ Hz, 1H), 7.38−7−28 (m, 5H), 5.04 (d, $J = 12.4$ Hz, 1H, part of AB), 5.01 (d, $J = 12.7$ Hz, 1H, part of AB),4.35 (br m, 1H), 3.74 (s, 3H), 3.12 (s, 3H), 1.92 (m, $J = 7.0$ Hz, 1H), 0.88 (d, $J = 6.7$ Hz, 3H), 0.84 (d, $J = 6.7$ Hz, 3H); ES-MS(+) *m/z* 295 (MH^+).

Magnesium turnings (0.113 g, 4.65 mmol, 3 equiv) were suspended in dry THF (5 mL) and an iodine crystal was added. The solution was brought to reflux and 4-benzyloxybromobenzene (1.35 g, 5.1 mmol, 3.3 equiv) in THF (5 mL) was added in small portions. After stirring at reflux for 3 h, the Grignard reagent was cooled to room temperature and added dropwise to a solution of the above Weinreb amide in THF (5 mL). The resulting mixture was stirred for 1 h at room temperature. THF was partially removed under reduced pressure and the residue taken up in CH_2Cl_2 (40 mL). The solution was washed with 1 N HCl (2 × 20 mL), dried ($MgSO_4$), and concentrated. The crude material was purified by flash chromatography using 10→20% EtOAc in hexane as eluent to give ketone **40** as an amber-colored oil that slowly crystallized on standing (0.419 g, 65% yield): TLC R_f 0.53 (33% EtOAc in hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, $J = 8.9$ Hz, 2H), 7.61 (d, $J = 8.6$ Hz, 1H), 7.50−7.27 (m, 10H), 7.13 (d, $J = 8.9$ Hz, 2H), 5.22 (s, 2H), 5.02 (s, 2H), 4.91 (dd, $J = 8.3$, 6.7 Hz, 1H), 2.10 (m, $J = 6.7$ Hz, 1H), 0.88 (d, $J = 7.0$ Hz, 3H), 0.84 (d, $J = 6.7$ Hz, 3H); ES-MS(+) *m/z* 418 (MH^+).

{(*R*)-1-[(4-Benzyloxyphenyl)hydroxymethyl]-2-methylpropyl}carbamic Acid Benzyl Ester 41. Ketone **40** (0.200 g, 0.48 mmol) was dissolved in MeOH (0.5 mL) containing 50 μL of DMF. Sodium borohydride (36 mg, 0.96 mmol, 2 equiv) was added in small portions and the mixture stirred for 2 h at room temperature. The reaction mixture was then partitioned between EtOAc (30 mL) and 1 N HCl (20 mL). The organic phase was washed with 1 N HCl (20 mL), dried ($MgSO_4$), and concentrated to give a single diastereomer of alcohol **41** in quantitative yield as a clear oil that solidified upon standing: TLC R_f 0.41 (33% EtOAc in hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47−7.19 (m, 10H), 7.05 (d, $J = 7.0$ Hz, 2H), 6.91 (d, $J = 8.5$ Hz, 2H), 6.83 (d, $J = 10.2$ Hz, 1H), 5.19 (d, $J = 5.4$ Hz, 1H), 5.07 (s, 2H), 4.93 (d, $J = 13.4$ Hz, 1H, part of AB), 4.78 (d, $J = 13.0$ Hz, 1H, part of AB), 4.30 (dd, $J = 9.5$, 5.4 Hz, 1H), 3.58 (dt, $J = 9.8$, 3.2 Hz, 1H), 2.24 (m, 1H), 0.85 (d, $J = 7.0$ Hz, 3H), 0.82 (d, $J = 7.0$ Hz, 3H); ES-MS(+) *m/z* 402 (M − OH).

4-((*S*)-2-Amino-3-methylbutyl)phenol Hydrochloride 42. Benzylic alcohol **41** (0.200 g, 0.48 mmol) was dissolved in AcOH (6 mL), and 10% Pd/C (200 mg) was added. The mixture was hydrogenolyzed in a Parr shaker (50 psi H_2 pressure) for 6 h. MS analysis of an aliquot indicated loss of Cbz and Bn protecting groups only. Concentrated HCl (1 mL) and fresh catalyst (200 mg) were added, and hydrogenolysis was resumed at 50 psi for an additional 18 h. The suspension was then filtered over Celite using MeOH for rinses, and volatiles were removed under high vacuum to give amine hydrochloride **42** as a yellow solid in quantitative yield: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.02 (br s, 3H), 7.07 (d, $J = 8.6$ Hz, 2H), 6.72 (d, $J = 8.2$ Hz, 2H), 4.11 (br m, 1H), 2.78 (dd, $J =$

14.0, 6.4 Hz, 1H, part of AB), 2.69 (dd, J = 14.0, 7.3 Hz, 1H, part of AB), 1.84 (m, 1H), 0.95 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H); ES-MS (+) *m/z* 180 (MH^+).

General Procedure for Coupling 2 with Amino Acid Derivatives and Deprotection of Ester Functionalities. Preparation of (*S*)-2-[(1-Cyclohexyl-2-furan-3-yl-1*H*-benzimidazole-5-carbonyl)amino]-3-(4-hydroxyphenyl)propionic Acid (Inhibitor 7). Carboxylic acid **2** (0.155 g, 0.5 mmol) and L-tyrosine methyl ester hydrochloride (0.154 g, 0.66 mmol, 1.3 equiv) were dissolved in DMSO (1.5 mL), and *N*,*N*-diisopropylethylamine (DIEA, 0.4 mL, 2.3 mmol, 4.6 equiv) was added. TBTU coupling reagent[24] (0.208 g, 0.65 mmol, 1.3 equiv) was added and the mixture stirred for 2 h at room temperature. The reaction mixture was then poured into water and acidified to pH 6 with dilute HCl. The precipitated solid was collected by filtration and washed with water. It was then redissolved in DMSO (1.5 mL) and 2.5 N NaOH (3 mL) was added. The mixture was stirred for 1 h after which point HPLC analysis showed complete hydrolysis of the methyl ester. The solution was acidified with excess TFA and the reaction mixture purified directly by preparative reversed-phase HPLC on a C18 column using 0.1% TFA in MeCN/0.1% aqueous TFA gradients. Compound **7** (0.117 g, 49% yield) was obtained as a white amorphous TFA salt after lyophilization: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, J = 8.0 Hz, 1H), 8.40 (s, 1H), 8.18 (s, 1H), 8.04 (d, J = 8.8 Hz, 1H), 8.01 (s, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.13 (d, J = 8.4 Hz, 2H), 7.00 (s, 1H), 6.63 (d, J = 8.4 Hz, 2H), 4.55 (m, 1H), 4.46 (tt, J = 12.3, 3.9 Hz, 1H), 3.09 (dd, J = 13.9, 4.1 Hz, 1H), 2.98 (dd, J = 13.5, 10.8 Hz, 1H), 2.35−2.21 (br m, 2H), 1.95 (br m, 2H), 1.86 (br m, 2H), 1.68 (br m, 1H), 1.44 (br m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 166.0, 155.8, 147.0, 144.9, 144.7, 138.7, 134.5, 130.0, 129.1, 128.2, 116.8, 114.9, 113.7, 113.5, 111.0, 57.4, 54.8, 35.5, 30.3, 25.3, 24.2; HRMS (FAB) *m/z* $C_{27}H_{28}N_3O_5$ (MH^+) calcd 474.2029, found 474.2020; HPLC homogeneity 99.8% (system A), 99.4% (system B).

Alternatively, on a larger scale, following isolation of the methyl ester intermediate, the material was dissolved in MeOH (0.516 g/5 mL), and 1 N LiOH (3 equiv) was added. After completion (~1.5 h as determined by HPLC analysis), MeOH was removed under reduced pressure and the residue was diluted with water (15 mL). The mixture is acidified by dropwise addition of 1 N HCl (3.17 mL) with vigorous stirring. The precipitated **7** was collected by filtration, washed with water, and dried. The material is obtained as a gray solid (0.504 g) in quantitative yield (HPLC homogeneity 98% in system A).

General Procedure for the Preparation of Amide Derivatives of Compound 7 (Compounds 11−20, Table 2). The Preparation of 1-Cyclohexyl-2-furan-3-yl-1*H*-benzimidazole-5-carboxylic Acid [(*S*)-2-(4-Hydroxyphenyl)-1-(2-morpholin-4-ylethylcarbamoyl)ethyl]amide (Inhibitor 15) Is Representative. Carboxylic acid derivative **7** (25 mg, 0.053 mmol), HOBt (8 mg, 0.058 mmol, 1.1 equiv), and EDC (11 mg, 0.058 mmol, 1.1 equiv) were dissolved in DMSO (0.3 mL), and DIEA (46 μL, 0.26 mmol, 5 equiv) was added. The mixture was stirred for 10 min and aminoethylmorpholine (10 μL, 0.079 mmol, 1.5 equiv) was added. The mixture was stirred overnight at room temperature (or until complete as determined by HPLC analysis), diluted with DMSO (0.3 mL), and acidified with TFA, and the reaction mixture was directly purified by preparative reversed-phase HPLC. Compound **15** (15.8 mg) was isolated as a white amorphous TFA salt after lyophilization: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.62 (br s, 1H), 9.18 (br s, 1H), 8.62 (d, J = 7.6 Hz, 1H), 8.33 (t, J = 5.7 Hz, 1H), 8.32 (s, 1H), 8.20 (d, J = 1.6 Hz, 1H), 7.98 (t, J = 1.8 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.73 (dd, J = 8.6, 1.6 Hz, 1H), 7.12 (d, J = 8.2 Hz, 2H), 6.96 (dd, J = 1.9, 0.6 Hz, 1H), 6.65 (d, J = 8.2 Hz, 2H), 4.55 (m, 1H), 4.46 (tt, J = 12.1, 3.5 Hz, 1H), 3.99 (br d, J = 12.1 Hz, 2H), 3.47 (m, 4H), 3.26−3.08 (m, 4H), 3.02 (dd, J = 14.0, 5.4 Hz, 1H), 2.96 (dd, J = 13.7, 9.8 Hz, 1H), 2.37−2.21 (br m, 2H), 1.98−1.82 (br m, 4H), 1.68 (br m, 1H), 1.51−1.38 (br m, 3H); HRMS (FAB) *m/z* $C_{33}H_{40}N_5O_5$ (MH^+) calcd 586.3029, found 586.3032; HPLC homogeneity 96.8% (system A), 95.0% (system B).

(*S*)-2-*tert*-Butoxycarbonylamino-3-(5-*tert*-butoxycarbonyloxy-1*H*-indol-3-yl)propionic Acid (43).[22] A 500 mL flask equipped with a pH meter was charged with L-5-hydroxytryptophan (5.510 g, 25.0 mmol) and water (160 mL). Di-*tert*-butyl dicarbonate (17.00 g, 77.9 mmol, 3.1 equiv) was added followed by 2-propanol (100 mL). The pH of the solution was adjusted to 11.5−12.0 by addition of 8 M KOH. The mixture was then stirred for 4 h, and pH was maintained in the range of 11.5−12.0 by slow addition of 8 M KOH. After 4 h, additional di-*tert*-butyl dicarbonate (2.00 g, 9.2 mmol, 0.37 equiv) was added and the mixture stirred another hour to complete the conversion (monitored by reversed-phase HPLC). A total of 30 mL of 8 M KOH was used to maintain the pH in the desired range. The reaction mixture was then concentrated under reduced pressure to remove the 2-propanol. Water (200 mL) was added and the aqueous phase was washed with *tert*-butylmethyl ether (TBME) (200 mL). The aqueous portion was neutralized by careful addition of AcOH (20 mL) (**Caution:** CO_2 evolution) and the product extracted into TBME (200 + 100 mL). The combined organic layers were washed with brine (2 × 100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The dark residue was diluted with TBME (40 mL), and hexane (~100 mL) was added slowly until a slight cloudiness persisted. Scratching and sonication caused a fine crystalline solid to form that was collected by filtration, washed with small portions of 30% TBME in hexane, and dried under vacuum (5.34 g). The filtrate and washings from above were concentrated to a foam that was suspended in hexane (180 mL). With vigorous stirring, TBME (20 mL) was added slowly, causing crystallization of a second crop that was collected, washed, and dried as above. The diprotected tryptophan derivative **43** (total yield of 9.24 g, 87% yield) was obtained as light mauve 25% TBME solvate: mp 106−112 °C (dec). R_f 0.12 (EtOAc). $[\alpha]_D$ −9.8 (*c* 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) indicated the presence of pseudo-cis- and trans-rotamers of the carbamate bond in a 8:1 ratio ((C*H*$_3$)$_3$C and Boc*H*N signals coalesced at 57 °C), δ 12.57 (br s, 1H), 10.96 (s, 1H), 7.31 (d, J = 8.6 Hz, 1H), 7.27 (d, J = 1.8 Hz, 1H), 7.21 (d, J = 1.4 Hz, 1H), 6.96 (d, J = 8.0 Hz, 0.9H, major BocHN trans-rotamer), 6.85 (dd, J = 8.6, 2.0 Hz, 1H), 6.61 (d, J = 8.0 Hz, 0.1H, minor BocHN cis-rotamer), 4.09 (m, 1H), 3.09 (dd, J = 14.7, 4.3 Hz, 1H, part of AB), 2.94 (dd, J = 14.5, 9.4 Hz, 1H, part of AB), 1.47 (s, 9H), 1.32 (s, 8H major Boc trans-rotamer), 1.21 (s, 1H, minor Boc cis-rotamer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 155.4, 152.2, 143.7, 133.8, 127.2, 125.3, 115.0, 111.7, 110.7, 110.0, 82.4, 78.0, 54.6, 28.1, 27.3, 26.7; HRMS (FAB) *m/z* $C_{21}H_{28}N_2O_7$ (M) calcd 420.1897, found 420.1886.

Carbonic Acid 3-((*S*)-4-Bromo-2-*tert*-butoxycarbonylamino-3-oxobutyl)-1*H*-indol-5-yl Ester *tert*-Butyl Ester 44. Bis-Boc-protected tryptophan derivative **43** (4.90 g, 11.65 mmol) was dissolved in anhydrous THF (30 mL) and the solution cooled in an ice−water bath under an argon atmosphere. Diisopropylethylamine (3.45 mL, 19.8 mmol, 1.7 equiv) was added followed by isobutylchloroformate (2.27 mL, 17.5 mmol, 1.5 equiv). The mixture was stirred for 5 h in the cooling bath. A solution of diazomethane (0.6 M in TBME, 100 mL) was added and the mixture stirred for an additional 1 h. Volatiles were removed under reduced pressure, and the residue was dissolved in TBME (300 mL). The solution was washed with 5% aqueous citric acid (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and brine (50 mL). The solution was then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using 40−50% EtOAc in hexane as eluent to give the intermediate diazomethyl ketone as a yellow foam (4.487 g, 86% yield): mp 75−79 °C (dec); R_f 0.20 (30% EtOAc−hexane); $[\alpha]_D$ +3.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) indicated the presence of pseudo-cis- and trans-rotamers of the carbamate bond in a 8:1 ratio, δ 10.96 (s, 1H), 7.34 (s, 1H), 7.31 (d, J = 5.5 Hz, 1H), 7.22 (m, 2H), 6.84 (dd, J = 8.6, 2.0 Hz, 1H), 6.16 (br s, 1H), 4.13 (br m, 1H), 3.05 (dd, J = 14.5, 4.3 Hz, 1H, part of AB), 2.85 (dd, J = 14.3, 10.0 Hz, 1H, part of AB), 1.48 (s, 9H), 1.30 (s, 8H, major trans-rotamer),

1.17 (s, 1H, minor cis-rotamer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.4, 155.2, 152.2, 143.7, 133.8, 127.2, 125.4, 115.1, 111.6, 110.5, 110.1, 82.3, 78.3, 58.2, 55.9, 28.1, 27.3; HRMS (FAB) *m/z* $C_{22}H_{29}N_4O_6$ (MH^+) calcd 445.2087, found 445.2094. Anal. Calcd for $C_{22}H_{28}N_4O_6$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.43; H, 6.37; N, 11.49.

The diazomethyl ketone from above (3.93 g, 8.84 mmol) was dissolved in EtOAc (50 mL) and the solution cooled to −45 °C in a dry ice−acetone bath. A solution of 45% HBr in AcOH (1.30 mL, 10.04 mmol, 1.14 equiv) was added dropwise over 75 min, the progress of the reaction being monitored by TLC. After completion, the cold reaction mixture was diluted with TBME (150 mL) and the solution washed with 5% aqueous citric acid (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and brine (50 mL). After drying (Na_2SO_4), the solution was concentrated under reduced pressure and the residue purified by flash chromatography on silica gel using 30% EtOAc in hexane as eluent. The desired bromomethyl ketone **44** was obtained as a yellow foam (3.614 g, 82% yield): mp 82−90 °C (dec); R_f 0.39 (50% EtOAc − hexane); $[\alpha]_D$ +0.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) indicated the presence of pseudo-cis- and trans-rotamers of the carbamate bond in a 8:1 ratio, δ 10.97 (br s, 1H), 7.35 (d, J = 1.6 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 7.21 (d, J = 1.4 Hz, 1H), 6.85 (dd, J = 8.8, 2.2 Hz, 1H), 4.47 (s, 1H), 4.46 (s, 1H), 4.39 (m, 1H), 3.13 (dd, J = 14.7, 4.7 Hz, 1H, part of AB), 2.87 (dd, J = 14.5, 9.4 Hz, 1H, part of AB), 1.48 (s, 9H), 1.31 (s, 8H, major trans-rotamer), 1.18 (s, 1H, minor cis-rotamer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.0, 155.4, 152.2, 143.8, 133.8, 127.2, 125.5, 115.1, 111.7, 110.3, 110.1, 82.3, 78.5, 58.7, 35.5, 28.1, 27.3; HRMS (FAB) *m/z* $C_{22}H_{29}BrN_2O_6$ (MH^+) calcd 496.1209, found 496.1209. Anal. Calcd for $C_{22}H_{29}BrN_2O_6$: C, 53.13; H, 5.88; N, 5.63. Found: C, 53.26; H, 5.96; N, 5.81.

General Procedure for the Preparation of Thiazolyl Derivatives of General Formula 45 from Bromomethyl Ketone 44. Carbonic Acid 3-[(*S*)-2-(2-Aminothiazol-4-yl)-2-*tert*-butoxycarbonylaminoethyl]-1*H*-indol-5-yl Ester *tert*-Butyl Ester (Compound 45, X = NH$_2$). Bromomethyl ketone **44** (1.180 g, 2.37 mmol) was dissolved in acetonitrile (10 mL), and thiourea (0.226 g, 2.97 mmol, 1.25 equiv) was added. The mixture was refluxed for 24 h. The reaction was then diluted with TBME (150 mL) and the solution washed with saturated $NaHCO_3$ (25 mL). After drying (Na_2SO_4), volatiles were removed under reduced pressure, and the residue was purified by flash chromatography on silica gel using 70−100% EtOAc in hexane to give the desired thiazolyl derivative as a white solid (1.050 g, 93% yield): mp 117−123 °C (dec); R_f 0.27 (70% EtOAc−hexane); $[\alpha]_D$ −14.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) indicated the presence of pseudo-cis- and trans-rotamers of the carbamate bond in a 8:1 ratio, δ 10.87 (br s, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.25 (d, J = 1.6 Hz, 1H), 7.08 (d, J = 1.3 Hz, 1H), 6.99 (d, J = 9.0 Hz, 1H), 6.90 (br s, 2H), 6.83 (dd, J = 8.6, 2.2 Hz, 1H), 6.20 (s, 1H)4.58 (m, 1H), 3.15 (dd, J = 14.7, 4.5 Hz, 1H, part of AB), 2.86 (dd, J = 14.7, 9.2 Hz, 1H, part of AB)1.49 (s, 9H), 1.29 (s, 8H, major trans-rotamer), 1.07 (s, 1H, minor cis-rotamer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 155.0, 153.8, 152.3, 143.6, 133.7, 127.6, 124.7, 114.8, 111.9, 111.5, 110.1, 100.3, 82.3, 77.6, 52.5, 30.3, 28.2, 27.3; HRMS (FAB) *m/z* $C_{23}H_{31}N_4O_5S$ (MH^+) calcd 475.2015, found 475.2015. Anal. Calcd for $C_{23}H_{30}N_4O_5S$: C, 58.21; H, 6.37; N, 11.81. Found: C, 58.41; H, 6.41; N, 11.35.

Carbonic Acid 3-[(*S*)-2-*tert*-Butoxycarbonylamino-2-(2-methylthiazol-4-yl)ethyl]-1*H*-indol-5 -yl Ester *tert*-Butyl Ester (compound 45, X = Me). Following a similar procedure to that described above but using thioacetamide instead of thiourea, the title compound was obtained as a white solid in 60% yield after purification by flash chromatography: mp 80−83 °C (dec); R_f 0.64 (70% EtOAc−hexane); $[\alpha]_D$ −9.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) indicated the presence of pseudo-cis- and trans-rotamers of the carbamate bond in a 8:1 ratio, δ 10.89 (s, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.22 (m, 2H), 7.12 (d, J = 8.6 Hz, 1H), 7.11 (s, 1H), 6.83 (dd, J = 8.8, 2.2 Hz, 1H), 4.84 (m, 1H), 3.19 (dd, J = 14.5, 5.1 Hz, 1H), 2.97 (dd, J = 14.5, 8.8 Hz, 1H), 2.63 (s, 3H), 1.49 (s, 9H), 1.29 (s, 8H, major trans-rotamer), 1.09 (s, 1H, minor cis-rotamer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 157.8, 155.0, 152,3, 143.6, 133.7, 127.5, 124.9114.9, 113.7, 111.5, 110.1, 82.3, 77.7, 52.3, 30.7, 28.2, 27.3, 18.8; HRMS (FAB) *m/z* $C_{24}H_{32}N_3O_5S$ (MH^+) calcd 474.2063, found 474.2058. Anal. Calcd for $C_{24}H_{31}N_3O_5S$: C, 60.87; H, 6.60; N, 8.87. Found: C, 60.71; H, 6.57; N, 8.67.

General Procedure for Coupling Thiazolyl Derivatives to Benzimidazole Carboxylic Acid 2. Preparation of 1-Cyclohexyl-2-furan-3-yl-1*H*-benzimidazole-5-carboxylic Acid [(*S*)-1-(2-Aminothiazol-4-yl)-2-(5-hydroxy-1*H*-indol-3-yl)ethyl]amide 30. The bis-Boc-protected aminothiazole derivative from above (0.160 g, 0.34 mmol, 1.4 equiv) was dissolved in CH_2Cl_2 (1 mL), and TFA (0.5 mL) was added. The mixture was stirred for 1 h at room temperature, and volatiles were removed under reduced pressure. The residue was dissolved in DMSO (2 mL), and carboxylic acid **2** (0.075 g, 0.24 mmol, 1 equiv) was added followed by triethylamine (0.28 mL, 2 mmol, 8.3 equiv) and TBTU (0.078 g, 0.24 mmol, 1 equiv). The reaction mixture was stirred for 1 h at room temperature (complete by RP-HPLC analysis) and acidified with TFA and the product isolated directly from the reaction mixture by preparative reversed-phase HPLC using 0.1% TFA−acetonitrile gradients. The TFA salt of inhibitor **30** (30 mg, 18% yield) was isolated as a yellow amorphous solid after lyophilization of pure fractions: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (d, J = 1.6 Hz, 1H), 8.86 (d, J = 7.8 Hz, 1H), 8.33 (s, 1H), 8.22 (d, J = 1.0 Hz, 1H), 7.96 (m, 2H), 7.78 (dd, J = 8.6, 1.0 Hz, 1H), 7.08 (d, J = 11.7 Hz, 1H), 7.02 (s, 1H), 6.97 (dd, J = 13.9, 2.0 Hz, 1H), 6.70 (s, 1H), 6.59 (dd, J = 8.6, 2.2 Hz, 1H), 5.27 (dt, J = 7.8, 6.9 Hz, 1H), 4.45 (tt, J = 12.3, 3.3 Hz, 1H), 3.24 (dd, J = 14.7, 6.3 Hz, 1H, part of AB), 3.17 (dd, J = 14.5, 8.8 Hz, 1H, part of AB), 2.32−2.19 (m, 2H), 1.97−1.80 (m, 4H), 1.68 (m, 1H), 1.50−1.35 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.83, 166.1, 150.3, 147.6, 144.6, 143.9, 135.3, 130.6, 128.2, 127.9, 124.1, 122.2, 117.8, 115.0, 112.9, 111.6, 111.3, 111.1, 109.2, 102.8, 102.4, 56.9, 48.5, 30.4, 29.5, 25.4, 24.3; HRMS (FAB) *m/z* $C_{31}H_{31}N_6O_3S$ (MH^+) calcd 567.2178, found 567.2197; HPLC homogeneity 99.6% (system A), 99.6% (system B).

Biological Assays. IC_{50} determinations were carried out as previously described[11] using an *N*-terminally tagged full-length NS5B construct. Reported values are the average of duplicate measurements carried out on two separate weightings. Specificity for HCV polymerase was evaluated using the rdrp of the poliovirus and a DNA-dependent RNA mammalian polymerase isolated from calf-thymus, also as previously described.[11,25] EC_{50} determinations in the cell-based replicon assay were performed in duplicates on two separate weightings using RT-PCR for RNA quantification as described elsewhere.[25]

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Supporting Information Available: Analytical data (^{1}H and ^{13}C NMR, HRMS, and HPLC homogeneities in two solvent systems) are provided for inhibitors **3**−**29** and **31**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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