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# Indole 5-carboxamide Thumb Pocket I inhibitors of HCV NS5B polymerase with nanomolar potency in cell-based subgenomic replicons (part 2): Central amino acid linker and right-hand-side SAR studies

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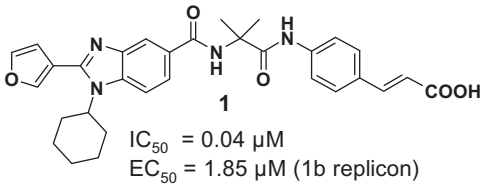
ABSTRACT

In this part 2, new indole 5-carboxamide Thumb Pocket 1 inhibitors of HCV NS5B polymerase are described. Structure–activity relationships (SAR) were explored at the central amino acid linker position and the right-hand-side of the molecule in an attempt to identify molecules with a balanced overall profile of potency ($EC_{50}$ <100 nM), physicochemical properties and ADME characteristics.

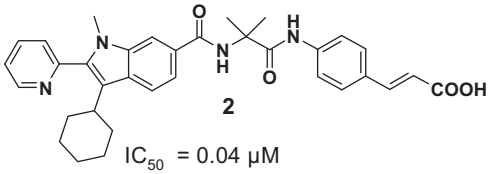


It was estimated more than a decade ago, that the hepatitis C virus (HCV) had infected 2–3% of the world population (about 170 million people). Small molecule therapeutics that are specific for HCV-encoded targets are in clinical development to treat this progressive life-threatening liver disease. The current standard of care, consisting of pegylated interferon in combination with ribavirin is either ineffective (50% of genotype 1 infections), contraindicated in some patients or associated with severe side effects.[1] HCV NS5B is a virally-encoded enzyme that is essential for viral replication and has been experimentally validated as a therapeutic target in HCV-infected patients.[2] This RNA-dependent RNA polymerase is responsible for replication of viral genetic information and has no mammalian counterpart. Like other polymerases (e.g., HIV-RT), NS5B function can be inhibited with chain-terminating nucleoside analogs[3] but in addition, at least four allosteric sites have been uncovered where small molecules can bind and inhibit RNA synthesis by interfering with protein conformational changes that are essential for enzymatic activity.[4] One of the allosteric sites, situated in the upper thumb domain of the enzyme (referred to as Thumb Pocket I), interacts intimately with the tip of a loop that extends from the finger domain. This interaction is vital for the formation of a productive complex between NS5B and the viral RNA template and subsequent RNA synthesis. We have discovered




1

$IC_{50}$ = 0.04 μM
$EC_{50}$ = 1.85 μM (1b replicon)
Calcd Log*P* = 4.4




2

$IC_{50}$ = 0.04 μM
$EC_{50}$ = 0.06 μM (1b replicon)
Calcd Log*P* = 4.9

**Figure 1.** Indole 5-carboxamide Thumb Pocket I inhibitors of HCV NS5B polymerase.

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0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
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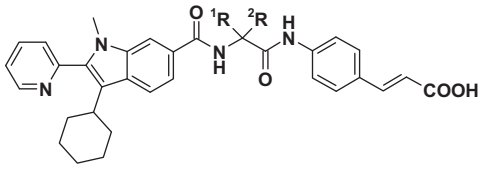
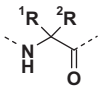
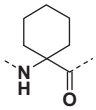
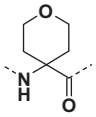
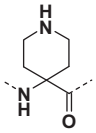
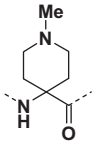
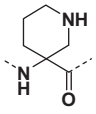
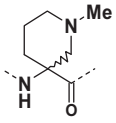
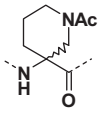
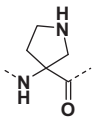
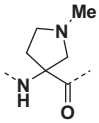
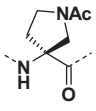
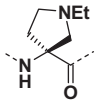
**Table 1**
2-(2-Pyridyl)indole 5-carboxamide central amino acid linker SAR



| Compd |  | $IC_{50}$[a] (nM) | $EC_{50}$[a] (nM) | HLM[b] $T_{1/2}$ (min) | Caco-2 ($\times 10^{-6}$ cm/s) | Solubility[8] pH 7.2 (μg/mL) | Calcd Log $P$[7] |
|---|---|---|---|---|---|---|---|
| **2** |  | 36 ± 8 | 57, 62 | 37 | 16 | 50 | 4.9 |
| **3** |  | 52 ± 18 | 43, 47 | 50 | 39 | 6 | 4.9 |
| **4** |  | 194 | 129 | 61 | | 5 | 5.7 |
| **5** |  | 68, 82 | 170, 180 | 41 | 5.7 | 15 | 3.9 |
| **6** |  | 13 ± 5 | 2000 | | | | −1.2 |
| **7** |  | 10 ± 5 | 60, 65 | 148 | 0.6 | 22 | 0.15 |
| **8** |  | (*R*) 40, 51<br>(*S*) 11 ± 6 | 220<br>20, 25 | 84 | 0.6 | 22 | −0.1 |
| **9** |  | 43, 69 | 66, 140 | | | | 0.9 |
| **10** |  | 230 | 230 | 256 | 5.5 | 24 | 3.8 |
| **11** |  | (*R*) 55, 81<br>(*S*) 23, 26 | 194<br>83, 154 | 191<br>>300 | 0.1<br>0.5 | 19<br>10 | −0.7 |
| **12** |  | (*R*) 66, 102<br>(*S*) 21 ± 4 | 78<br>34, 37 | 46<br>456 | 7.8<br>8.1 | 20<br>15 | 1.0 |
| **13** |  | 24 ± 9 | 212, 217 | | | | 3.3 |

(*continued on next page*)

**Table 1** (*continued*)

| Compd | | $IC_{50}$[a] (nM) | $EC_{50}$[a] (nM) | HLM[b] $T_{1/2}$ (min) | Caco-2 ($\times 10^{-6}$ cm/s) | Solubility[8] pH 7.2 (μg/mL) | Calcd Log $P$[7] |
|---|---|---|---|---|---|---|---|
| **14** |  | 23, 24 | 50, 52 | 61 | 5.6 | | 1.1 |

[a] $n \geqslant 2$: arithmetic mean ± standard deviation is reported.
[b] $T_{1/2}$ measured in human liver microsomes at 10 μM initial concentration.

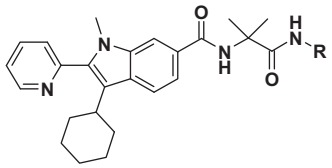
benzimidazole derivatives that bind to this site and prevent the interaction between the thumb domain and the finger-loop.[5] We recently reported the optimization of these *finger-loop* inhibitors from benzimidazoles to indole derivatives with improved cell-based subgenomic replicon potencies. In the accompanying paper we described how replacement of the benzimidazole core of carboxamide derivatives such as **1** (Fig. 1) with a more lipophilic indole scaffold resulted in inhibitors with profoundly improved cell culture activity.[6] Through optimization of the C-2 indole substituent, potent analogs such as **2** were identified that retained comparable intrinsic potency to the benzimidazoles, yet inhibited cellular HCV RNA replication at much lower concentrations ($EC_{50}$ = 60 nM; 30-fold improvement) with acceptable apical to basolateral Caco-2 permeability, lipophilicity (Calcd Log $P$ = 4.9)[7] and solubility (50 μg/mL in pH 7.2 buffer).[8] However, stability to CYP450-induced metabolism of this class of compounds in human and rat liver microsomes was moderate (HLM/RLM $T_{1/2}$ = 37/9 min for **2**) contributing, at least in part, to the modest plasma exposure and rapid clearance of the compound following oral administration in rat. In this account, we describe SAR at the central amino acid linker position and the right-hand-side of the molecules, with the ultimate goal of improving the pharmacokinetic profile of these inhibitors while maintaining potency in the desired range ($EC_{50}$ <100 nM).

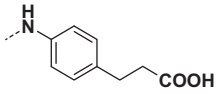
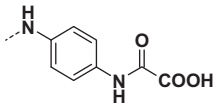
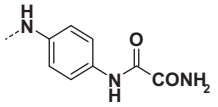
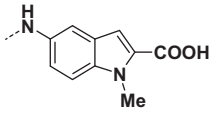
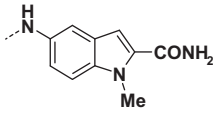
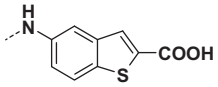
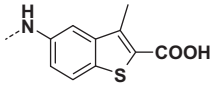
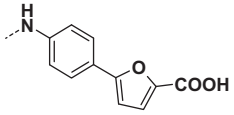
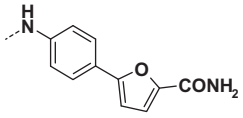
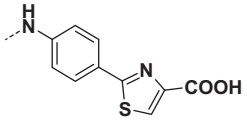
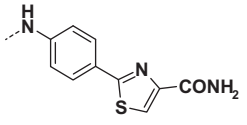
The synthesis of inhibitors was performed as previously reported.[6,9] Studies on modification of the central amino acid linker are presented in Table 1.[10] Structural studies of inhibitors bound to NS5B using X-ray crystallography and solution NMR suggested that the central amino acid serves as a conformational rigidifier between the two halves of the ligands.[11] α,α-Disubstituted amino acids provide the best potency in that respect, and since the substituents are solvent exposed, this position can also be used to manipulate the overall physicochemical and pharmacokinetic properties of the molecules with minimal impact on potency. As was observed in the case of benzimidazole diamides,[11] cyclobutane analog **3** had comparable activity to the *gem*-dimethyl compound **2**, whereas increasing the size of the ring to cyclohexane (**4**) reduced potency fivefold against the enzyme and somewhat less in cell culture due to increased lipophilicity. The increase in Caco-2 permeability for compound **3** is noteworthy and does not appear to result from an increase in lipophilicity since the Calcd Log $P$ values are identical. We suspected that inhibitor **4** was intrinsically less potent due to unfavorable solvation penalties that result from exposure of the hydrophobic cyclohexane ring to an aqueous environment. Indeed, introduction of an oxygen atom in the ring (**5**) partially offset these unfavorable effects, but the resulting increase in polarity (Calcd Log $P$ = 3.9) also had a negative impact on cell culture activity and Caco-2 permeability. Some of the most potent analogs made in this class incorporate a basic nitrogen atom in the amino acid linker.[11] Piperidine analog **6** displayed excellent intrinsic potency toward the enzyme, however, the high polarity of this analog (Calcd Log $P$ = −1.2) was not conducive to cellular permeation and replicon potency was lost. Attempts were then made to modulate the lipophilicity of these very potent analogs, with the aim of striking an appropriate balance between polarity, potency and ADME parameters. Methylation of the piperidine ring (**7**) was well tolerated and cell-based potency was recovered ($EC_{50}$ = 60 nM). However, Caco-2 permeability was low, presumably as a result of the zwitterionic nature of the molecule. The improved metabolic stability compared to non-basic analogs is consistent with a decrease in lipophilicity (Calcd Log $P$ = 0.15). Unsymmetrical piperidine analog **8** features a somewhat less solvent-exposed nitrogen atom. Interestingly, the two enantiomers, displayed characteristically different levels of potency, (*R*)-**8** being 4-fold less active than (*S*)-**8** against the enzyme. In cell culture, the potency disparity between the two antipodes was further accentuated (10-fold difference in $EC_{50}$). With a replicon $EC_{50}$ = 20–25 nM, (*S*)-**8** was the most potent compound so far in this series. Unfortunately, Caco-2 permeability was still compromised due to the highly ionized nature of the inhibitor. In contrast to **6**, methylation of the racemic unsymmetrical piperidine did not improve potency. While acetylation of the nitrogen atom (**10**) restored permeability, maintained acceptable solubility, lipophilicity and metabolic stability, potency was reduced. Similarly to the six-membered rings, pyrrolidine analogs also showed a consistent eutomer effect and preference for the (*S*)-enantiomer. Unsubstituted analog (*S*)-**11** was less potent than the corresponding piperidines **8**, however, in this case, methylation of the nitrogen atom returned cell-based potency to the desired level (**12**, $EC_{50}$ = 36 nM). Interestingly, both enantiomers of *N*-methylpyrrolidine **12** had acceptable Caco-2 permeability (~$8 \times 10^{-6}$ cm/sec), metabolic stability and moderate solubility at pH 7.2. Overall, compound (*S*)-**12** displayed a balance between potency and in vitro ADME profile. Further attempts to improve overall properties such as acetylation of the pyrrolidine ring (compound **13**) or alkylation of the nitrogen atom with a larger ethyl group (compound **14**) were unsuccessful.

Having completed exploration of the central amino acid position, we next turned our focus to the right-hand-side of the inhibitors. In this study, which is summarized in Table 2, efforts were directed at identifying alternatives for the cinnamic acid moiety, modulating the p$K_a$ of the carboxylic acid function and exploring replacements for this ionizable function.

Cinnamic acid derivatives such as those described so far are only weakly acidic due to conjugation with the double bond. This is consistent with the good permeability and potency of these inhibitors in cell-based assays, despite the presence of an exposed ionizable group. Hydrocinnamic acid **15** resulting from hydrogenation of the double bond of compound **3** was 4-fold less active against the enzyme, and in the cell-based assay. In this case, permeability remained excellent, solubility at pH 7.2 was increased significantly and metabolic stability was satisfactory. Oxamic analog **16** was well tolerated as a cinnamic acid replacement, but the compound was inactive in cell culture, presumably as a consequence of increased acidity. Notably, the corresponding primary

**Table 2**
2-(2-Pyridyl)indole 5-carboxamide right-hand-side SAR



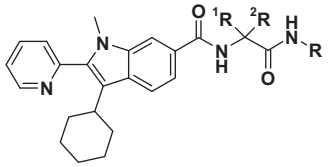
| Compd | | $IC_{50}$ [a] (nM) | $EC_{50}$[a] (nM) | HLM[b] $T_{1/2}$ (min) | Caco-2 ($\times 10^{-6}$ cm/s) | Solubility[8] pH 7.2 (μg/mL) | Calcd Log $P$[7] |
|---|---|---|---|---|---|---|---|
| **15** |  | 132 | 267 | 63 | 12.7 | 244 | 5.0 |
| **16** |  | 34, 40 | 2500 | | | | 3.1 |
| **17** |  | 124 | 139, 180 | 25 | 12.9 | 1 | 3.9 |
| **18** |  | 75, 119 | 143, 179 | 36 | 0.7 | 945 | 4.1 |
| **19** |  | 165 | 97 | 21 | | <0.1 | 4.3 |
| **20** |  | 23, 59 | 88 | 49 | 0.4 | 468 | 4.9 |
| **21** |  | 35, 48 | 46 | 34 | 0.8 | 202 | 5.2 |
| **22** |  | 152 | 145 | 43 | 0.2 | >564 | 4.2 |
| **23** |  | 330 | 96 | 19 | | <0.1 | 4.6 |
| **24** |  | 154 | 420 | 55 | <0.1 | 662 | 4.5 |
| **25** |  | 317 | 150 | 16 | | <0.1 | 4.9 |

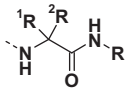
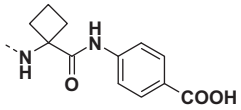
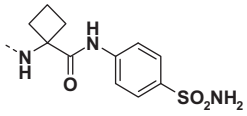
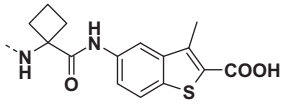
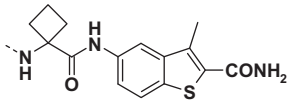
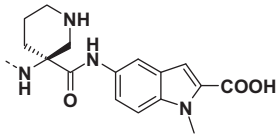
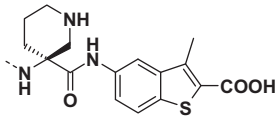
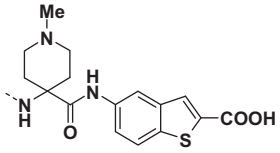
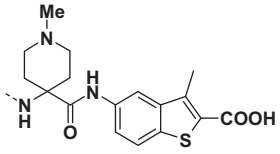
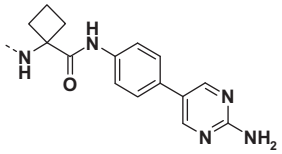
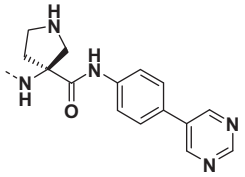
[a] $n \geqslant 2$: arithmetic mean ± standard deviation is reported.
[b] $T_{1/2}$ measured in human liver microsomes at 10 μM initial concentration.

amide analog **17** retained some activity against the enzyme, but this derivative was also active in the replicon assay, providing evidence that the carboxylic acid function is not essential for antiviral activity. Not unexpectedly however, this compound lacked aqueous solubility.

We next explored a set of heterobicyclic carboxylic acid derivatives which we envisioned as conformationally restricted cinnamic acid derivatives (compounds **18**–**21**). Benzothiophene carboxylic acid **21** stood out as the most potent of these analogs, with potency comparable to cinnamic acid derivative **2**. Although solubility was

**Table 3**
2-(2-Pyridyl)indole 5-carboxamide linker/right-hand-side combination SAR



| Compd |  | $IC_{50}$[a] (nM) | $EC_{50}$[a] (nM) | HLM $T_{1/2}$[b] (min) | Caco-2 ($\times 10^{-6}$ cm/s) | Solubility[8] pH 7.2 (μg/mL) | Calcd Log $P$[7] |
|---|---|---|---|---|---|---|---|
| **26** |  | 93 | 110 | 21 | 9.7 | 336 | 4.5 |
| **27** |  | 312 | 350 | 15 | | <0.1 | 4.2 |
| **28** |  | 122 | 68 | 79 | 0.6 | 112 | 5.2 |
| **29** |  | 86 | 76 | 71 | | | 5.6 |
| **30** |  | 44, 65 | 245 | 28 | <0.1 | 14 | -0.1 |
| **31** |  | 35, 53 | 60 | 93 | <0.1 | 1 | 1.0 |
| **32** |  | 17, 32 | 220 | | | | 0.4 |
| **33** |  | 27, 44 | 58 | 185 | 0.8 | 1 | 0.8 |
| **34** |  | 745, 871 | 180, 218 | | | 0.9 | 5.0 |
| **35** |  | 64 ± 19 | 130, 172 | 64 | <0.1 | 9 | 3.9 |

[a] $n \geqslant 2$: arithmetic mean ± standard deviation is reported.
[b] $T_{1/2}$ measured in human liver microsomes at 10 μM initial concentration.

excellent, Caco-2 permeability was poor and this parameter was previously shown to correlate with poor oral exposure in animals (vide infra).[6] Other variations on this theme such as 5-aminophenyl-2-furoic acid **22** or thiazole carboxylic acid **24** were less active. The corresponding primary carboxamides **23** and **25** had improved cell-based potency but predictably lacked aqueous solubility. Even though some alternatives were identified, cinnamic acids (such as analogs **2** and **3**) provided the most attractive overall profiles.

The results of investigations exploring various combinations of the central amino acid and right-hand-side moieties are described in Table 3. Attempts were made to balance polarity/lipophilicity at the two positions, with the aim of maintaining potency and achieving an in vitro ADME profile predictive of good oral absorption and reduced clearance relative to earlier compounds. For this study, 2-pyridyl was retained as the C-2 substituent on the indole core as it was previously shown to confer good activity and drug-like properties to this class of inhibitors.[6] Extrusion of the double bond in **3** gave benzoic acid derivative **26** and a compound with good permeability and solubility but with borderline potency and modest metabolic stability. Apparently, phenylsulfonamide **27** was substantially less potent but this interpretation is questionable in light of the poor solubility of this compound. Replacement of the *gem*-dimethyl amino acid linker with the cyclobutyl analog had no impact on the profile of benzothiophene carboxylic acids (**21** vs **28**) and Caco-2 permeability remained problematic in this case. Amide **29** also maintained potency similar to acid **28** but lipophilicity was high (Calcd Log $P = 5.6$). Combinations of a basic central amino acid with heterobicyclic carboxylic acids were investigated as a means of reducing lipophilicity (compounds **30**–**33**). Only benzothiophene carboxylic acids bearing a shielding methyl group in the 3-position provided inhibitors with cell-based activity in the desired range. However, these zwitterionic species lacked permeability in the Caco-2 cell model. Furthermore, these compounds also lacked solubility, as often observed with zwitterions. Finally, since the carboxylic acid function was not essential for activity, a few basic right-hand-sides were also evaluated. The two compounds shown in Table 3 (compounds **34** and **35**) are representative and were less active ($EC_{50}$ >150 nM). Consequently, this approach was not pursued further.

At this stage of our optimization program, we had succeeded in discovering a diverse collection of potent inhibitors of gt1b HCV subgenomic replication in cell culture. They included neutral, acidic, basic and zwitterionic species, with distinctive in vitro ADME (particularly with respect to metabolic stability and Caco-2 permeability) and solubility profiles. In order to assess the impact of these parameters on the pharmacokinetic properties of these compounds, a subset of inhibitors was evaluated for oral absorption in rat.[12] The data in Table 4, includes in vitro metabolic stability in rat liver microsomes when available. None of the compounds evaluated (Table 4) showed improved plasma exposure compared to inhibitor **2**. Despite a significant increase in permeability and comparable metabolic stability, cyclobutyl analog **3** was less absorbed, perhaps as a consequence of reduced aqueous solubility. Consistent with their low Caco-2 permeability, zwitterionic species (**7**, **8**, and **11**, Caco-2 $<0.6 \times 10^{-6}$ cm/s) lacked oral exposure, except in the case of **12** (Caco-2 $= 8.1 \times 10^{-6}$ cm/s) for which modest plasma levels and a longer oral MRT were measured. Compounds **15** and **26**, both permeable and water soluble, also showed modest exposure. Poor metabolic stabilities in rat liver microsomes, low AUC and short oral MRT values were all consistent with rapid clearance in this species.

Several representative inhibitors were tested for specificity against another RNA-dependent RNA polymerase from polio virus and a mammalian DNA-dependent RNA polymerase II isolated from calf thymus.[10a] Selectivity index (S.I.) was >150-fold in all cases.

In summary, SAR studies with the central amino acid linker and the right-hand-side of indole 5-carboxamide Thumb Pocket 1 inhibitors generated a diverse collection of compounds with adequate cell-based HCV subgenomic replicon potency. In the course of these studies, we established that a carboxylic acid function on the right-hand-side of the molecules was not essential for activity, but was necessary to provide some aqueous solubility. The central linker amino acid was tolerant of diverse functionality (including strongly basic groups), consistent with previous analyses that suggested this position is solvent exposed. Nevertheless, *gem*-dimethyl or small cyclic amino acids (e.g., cyclobutyl) were preferred to provide permeability. Oral absorption correlated relatively well with Caco-2 cell permeability and aqueous solubility. However, compounds in this class were rapidly cleared following oral administration in rat, and reflect modest metabolic stability. Future optimization of this class of HCV polymerase inhibitors will focus on addressing this issue, while maintaining potency and the other generally favorable drug-like characteristics.

**Table 4**
Rat oral PK parameters for selected indole 5-carboxamides

| Compd | RLM $T_{1/2}$[a] (min) | $C_{max}$ (μM) | $T_{max}$ (h) | AUC (μM h) | MRT (h) |
|---|---|---|---|---|---|
| **2** | 9 | 1.23 | 0.25 | 0.87 | 0.5 |
| **3** | 17 | 0.29 | 0.5 | 0.35 | N.A. |
| **7** | 34 | <0.1 | | | |
| **8**-(*S*) | 17 | <0.1 | | | |
| **11**-(*S*) | | BLD[b] | | | |
| **12**-(*S*) | 18 | 0.32 | 0.5 | 0.81 | 3.8 |
| **15** | N.A. | 0.63 | 0.5 | 0.81 | 1.7 |
| **21** | | BLD | | | |
| **26** | 9 | 0.5[b] | | | |
| **29** | | BLD[b] | | | |
| **35** | N.A. | BLD[b] | | | |

[a] $T_{1/2}$ measured in rat liver microsomes at 10 μM initial concentration.
[b] Plasma concentration at 1 h following oral dosing of a mixture of four compounds (4 mg/kg each). N.A.: not available. BLD: below limit of detection.

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6. Part 1: Beaulieu, P. L. et al., Bioorg. Med. Chem. Lett. (2011), doi:10.1016/j.bmcl.2011.04.082. All α,α-dialkylamino acids used in this work were obtained from commercial sources. See WO 2003/010141 and WO 2004/065367 for experimental procedures detailing the synthesis of diamide inhibitors.
7. JChem 5.0.0 (http://www.chemaxon.com) was used for Log *P* predictions. The prediction method is based on the algorithm described in Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. R.; Robins, R. K. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163.
8. Performed on amorphous TFA salts using the 24 h shaking flask method (pH 7.2 phosphate buffer).
9. All inhibitors in this study were purified to >95% homogeneity by reversed-phase HPLC and isolated as TFA salts. All compounds were characterized by mass spectrometry and gave $^1$H NMR spectra consistent with expected structures.
10. $IC_{50}$ values were determined as described previously: (a) McKercher, G.; Beaulieu, P. L.; Lamarre, D.; LaPlante, S.; Lefebvre, S.; Pellerin, C.; Thauvette, L.;

Kukolj, G. *Nucleic Acids Res.* **2004**, *32*, 422; (b) $EC_{50}$ determinations were performed using Huh-7 cells with a stable subgenomic HCV 1b replicon. The HCV RNA levels were normalized to total cellular RNA in this 72 h assay. The quantification of total RNA recovery allowed for an assessment of cellular homeostasis to eliminate the possibility of antiviral activity due to subtle toxic effects. For an overview and basic protocols on the use of HCV replicons, see V. Lohmann, in: H. Tang (Ed.), Methods in Mol. Biol., Hepatitis C: Methods and Protocols, 2009, 510, 145, Second Edition, Humana Press.

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12. Male Sprague–Dawley rats were fasted overnight and dosed by oral gavage at 10 mg/kg using 0.5% methocel and 0.3% Tween-80 as vehicle. Plasma samples from three animals were pooled at each time points (0–8 h) for analysis. Compound detection in plasma samples was performed following liquid–liquid extraction and HPLC analysis using UV detection.