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# Improved replicon cellular activity of non-nucleoside allosteric inhibitors of HCV NS5B polymerase: From benzimidazole to indole scaffolds

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**Abstract**—Benzimidazole-based allosteric inhibitors of the hepatitis C virus (HCV) NS5B polymerase were diversified to a variety of topologically related scaffolds. Replacement of the polar benzimidazole core by lipophilic indoles led to inhibitors with improved potency in the cell-based subgenomic HCV replicon system. Transposing the indole scaffold into a previously described series of benzimidazole–tryptophan amides generated the most potent inhibitors of HCV RNA replication in cell culture reported to date in this series ($EC_{50} \sim 50$ nM).


Chronic infection by the hepatitis C virus afflicts an estimated 2–3% of the world population and represents a health issue of increasing concern. While it can remain asymptomatic for decades, the infection may progress to life-threatening liver diseases such as cirrhosis (20% of cases) and hepatocellular cancers (2.5%). HCV infection is the major reason for liver transplantation in the western world and causes more than 10,000 deaths annually in the US alone.[1] The last 10 years have witnessed major advances in our understanding of this pathogen from the identification of essential viral functions,[2] to the development of subgenomic replicons that allow HCV RNA replication in Huh-7 cells,[3] to replicating specific viral strains in the laboratory.[4] As a result, several drug candidates that target specific viral enzymes have recently been described[1b] and clinical testing in HCV patients is producing unprecedented results.[5]

Recently, we reported the discovery, preliminary optimization, and mechanism of action of non-nucleoside, allosteric inhibitors of the NS5B polymerase of the HCV virus.[6] These compounds are characterized by a benzimidazole scaffold harboring a critical cyclohexyl or cyclopentyl ring at the $N^1$-position, a small aromatic or heteroaromatic ring at C-2 and a carboxylic acid function at C-5 (e.g., compound **1**, Fig. 1).[6b] While low micromolar potency in a truncated genotype 1b HT-NS5BΔ21 enzymatic assay[6a] was attained, this class of inhibitors did not inhibit HCV RNA replication in the cell-based replicon assay.[7] Significant improvements in potency (58-fold) were attained by elaborating the right-hand side of the inhibitors through attachment of functionalized amino acid residues[6c] (e.g., compound **2**). Adjustments to the physicochemical properties of the molecules by replacement of the ionizable carboxylic acids eventually led to the discovery of inhibitors (e.g., compound **3**) active in cell culture at low micromolar concentrations.[8]

At this stage of the optimization process, much of the SAR surrounding the benzimidazole scaffold had been exploited and further improvements in potency (enzymatic and cell-based) required modification of the benzimidazole core itself. In particular, we hypothesized that

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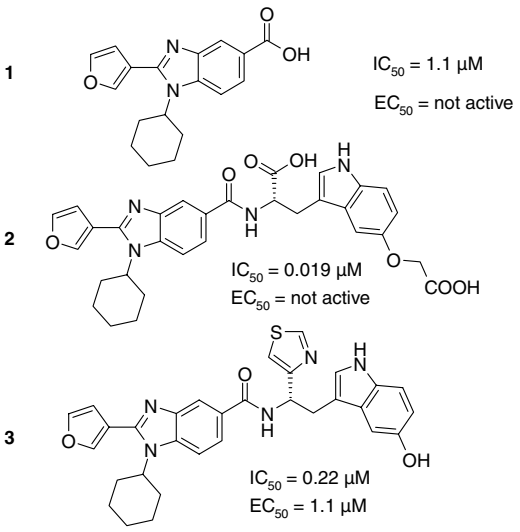



**Figure 1.** Initial SAR in the benzimidazole series.

replacement of the polar benzimidazole system by more lipophilic, topologically related variants may favor cellular permeation and thereby, improve the modest cell-based potency achieved so far in this series.

Retaining the critical pharmacophores of **4A**, we prepared a series of indole derivatives as illustrated in Table 1. Replacement of the benzimidazole core of **4A** (calcd log $P$ = 3.4) with the more lipophilic indole moiety generated a compound (**4B**, calcd log $P$ = 4.4) which displayed a 37-fold improvement in enzymatic potency ($IC_{50}$ = 0.030 vs 1.1 μM).[6a] Furthermore, indole **4B** was active in the replicon ($EC_{50}$ = 6.7 μM),[7] presumably reflecting the expected increase in lipophilicity and improved cellular permeation.[9] Encouraged by these results, we proceeded with N-methylation of the indole nitrogen, a newly accessible position for further elaboration of the scaffold, achieving an additional 2-fold improvement in enzymatic potency (**4C**) while retaining similar replicon activity. The isomeric 5-indole carboxylic acid analog **4D** was slightly less potent (2-fold). In an attempt to improve cellular permeation by reducing ionization of the carboxylic acid by modifying its p$K_a$, 7-azaindole analogs **4E** and **5E** were prepared. Even though enzymatic potency was slightly reduced (2- to 3-fold) compared to indole **4C**, 7-aza analog **5E** was 3-fold more potent in cell culture, reflecting the contribution of the p$K_a$/ionization of the carboxyl function to cellular permeation and replicon activity.[9c]

Evaluation of the scaffolds in a small combination matrix with representative C-2 substituents (identified in our previous work on benzimidazoles)[6b] confirmed the advantage of the *N*-methyl-6-indolecarboxylic acid scaffold in both enzymatic and cell culture assays (Table 1). Notably, the 2-pyridyl analog **6C** had comparable replicon potency to the furyl derivative **4C** despite a 5.5-fold decrease in intrinsic potency, a possible indication of improved cellular permeation.

With the aim of further reducing compound size and probing the effect of conformational restriction, pyrroles **6F** and pyrrazole **6G** were prepared. The compounds were >500-fold less potent than the corresponding indole **6C**. This dramatic drop in potency may be due to the loss of critical hydrophobic interactions between the indole benzene ring and the protein. Since the carboxylic acid group interacts with a critical basic protein residue,[10] alterations to the p$K_a$ and changes in the orientation of the carboxyl function in **6F** and **6G** may also contribute to the observed decrease in potency.[11]

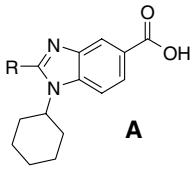
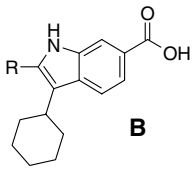
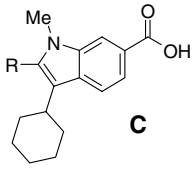
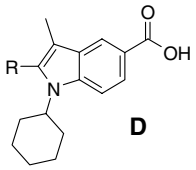
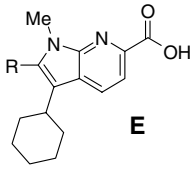
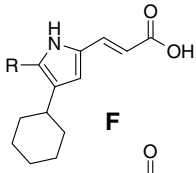
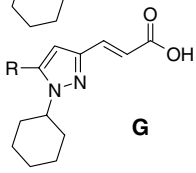
The scaffold study was extended to 3-cyclopentyl derivatives (Table 2), which were previously shown to have comparable potency to cyclohexyl analogs.[6b] 6-Indolecarboxylic acids (**8** and **9**) were more potent than the reference benzimidazole **7**, showing improved enzymatic potency (~100-fold) and cellular activity comparable to the cyclohexyl derivatives (**4B** and **4C**). As previously noted, N-methylation did not affect potency (**8** vs **9**) and 6-indolecarboxylic acids were superior (7-fold in this case) to the 5-isomer (**9** vs **10**). Benzofuran **11** and benzothiophene **12**, which are topologically similar to indoles (see Fig. 2 depicting 2-phenyl derivatives), had reduced enzymatic ($IC_{50}$ = 0.08 μM, a 4-fold drop compared to unsubstituted indole **8**) and replicon potency. Figure 2 shows a superposition between key pharmacophores (cyclopentyl ring and carboxylic acid function) within the three systems, the largest deviation resulting from the longer C–S bonds in the benzothiophene analog. In the latter compound, a slight change in orientation of the C-2 substituent is apparent but the effect is not reflected in the potency of the analogs. This is in agreement with the observation that the C-2 position tolerates a variety of structural modifications.[6b]

Finally, oxidation of the thiophene ring (compound **13**) resulted in complete loss of activity. The presence of the two polar oxygen atoms may interfere with stacking of the scaffold within the hydrophobic enzyme pocket and account for the loss of activity.[10]

As mentioned previously, the improved cellular potency of azaindole **5E** over the corresponding carbo-analog **5C** (See Table 1) suggested that the p$K_a$ of the carboxylic acid function and the overall ionization state of the molecule may be contributors to cellular permeation and cell culture activity. Previous work on benzimidazoles (unpublished results) had shown that addition of a methyl group was well tolerated in the 4-position of that scaffold (7-position in **4C** or **9**) but was detrimental to potency at the 6-position of **4A** (5-position on indole **9**). The effect on potency of introducing substituents at the 7-position of the indole scaffold was thus examined, with the intention of probing electronic and steric effects on ionization of the nearby critical carboxylic acid function and replicon potency. We also hoped that introduction of functional groups on the indole scaffold would lead to the discovery of unexploited interactions with the protein and further improvements in overall potency (see Table 3).

Unfortunately, apart from the 7-hydroxy analog **14** which was 3 times less potent than unsubstituted indole

**Table 1.** Benzimidazole scaffold replacements ($IC_{50}$ values determined using a truncated genotype 1b HT-NS5B-Δ21 construct)[6a]

| Compound | | R =  4 | |  5 | |  6 | |
|---|---|---|---|---|---|---|---|
| | | $IC_{50}$[a] (μM) | $EC_{50}$[a] (μM) | $IC_{50}$[a] (μM) | $EC_{50}$[a] (μM) | $IC_{50}$[a] (μM) | $EC_{50}$[a] (μM) |
|  A | 1.1 | NA | 3.1 | NA | 2.7 | NA | |
|  B | 0.030 | 6.7 | 0.05 | >10 | 0.23 | >10 | |
|  C | 0.016 | 4.2 | 0.03 | 6.6 | 0.09 | 3.9 | |
|  D | 0.044 | >10 | 0.18 | >10 | 0.82 | >10 | |
|  E | 0.051 | Not tested | 0.08 | 2.2 | | | |
|  F | | | | | >50 | Not tested | |
|  G | | | | | >100 | Not tested | |

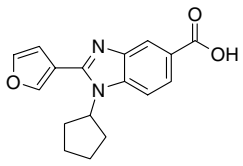
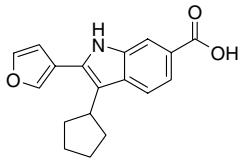
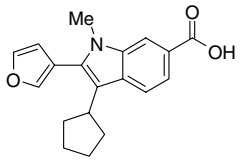
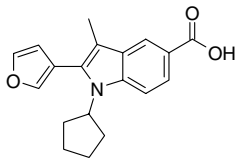
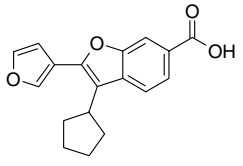
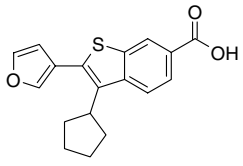
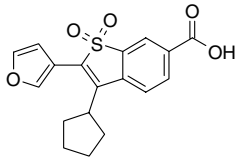
[a] Values are means of duplicate experiments on two separate weightings. NA (not active).

**9**, all other derivatives were >13-fold less potent. The observed trends were consistent with a steric rather than electronic signature (cf. **15**, **16** vs **17**). The low potency of diacid **18** may be due to an unfavorable intramolecular interaction between the two carboxyl functions that preclude the 6-COOH from engaging in a productive interaction with the polymerase.

Selected compounds (**4B** and **4C**) were also evaluated in a poliovirus RNA-dependent RNA polymerase and DNA-dependent RNA polymerase II (derived from mammalian calf thymus as previously described)[6a] assay and were found to be inactive ($IC_{50}$ > 125–250 μM).

We and others[9b] have established the superiority of indole scaffolds over the original benzimidazole-based leads, with respect to intrinsic potency against the NS5B enzyme. However, the presence of the carboxylic acid function that is ionized at physiological pH in small indole derivatives such as **4C** results in relatively modest

**Table 2.** Scaffold replacements in 3-cyclopentyl analogs ($IC_{50}$s determined using a genotype 1b HT-NS5B-Δ21 construct)[6a]

| Compound | | HT-NS5B-Δ21 $IC_{50}$,[a] (μM) | Replicon $EC_{50}$,[a] (μM) |
|---|---|---|---|
| **7** |  | 2.2 | NA |
| **8** |  | 0.025 | 6.6 |
| **9** |  | 0.021 | 6.6 |
| **10** |  | 0.15 | 39 |
| **11** |  | 0.081 | 21 |
| **12** |  | 0.078 | 22 |
| **13** |  | >100 | Not tested |

[a] Values are means of duplicate experiments on two separate weightings. NA (not active).

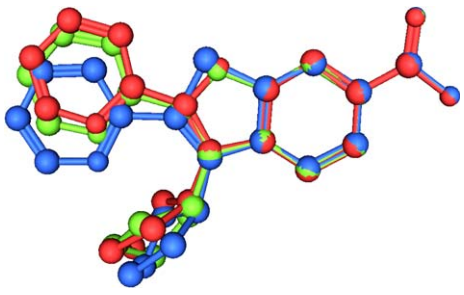
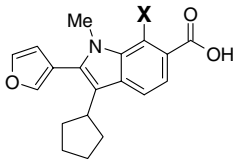


**Figure 2.** Overlay of 3-cyclopentyl-2-phenyl-6-carboxy-indole (green), benzofuran (red), and benzothiophene (blue) scaffolds.

**Table 3.** Effect of substituents at the 7-position of 6-indolecarboxylic acid derivatives. ($IC_{50}$s determined using a genotype 1b HT-NS5B-Δ21 construct)[6a]

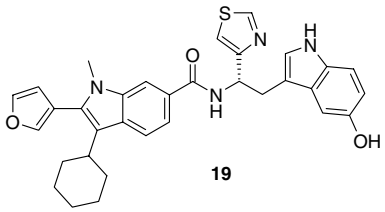


| Compound | X | HT-NS5B-Δ21 $IC_{50}$,[a] (μM) | Replicon $EC_{50}$,[a] (μM) |
|---|---|---|---|
| **9** | H | 0.021 | >10 |
| **14** | OH | 0.075 | 19 |
| **15** | OMe | 0.34 | 40 |
| **16** | Cl | 0.29 | 37 |
| **17** | COOMe | 0.48 | 22 |
| **18** | COOH | 5.3 | Not tested |

[a] Values are means of duplicate experiments on two separate weightings.

inhibition in the replicon assay ($EC_{50}$ = 4 μM), despite high levels of inhibition in enzymatic assays ($IC_{50}$ = 16 nM). Compound **19** (Fig. 3), the indole version of amide **3**,[8] showed a 2-fold improvement in potency in the enzymatic assay and was 6-fold less potent than carboxylic acid **4C**. However, the improved cellular permeation resulting from removal of the carboxylic acid charge led to a dramatic increase in cellular potency and **19** is the most potent inhibitor of HCV RNA replication in the cell-based replicon that has been reported to date from this class of non-nucleoside allosteric NS5B inhibitors. The simple replacement of a nitrogen by a carbon atom in compound **3** resulted in a 22-fold improvement in cell culture activity ($EC_{50}$ ~ 50 nM; the value was derived after correcting for actual compound concentration in assay medium as determined by HPLC analysis, due to the very poor aqueous solubility of this compound). Efforts toward further optimization of this series will be reported in due course.

The synthesis of indole-6-carboxylic acid analogs is shown in Scheme 1 for the cyclohexyl series:[9,11] condensation of commercially available 6-indolecarboxylic acid **20** with excess cyclohexanone under basic conditions followed by hydrogenation produced the 3-cyclohexyl acid intermediate **21**. The carboxyl function was esterified to give indole **22**, or **21** was per-methylated to *N*-methylindole ester **23**. Alternatively, ester **22** was N-methylated to **23** in a separate step. Bromination of the 2-position using pyridinium perbromide gave bromoindoles **24** or **25** that served as substrates for a palladium-catalyzed

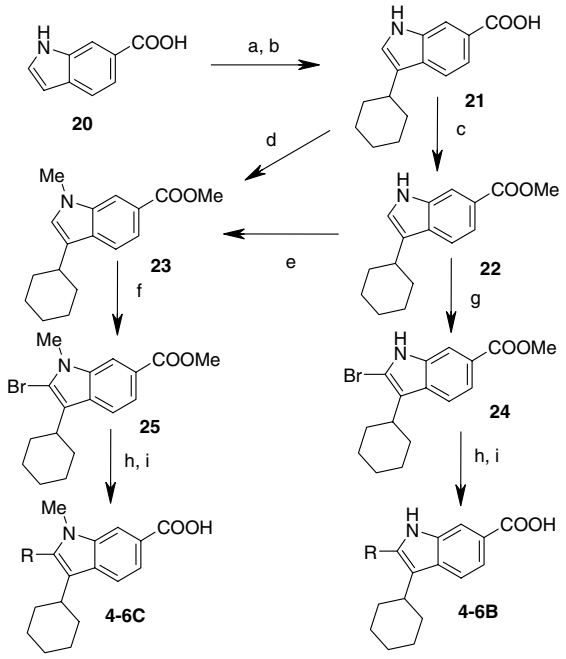


**19**

$IC_{50}$ = 0.1 μM

$EC_{50}$ = 0.05 μM

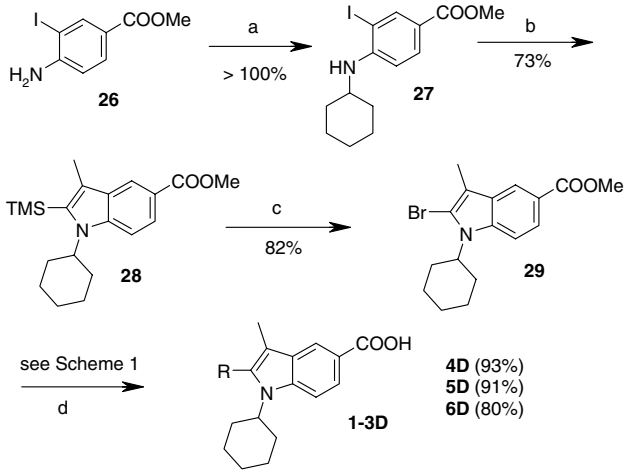
**Figure 3.** HCV NS5B polymerase indole-based derivative with submicromolar potency in the cell-based replicon.




**Scheme 1.** Synthesis of indole 6-carboxylic acid derivatives. Reagents and conditions: (a) cyclohexanone (3 equiv), NaOMe (6.5 equiv), MeOH, reflux for 36 h (>100%). For the 3-cyclopentyl analogs: cyclopentanone (5 equiv slow addition), KOH (10 equiv), MeOH, water, 75 °C, 18 h (88%). (b) 20% $Pd(OH)_2/C$, $H_2$ gas (55 ψ), MeOH/THF 1:1 (100%). (c) MeI (1.1 equiv), $K_2CO_3$ (1.4 equiv), DMF, rt, 18 h (90%). (d) MeI (2.5 equiv), $K_2CO_3$ (2.5 equiv), DMF, rt, 2 h; then NaH (1.5 equiv), 0 °C to rt, 4 h. (e) NaH (1.3 equiv), MeI (1.5 equiv), DMF, rt, 18 h (86%). (f) $Br_2$ (1.3 equiv), NaOAc (1.3 equiv), *i*-PrOAc, 5 °C, 30 min (76%). (g) $PyrBr_3$ (1.4 equiv), THF/$CHCl_3$ 1:1, 0 °C to rt, 2.5 h (71%). (h) For R = 3-furyl (99%) or Ph (88%): 3-furylboronic acid or phenylboronic acid (1.3 equiv), LiCl (2 equiv), $Na_2CO_3$ (2.5 equiv), $Pd(PPh_3)_4$ (0.04 equiv), toluene/EtOH/water 1:1:1, reflux overnight. For R = 2-pyridyl: *n*-BuLi (1.3 equiv), THF, −78 °C, 1.5 h, then $Bu_3SnCl$ (1.4 equiv) (79%) followed by: 2-bromopyridine (1.3 equiv), $PPh_3$ (0.2 equiv), CuI (0.1 equiv), LiCl (2 equiv), $Pd(PPh_3)_4$ (0.05 equiv), DMF, 100 °C, overnight (71%). (i) NaOH then HCl.

Suzuki cross-coupling with 3-furyl and phenylboronic acid to provide final inhibitors **4**–**5B** and **4**–**5C** following saponification of the ester group. In the case of the 2-pyridyl analog **6C**, 2-bromoindole **25** was *trans*-metallated at low temperature and the 2-lithioindole was stannylated with chlorotributyltin to provide an acid-sensitive 2-stannylindole intermediate. Stille coupling with 2-bromopyridine provided the desired indole **6C** in good yield following deprotection of the ester function. For **6B**, indole **22** was protected as the *N*-Boc derivative and stannylated in the 2-position (LDA/*n*-$Bu_3SnCl$). Cross-coupling with 2-bromopyridine under standard Stille conditions followed by removal of protecting groups provided inhibitor **6B**. Cyclopentyl analogs **8** and **9** were prepared in a similar fashion.

The synthesis of isomeric 5-carboxyindole derivatives is depicted in Scheme 2. Reductive amination of methyl 4-amino-3-iodobenzoate **26** with cyclohexanone provided iodoaniline **27**. Palladium-catalyzed cross-coupling with 1-trimethylsilylpropyne gave 2-trimethylsilylindole **28** which was brominated to provide 2-bromoindole **29**. Suzuki cross-couplings and metallation–stannylation

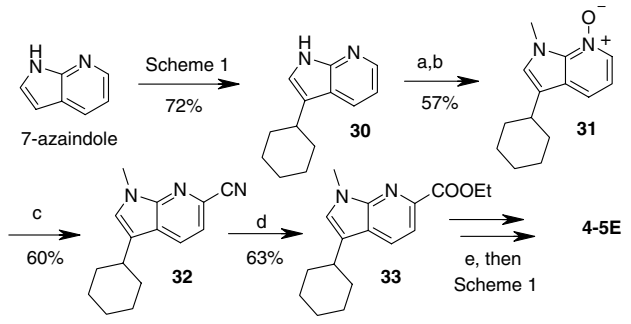



**Scheme 2.** Synthesis of indole 5-carboxylic acid derivatives. Reagents and conditions: (a) cyclohexanone (6 equiv), $MgSO_4$ (10.5 equiv), $NaBH(OAc)_3$ (3.5 equiv), AcOH, rt overnight; (b) TMS-C≡$CCH_3$ (2.8 equiv), LiCl (1 equiv), KOAc (2 equiv), $Pd(OAc)_2$ (0.05 equiv), DMF, 100 °C overnight; (c) $Br_2$ (0.9 equiv), $CH_2Cl_2$, 0 °C, 30 min; (d) For **6D**: *n*-BuLi (1.05 equiv), THF, −78 °C, 45 min then $B(OMe)_3$ (1.2 equiv), −70 °C, 1 h then $Pd(OAc)_2$ (0.01 equiv), P(*p*-tolyl)$_3$ (0.06 equiv), $K_2CO_3$ (2 equiv), 2-bromopyridine (1.2 equiv), MeOH, reflux, 18 h.

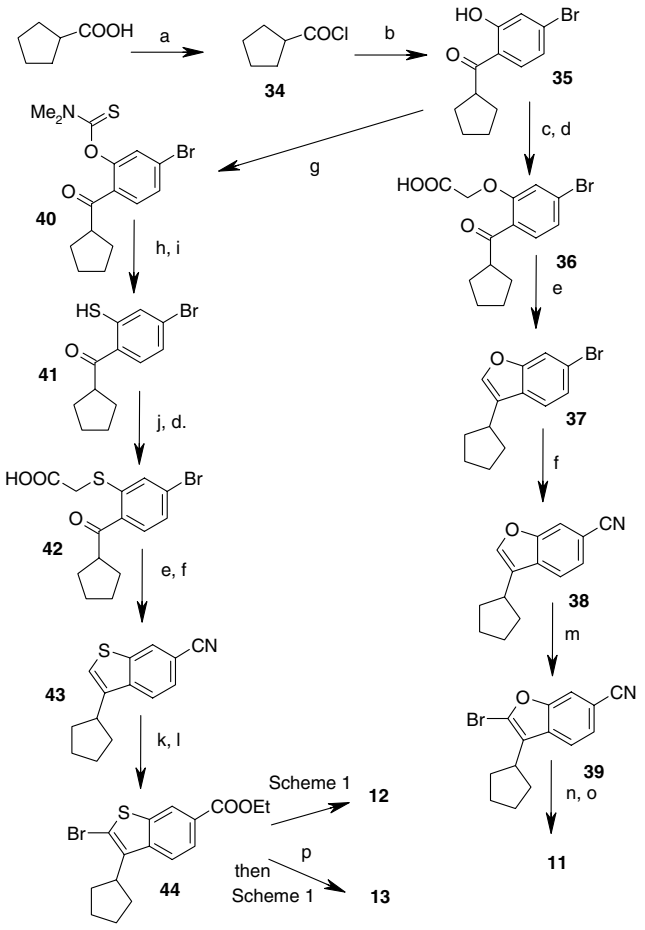
followed by Stille couplings were employed as described in Scheme 1 to provide the desired inhibitors **4**–**6D**. A similar sequence replacing cyclohexanone by cyclopentanone provided inhibitor **10**.

7-Azaindole analogs **4E** and **5E** were prepared following the route depicted in Scheme 3: commercially available 7-azaindole was condensed with cyclohexanone and hydrogenated as described in Scheme 1 to give **30**. Oxidation of the pyridine to the *N*-oxide directed subsequent N-methylation to the indole nitrogen to form **31**. Treatment with TMSCN under basic conditions provided nitrile **32**. After conversion to ethyl ester **33** the desired inhibitors were obtained following the usual bromination, cross-coupling, and saponification sequence.

The benzofuran **11** and benzothiophene **12** analogs were prepared as described in Scheme 4: Friedel–Craft acylation of 3-bromophenol with acid chloride **34** derived




**Scheme 3.** Synthesis of 7-azaindoles. Reagents and conditions: Scheme 1, steps a–b. (a) MCPBA (2 equiv), DME, rt, 2 h; (b) NaH (2 equiv), MeI (1 equiv), DMF, rt, 3 h; (c) $Et_3N$ (2.5 equiv), TMSCN (5 equiv), MeCN, reflux 19 h; (d) EtOH, HCl gas, 15 min at rt then reflux for 1.5 h; (e) $PyrHBr_3$ (1.5 equiv), THF, 65 °C, 16 h (96%).




**Scheme 4.** Synthesis of benzofuran and benzothiophene analogs. Reagents and conditions: (a) $SOCl_2$, pyridine (cat.), reflux; (b) 3-bromophenol (1 equiv), $AlCl_3$ (1.5 equiv), 1,2-dichloroethane, reflux, 2 h (78%); (c) NaH (1.5 equiv), ethyl bromoacetate (1.5 equiv), DMF, 1 h; (d) NaOH, THF–water; (e) NaOAc (2 equiv), $Ac_2O$, reflux 5 h (80%); (f) CuCN (1.2 equiv), DMF reflux, 7 h (72%, 3 steps); (g) DABCO (2 equiv), dimethylthiocarbamyl chloride (2 equiv), DMF, rt, 3 h (100%); (h) 180–190 °C, neat, 5 h; (i) KOH, MeOH, reflux, 1.5 h (91%, 2 steps); (j) ethylbromoacetate (1.2 equiv), $K_2CO_3$ (3 equiv), acetone, rt overnight (82%); (k) $Br_2$ (4 equiv), AcOH, rt overnight; (l) EtOH–concd $H_2SO_4$, reflux 1–2 days (58%, 2 steps); (m) NBS (3.2 equiv), DMF, rt overnight (53%); (n) cross-coupling: see conditions in Scheme 1; (o) concd $H_2SO_4$/AcOH/water 5:4:2, reflux 1.5 h; (p) 30% $H_2O_2$ (6 equiv), AcOH (68%), 60 °C, 24 h.

from cyclopentanecarboxylic acid gave ketone **35**. Alkylation with bromoacetate followed by saponification (**36**) and ring closure gave 6-bromobenzofuran **37** which was subjected to cyanation to provide nitrile **38**. Bromination (**39**) followed by Suzuki cross-coupling with 3-furylboronic acid and hydrolysis of the nitrile to the carboxylic acid gave analog **11**. Conversion of phenol **35** to thiol **41** was effected through a thermal rearrangement of *O*,*S*-thiocarbamate **40**. Thiol **41** was then elaborated (**42**–**44**) to bromobenzothiophene **44** that was converted to analog **12** in the usual manner. Benzothiophene dioxide **13** was obtained by oxidation of intermediate **44** using $H_2O_2$/AcOH,[12] and further elaboration as described in Scheme 1.

Substituted indoles **14**–**18** were prepared from the corresponding 7-substituted-6-indolecarboxylic acids using the protocols described in Scheme 1 or the Supplementary data section.[13,14] The synthetic approaches to pyrrole **6F** and pyrrazole **6G** are also provided in the Supplementary data section.[14]

In conclusion, we have shown that replacement of the polar benzimidazole scaffold of allosteric HCV NS5B polymerase inhibitors by a more lipophilic indole core provides significant potency improvements in a cell-based replicon assay of HCV RNA replication. Combination of an indole-based scaffold with a lipophilic tryptophan-derived right-hand side has led to the most potent inhibitor in cell culture reported to date in this series.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.07.074

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