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# Design and synthesis of spirocyclic compounds as HCV replication inhibitors by targeting viral NS4B protein

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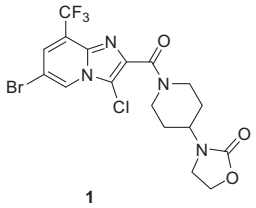
ABSTRACT

Two novel series of spirocyclic piperidine analogs appended to a pyrazolo[1,5-*a*]pyridine core were designed, synthesized and evaluated for their anti-HCV activity. A series of piperidine ketals afforded dispiro **6p** which showed excellent in vitro anti-HCV activities ($EC_{50}$ of 1.5 nM and 1.2 nM against genotype 1a and 1b replicons, respectively). A series of piperidine oxazolidinones afforded **27c** which showed $EC_{50}$'s of 10.9 nM and 6.1 nM against 1a and 1b replicons, respectively. Both compounds **6p** and **27c** bound directly to non-structural NS4B protein in vitro ($IC_{50}$'s = 10.2 and 30.4 nM, respectively) and exhibited reduced potency in replicons containing resistance mutations encoding changes in the NS4B protein.

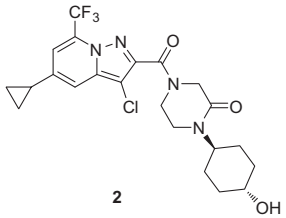

Hepatitis C is a blood-borne disease of the liver transmitted by the Hepatitis C Virus (HCV) and is a leading cause of chronic liver disease and liver transplantations.[1,2] The World Health Organization (WHO) estimates that about 150 million people are chronically infected with HCV, and more than 350,000 people die every year from hepatitis C-related liver diseases worldwide.[3] Unlike hepatitis A and B viruses, no vaccine against HCV is available. The current standard of care for treating genotype 1 HCV patients involves the combination of an oral protease inhibitor, boceprevir or telaprevir, along with pegylated IFN (PegIFN) and ribavirin.[4–6] These triple therapy regimens offer an improvement in cure rate and shorten the duration of treatment in comparison to PegIFN and ribavirin alone. However, these therapies suffer from significant side effects, such as anemia, serious skin reactions/rash, and fatigue as well as drug–drug interactions that require close monitoring. Opportunities remain for improvement of the first generation of direct-acting antiviral (DAA) agents in areas such as resistance, tolerability, and pan-genotypic efficacy. Furthermore, identification of a regimen with all oral agents that removes the need for IFN remains a long sought after goal. The recent FDA approval of Sovaldi™ (sofosbuvir) is a significant step towards these goals.

HCV is a single-stranded RNA virus in the *Flaviviridae* family, encoding a polyprotein of ~3000 amino acids that is processed into 11 proteins, including 4 structural proteins (C, E1, E2, and p7) and 6 nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) and the so-called 'F' protein.[7,8] Several of the non-structural proteins have proven to be viable targets for clinical HCV intervention.[9,10] The non-structural protein 4B (NS4B) is less well studied; however recent reports have shown this protein is an integral part of the replication complex, and therefore represents a novel target for HCV therapy.[11–16] Recently, we[17–19] and others[20–22] have described small molecule inhibitors of HCV that target NS4B. These compounds generate resistant mutations in the HCV replicon, including H94N, F98L, and V105M in the NS4B sequence of genotype 1b, and have been shown to directly bind the NS4B protein. The current work extends these chemical series,[17–19] resulting in compounds of novel structure that exhibit low-nM potency against HCV replicons.




1

$EC_{50}$ HCV 1a = 31.4 nM
$EC_{50}$ HCV 1b = 45.5 nM




2

$EC_{50}$ HCV 1a = 0.3 nM
$EC_{50}$ HCV 1b = 2.0 nM

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0960-894X/

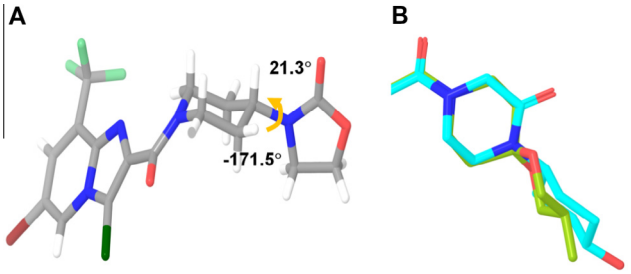
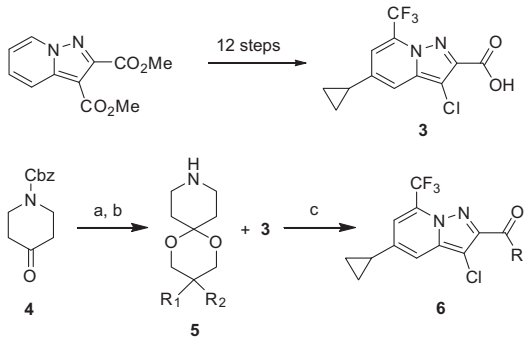



**Figure 1.** (A) X-ray crystal structure of compound **1**. (B). Overlay of models for compounds **2** (cyan) and **6e** (green).

Compounds **1** and **2** are potent HCV replication inhibitors targeting NS4B (Fig. 1).[17,18] An X-ray crystal structure of compound **1**[23] revealed that the piperidine and oxazolidinone rings are orthogonal to each other (Fig. 1A). The orthogonal orientation of the two rings is also favored in the energy minimized molecular modeling calculations for analog **2**. As part of our efforts to optimize this class of inhibitors, new chemotypes in the amide region were explored. Spiro-bicyclic motifs offer unique rigid 3-dimensional frameworks wherein the two rings are constrained to perpendicular planes (Fig. 1B). Furthermore, spirocyclic ketal substructures such as spiroketals are found in numerous natural products from diverse sources such as insects, microbes, plants, fungi and marine organisms.[24] Two classes of spirocyclic compounds based on the potent pyrazolo[1,5-*a*]pyridine core are reported herein as HCV replication inhibitors.

The pyrazolopyridine core **3** was prepared in 12 steps from dimethyl pyrazolo[1,5-*a*]pyridine-2,3-dicarboxylate with 35% overall yield.[18] The amines **5** are either commercially available or can be readily synthesized by a two-step procedure involving ketalization

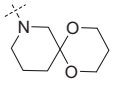
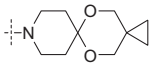
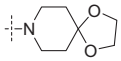
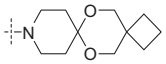
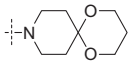
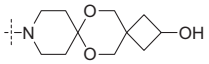
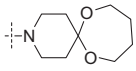
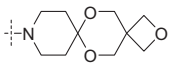
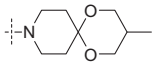
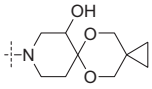
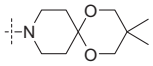
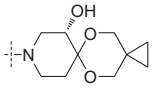
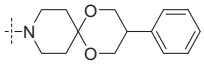
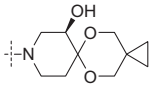
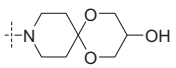
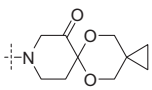
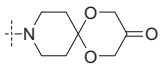



**Scheme 1.** General synthetic scheme for spiro analogs. Reagents and conditions: (a) diol (1 equiv), *p*TsOH (0.05 equiv), toluene, 135–140 °C, Dean–Stark apparatus; (b) $H_2$ (1 atm), 10% Pd/C Degussa type, MeOH; (c) Method A: amine (1–1.5 equiv), T3P (1-propanephosphoric acid anhydride, 50% wt solution in EtOAc, 1.5 equiv), Hunig's base (3 equiv), DMF; or Method B: amine (1–1.5 equiv), HATU (1.2 equiv), Hunig's base (3 equiv), DMF. (In **6h**, 2-benzyloxy-1,3-propanediol was used as starting material which was removed during hydrogenation of Cbz group.)

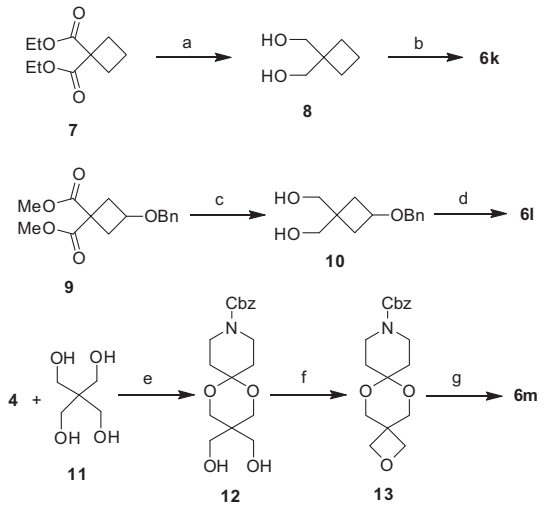
of 1-Cbz-4-piperidone **4** with the corresponding diol followed by hydrogenolysis (Scheme 1). The core acid **3** was coupled to amines **5** using standard amide coupling conditions such as HATU or T3P.[25] Compounds **6a**–**6h** (Table 1) were readily prepared using this general protocol. Ketone **6i** was prepared in 81% yield by oxidation of compound **6h** with Dess–Martin periodinane (DMP).

The synthesis of simple dispiro compounds **6j**–**6m** is shown in Scheme 2. The diols are either commercially available or can be prepared from reduction of the 1,1-dialkyl esters.[26] Oxetane analog **6m** was synthesized based on a cyclization strategy employing diol **12**, which was prepared using a modified ketalization procedure with benzene/DMF as solvents to aid solubility of

**Table 1**
HCV 1a and HCV 1b replicon efficacy of spiro ketal series of compounds[a]

| Compound | R | $EC_{50}$ 1a (nM) | $EC_{50}$ 1b (nM) | Compound | R | $EC_{50}$ 1a (nM) | $EC_{50}$ 1b (nM) |
|---|---|---|---|---|---|---|---|
| **6a** |  | >500 | >500 | **6j** |  | 9.0 | 4.1 |
| **6b** |  | 299 | 161 | **6k** |  | 5.1 | 7.6 |
| **6c** |  | 33.8 | 6.1 | **6l** |  | 2.2 | 14.2 |
| **6d** |  | 46.3 | 8 | **6m** |  | 14.4 | 3.2 |
| **6e** |  | 7.4 | 1.2 | **6n** |  | 4.3 | 2.2 |
| **6f** |  | 23.8 | 9.8 | **6o** |  | 32.5 | 8.2 |
| **6g** |  | 42.2 | 209 | **6p** |  | 1.5 | 1.2 |
| **6h** |  | 19.5 | 13.8 | **6q** |  | 5.1 | 3.8 |
| **6i** |  | 18 | 19.1 | | | | |

[a] All compounds in Tables 1 and 2 were tested in a cell based cytotoxicity assay in Huh-7 cells (genotype 1b) and showed $CC_{50}$s >50 μM except compounds **6d**, **6f** with $CC_{50}$s >25 μM.




**Scheme 2.** Synthesis of compounds **6k**, **6l**, **6m**. Reagents and conditions: (a) LAH (4 equiv), $Et_2O$, 0 °C to rt (88%); (b) same steps as in Scheme 1 (66% for 3 steps); (c) LAH (4 equiv), $Et_2O$, 0–45 °C (47%); (d) same steps as in Scheme 1 (45% for 3 steps); (e) pentaerythritol (1.5 equiv), *p*TsOH (0.05 equiv), benzene/DMF (4:6 v/v) heated to 80 °C for 30 min, 1-*Z*-piperidone (1 equiv) in benzene/DMF (4:6 v/v) added, 125 °C for 4 days (67%); (f) *n*BuLi (1.1 equiv), THF, −25 °C to rt, 30 min; *p*-TsCl, −20 °C to 0 °C for 1.5 h; *n*BuLi (1.1 equiv) 0 °C to rt, overnight (40% based on 11% recovery of starting material); (g) last 2 steps as in Scheme 1 (86% for 2 step).

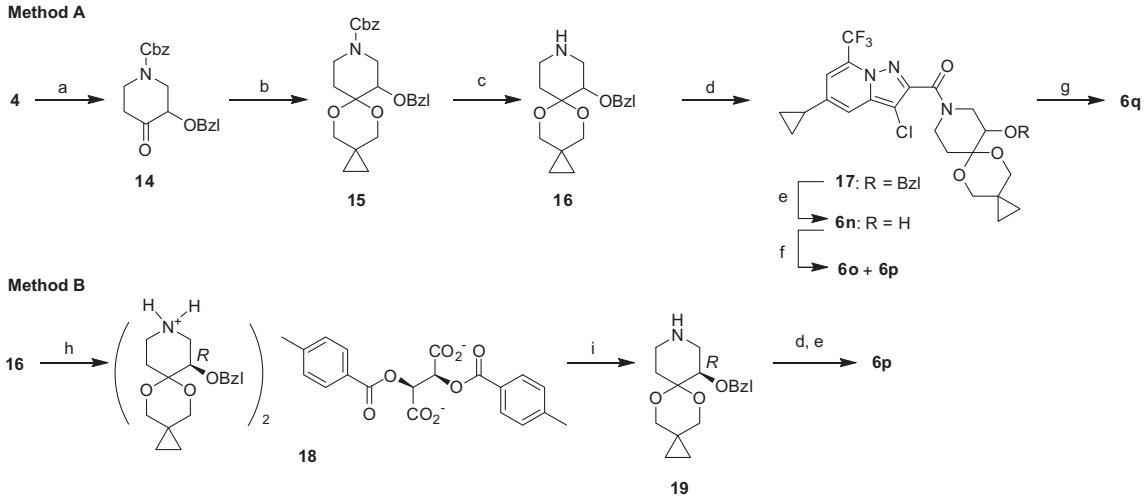
pentaerythritol.[27] The diol **12** was converted to mono-tosylate followed by treatment with *n*BuLi in the same pot to give oxetane **13** in 27% yield.[28]

Hydroxyl piperidine analogs **6n**, **6o**, **6p** and keto analog **6q** were prepared as shown in Scheme 3. Benzoylation of 1-Cbz-4-piperidone **4** at the 3-position was carried out using Tomkinson's protocol[29] with *N*-methyl-*O*-benzoylhydroxylamine hydrochloride in DMSO to give **14** in 97% yield. Ketalization of ketone **14** with commercially available 1,1-bis(hydroxymethyl)cyclopropane followed by hydrogenolysis gave racemic amine **16** in 81% yield over 2 steps. Coupling of core acid **3** with amine **16** gave compound **17** which was deprotected to give racemic compound **6n**. The racemate **6n** was resolved by chiral HPLC to give enantiomers **6o** and **6p**. Additionally, the hydroxyl group in racemate **6n** was oxidized by DMP to give ketone **6q**. An alternative method was also developed to provide larger quantities of the desired *R*-isomer **6p** required for in vitro and in vivo studies. The availability of chiral amine **19** also allowed quick access to analogs with different cores. Chiral resolution of amine **16** was achieved by crystallization of the diastereomeric salts[30] of amine **16** and (+)-*O*,*O*′-di-*p*-toluoyl-D-tartaric acid in ethanol (Scheme 3, Method B). The desired *R*-isomer was obtained as a 2:1 stoichiometry of amine/acid in 26% yield. The absolute stereochemistry of the 3-*O*-benzoate group was determined by X-ray crystallography of diastereomeric salt **18** (Fig. 2).[23] Washing with aqueous sodium bicarbonate provided the free base **19** in 88% yield with 97% enantiomeric excess. Using this diastereomeric crystallization methodology, 10 g of chiral amine **19** was readily obtained. Similar to Method A, the amine **19** was coupled to acid **3** and the benzoate saponified to give compound **6p** in 93% yield over 2 steps.

A second series of spiro compounds with five-membered oxazolidinone and lactam rings was also identified through focused screening efforts.[31] Amines **21a** and **21b** were obtained by deprotection of commercially available *N*-Boc derivatives **20a** and **20b** respectively. The amines **21a** and **21b** were coupled to acid **3** under T3P conditions to give compounds **22a** and **22g** in 89% and 85% yield, respectively (Scheme 4). The *N*-alkyl analogs **22b**–**22f**, **22h** were prepared by simple alkylation of compounds **22a** and **22g** with the corresponding halides.

Hydroxyl piperidine analogs **27a**–**d** were prepared as shown in Scheme 5. Treatment of ketone **14** with potassium cyanide under basic conditions gave cyanohydrin **23** as a mixture of diastereoisomers in 84% yield. The nitrile group in **23** was reduced under hydrogen in the presence of platinum oxide and Boc anhydride to give a mixture of *syn*- and *anti*-isomers (***rac*-24a** and ***rac*-24b**) which were easily separated by silica gel chromatography. The relative stereochemistry of each compound was determined at a later stage (vide infra). Acid hydrolysis of the Boc group in ***rac*-24a** followed by oxazolidinone formation with carbonyldiimidazole (CDI) gave spiro oxazolidinone ***rac*-25a** in 77% yield.

Methylation of ***rac*-25a** followed by purification using chiral supercritical fluid chromatography (SFC) gave enantiomers **26a** and **26b** each in 25% yield. The absolute stereochemistry of compounds **26a** and **26b** was determined by vibrational circular dichroism (VCD) and infrared (IR) analyses (vide supra). Removal of the protecting groups in **26a** and **26b** followed by coupling with core acid **3** gave compounds **27a** and **27b**, respectively. Similarly,




**Scheme 3.** Reagents and conditions: Method A: (a) MeHNOC(O)Ph (1 equiv), DMSO, rt (97%); (b) 1,1-bis(hydroxymethyl)cyclopropane (2.5 equiv), *p*TsOH (0.05 equiv), toluene, 140 °C, Dean–Stark apparatus (83%) ; (c) $H_2$ (1 atm), 10% Pd/C Degussa type, MeOH (98%); (d) **3** (1 equiv), **16** (1.4 equiv), T3P (1.5 equiv), Hunig's base (4 equiv), DMF, 0 °C (97%); (e) LiOH.$H_2O$ (3 equiv), THF/MeOH/$H_2O$ (92%); (f) separation by chiral column, RegisCell™ ODH (25 cm × 30 mm), 20% IPA in hexanes, **6o** (36%), **6p** (39%); (g) DMP (1.2 equiv), DCM, rt (49%). Method B: (h) di-*O*,*O*′-di-*p*-toluoyl-D-tartaric acid (1 equiv), EtOH, 50 °C to room temperature (26%); (i) aq $NaHCO_3$, EtOAc (23%, 97% ee); (d) **3** (1 equiv), **19** (1.4 equiv), T3P (1.5 equiv), Hunig's base (4 equiv), DMF (98%); (e) LiOH (3 equiv), THF, MeOH, water (95%).

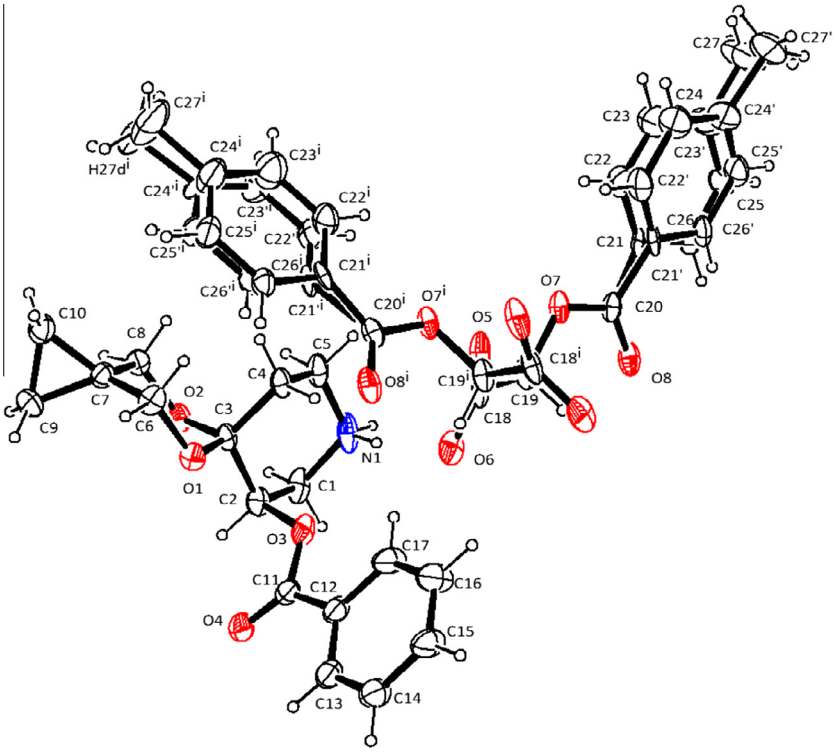
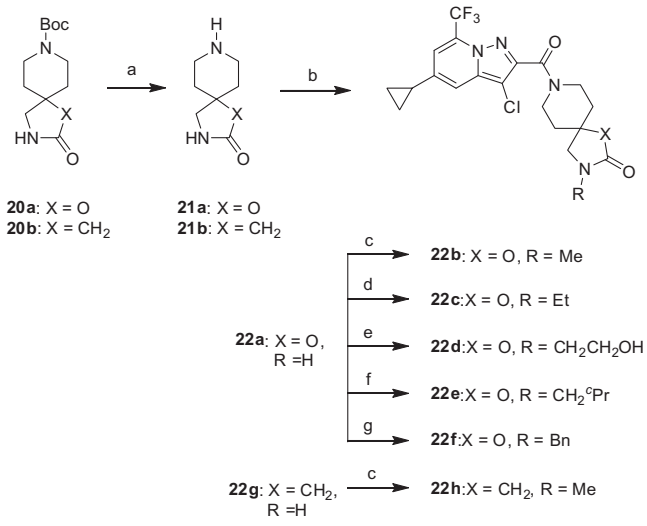


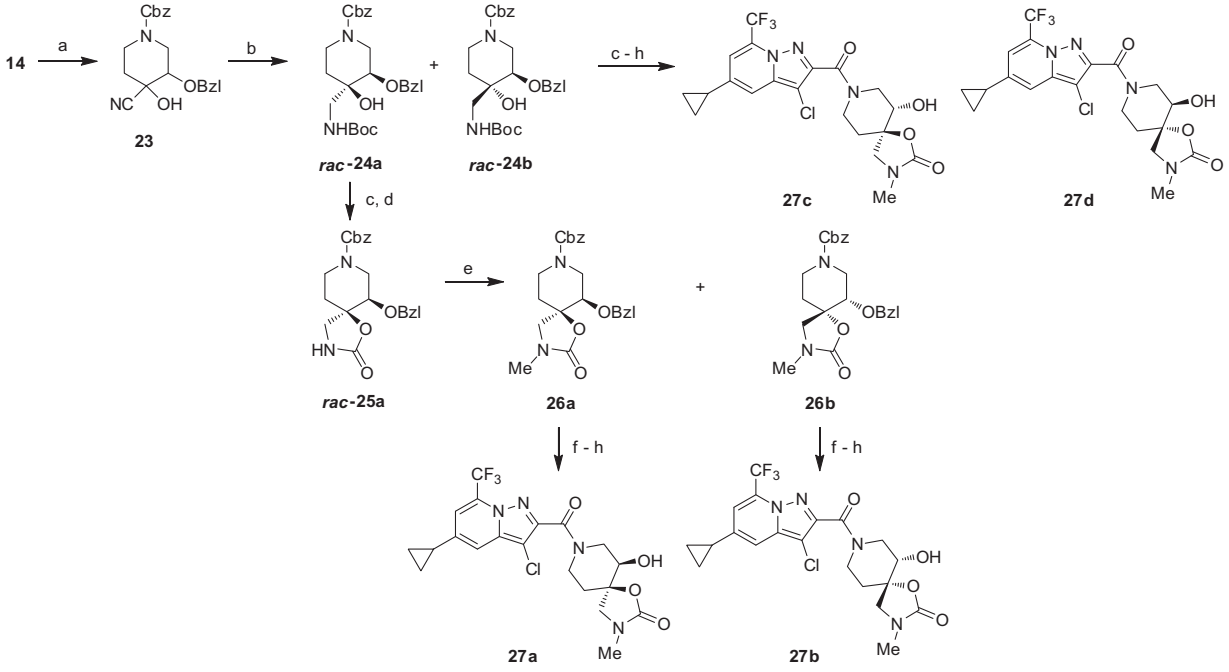

**Figure 2.** An X-ray crystal structure showing the cation and anion of compound **18**. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 20% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.




**Scheme 4.** Reagents and conditions: (a) HCl in 1,4-dioxane (10 equiv), DCM (**21a**, quantitative; **21b**, quantitative); (b) **3** (1 equiv), **20a** or **21b** (1.5 equiv), T3P (1.5 equiv), Hunig's base (4 equiv), DMF, 0 °C (89% for **22a**, 85% for **22g**); (c) NaH (1.5 equiv), MeI (1.5 equiv), DMF, 0 °C (88% for **22b**, 85% for **22h**); (d) NaH (1.2 equiv), EtI (1.5 equiv), DMF, 0 °C (79%); (e) (i) NaH (1.2 equiv), $TBDMSO(CH_2)_2$-Br (1.5 equiv), DMF, 0 °C; (ii) TBAF (2 equiv), THF (32%); (f) NaH (1.2 equiv), $^cPrCH_2Br$ (1.5 equiv), DMF, 0 °C (75%); (g) NaH (1.2 equiv), BnBr (1.5 equiv), DMF, 0 °C (81%).

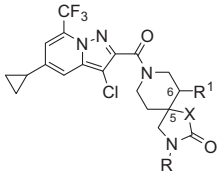
compounds **27c** and **27d** were obtained in a 6-step sequence from ***rac*-24b**.

Compounds were evaluated using subgenomic replicons of genotypes 1a (HCV 1a) and 1b (HCV 1b).[32] As shown in Table 1, spiro ring attachment at the 4 position of the piperidine amide was preferred relative to the 3-position (compound **6c** vs **6a**). A quick evaluation of the ring size of the spiro system demonstrated that the six- and seven-membered rings (**6c** and **6d**) were equipotent and superior to the five-membered ring (**6b**). The six-membered ring system was chosen for the development of further SAR. Simple methyl substitution on the terminal spiro ring (**6e**) improved the potency on both 1a and 1b replicons, while the dimethyl and phenyl analogs (**6f**, **6g**) were less active. Compounds with polar groups, such as hydroxyl- (**6h**) and keto-groups (**6i**), were tolerated. The interesting structural feature of the dispiro cyclopropyl compound **6j** prompted us to further examine other dispiro analogs. Both the cyclopropyl and cyclobutyl dispiro compounds (**6j**, **6k**) gave excellent potency versus genotype 1a and 1b replicons ($EC_{50}$ <10 nM). Compound **6j** was then tested in a pharmacokinetic (PK) study in rats, revealing a moderate rate of clearance (37.8 mL/min/kg) (Table 4). A reactive metabolite was also detected upon incubation in rat microsomes although it was not observed in human microsomes.[33] We envisioned that addition of polar functionalities at the amide region may help improve the PK profile, as previously reported.[19] Oxetane compound **6m** and the hydroxyl cyclobutyl analog **6l** were tolerated similarly to the cyclobutyl analog **6k**. In a related effort, a polar substituent (hydroxyl or ketone) was installed at the 3-position of the piperidine ring, reminiscent of the carbonyl in piperazinone **2**. Racemic-**6n** and ketone **6q** showed similar potency in genotype 1a and 1b replicons. Racemic-**6n** was further resolved to give two enantiomers in which the *R*-isomer **6p** ($EC_{50}$ 1a = 1.5 nM, $EC_{50}$ 1b = 1.2 nM) was similar in potency to our previously reported lead molecule **2**.[18] One of the oxygen atoms in the spiroketals is proposed to act as a hydrogen bond acceptor, similar to the carbonyl in oxazolidinone as in compound **1** or the carbonyl in piperazinone as in compound **2**. Addition of a *R*-hydroxy group at the 3-position of the piperidine ring (**6p**) may provide an alternative oxygen atom as hydrogen bond acceptor. The superior HCV 1a potency of compound **2** is attributed to the hydroxy group on the cyclohexyl ring which is also observed in hydroxyl cyclobutyl analog **6l**.[34] Compound **6p** was further evaluated in rat PK studies (Table 4).




**Scheme 5.** Reagents and conditions: (a) KCN (1.2 equiv), $NaHCO_3$ (2 equiv), diethyl ether/water (84%); (b) $H_2$ (50 psi), $Pt_2O$ (0.22 equiv), $Boc_2O$ (2.5 equiv), $^{i}$PrOH (22% for ***rac*-24a**, 17% for ***rac*-24b**); (c) HCl in 1,4-dioxane (55 equiv), DCM; (d) CDI (6 equiv), DMF (77% for ***rac*-25a**, 64% for ***rac*-25b**); (e) (i) NaH (1.1 equiv), MeI (1 equiv), DMF, 0 °C; (ii) chiral separation of **26a** and **26b** by OJH chiral column (25 cm × 30 mm), 20% *iso*-propanol in hexanes (25% for **26a**, 25% for **26b**), chiral separation of **26c** and **26d** by ADH chiral column under SFC condition with 20% MeOH in $CO_2$ (26% for **26c**, 28% for **26d**); (f) LiOH·$H_2O$ (3 equiv), THF/MeOH/water; (g) $H_2$ (1 atm), 10 wt% Pd/C (Degussa type) (0.05 equiv); (h) **3** (1 equiv), spirocyclic amine (1.1), HATU (1.2 equiv), Hunig's base (4 equiv), DMF (yields for 3 steps: 78% for **27a**, 80% for **27b**, 68% for **27c**, 72% for **27d**).

**Table 2**
HCV 1a and HCV 1b replicon efficacy of spiro oxazolidinone and spiro lactam series of compounds




| Compound | R | Stereo-chemistry | X | $R^1$ | $EC_{50}$ 1a (nM) | $EC_{50}$ 1b (nM) |
|---|---|---|---|---|---|---|
| **22a** | H | | O | H | 166 | 55.4 |
| **22b** | Me | | O | H | 2.0 | 3.4 |
| **22c** | Et | | O | H | 20.6 | 5.9 |
| **22d** | $CH_2CH_2OH$ | | O | H | 23.2 | 78.5 |
| **22e** | $CH_2{}^{c}Pr$ | | O | H | 18.1 | 23.4 |
| **22f** | Bn | | O | H | 427 | 457 |
| **22g** | H | | $CH_2$ | H | 441 | 1575 |
| **22h** | Me | | $CH_2$ | H | 184 | 69.2 |
| **27a** | Me | (5*R*,5*R*) | O | OH | 14.5 | 7.0 |
| **27b** | Me | (5*S*,6*S*) | O | OH | 3558 | 3992 |
| **27c** | Me | (5*R*,6*S*) | O | OH | 10.9 | 6.1 |
| **27d** | Me | (5*S*,6*R*) | O | OH | 16.7 | 7.2 |

In the spiro [6.5] series, an initial assessment of alkyl substitution on the lead compound **22a** identified the *N*-methyl derivative **22b** ($EC_{50}$ 1a = 2 nM, $EC_{50}$ 1b = 3.4 nM) as the most potent compound in the spiro oxazolidinone series (Table 2). SAR revealed that the methyl substitution appears optimal. Replacement of the oxazolidinone ring with a lactam ring (O → C) resulted in significant loss of potency, in particular for compound **22h**. This is consistent with previous amide modifications that demonstrate the need for at least one oxygen atom between the piperidine and the adjacent ring.[18,19] Unfortunately, metabolite ID studies of **22b** showed a significant amount of *N*-demethylation. In an analogous effort to improve the PK in the spiro ketal series, a hydroxyl group was incorporated at the 3-position of the piperidine ring. All four diastereoisomers were prepared and three (**27a**, **27c**, **27d**) of them were tolerated. The most potent diastereoisomer **27c** was further profiled in a rat PK study.

Two sets of compounds (**6j** and **6p**, **22b** and **27c**) were selected for further in vitro profiling. In addition to their potent binding ($EC_{50}$'s = 10.2–32 nM) to NS4B as measured by displacement of a radioligand (Table 3), all four compounds showed shifts in replicon potency versus two stable replicons bearing mutations (H94N and V105M) previously shown to confer resistance to analogs in a related chemical series. These data suggest the analogs are interacting directly with NS4B. While these compounds showed excellent potency in HCV 1a and HCV 1b replicons, the potency against a genotype 2a replicon remained poor (in the micro-molar range).

In general, this class of inhibitors showed excellent drug-likeness, as demonstrated by the potencies and physicochemical properties such as low *c*log*P*, good solubility and permeability (Table 3). In vivo profiling of the two pairs of compounds suggested improved PK properties with the addition of the hydroxyl group at the 3-position of the piperidine ring (Table 4). In the spiro ketal series (**6j** vs **6p**), addition of the hydroxyl group in compound **6p** had little effect on the moderate clearance of compound **6j** after IV administration to rats, but it resulted in significant improvement of the oral exposure (greater than 4-fold improvement in $C_{max}$ and AUC). In addition, the reactive metabolite observed in compound **6j** was not observed in compound **6p**. In the spiro oxazolidinone series (**22b** vs **27c**), addition of hydroxyl group led to improvement in clearance (~2×) while oral exposure only increased by 1.5-fold.

In summary, two new series of spiro piperidine analogs were rationally designed which inhibit HCV replication with $EC_{50}$s <11 nM for genotypes 1a and 1b replicons. However, the emergence of more potent analogs from other series[18] and the low HCV 2a potency for these compounds has put these series on hold.

**Table 3**
In vitro and physical properties of matched compound pairs

| Compound | 6j | 6p | 22b | 27c |
|---|---|---|---|---|
| $EC_{50}$ 1a (nM) | 9 | 1.5 | 2 | 10.9 |
| $EC_{50}$ 1b (nM) | 4.1 | 1.2 | 3.4 | 6.1 |
| $EC_{50}$ 1b H94N (nM) | 24.3 | 35.9 | 42.7 | 175 |
| (fold shift) | (5.9×) | (29.9×) | (12.5×) | (27.3×) |
| $EC_{50}$ 1b V105M (nM) | 67.6 | 171.8 | 20.4 | 1,128 |
| (fold shift) | (16.5×) | (143×) | (6×) | (185×) |
| $EC_{50}$ 2a (nM) | 6,839 | 11,253 | 6,839 | 38,019 |
| $IC_{50}$ NS4B 1b (nM)[a] | 32 | 10.2 | 23.4 | 30.4 |
| Solubility (μg/mL)[b] | 105 | 323 | 267 | 310 |
| Papp MDCK (nm/s)[c] | 432 | 407 | N/A | N/A |
| MW | 469.9 | 485.9 | 456.9 | 472.9 |
| $T_{½}$ human (min)[d] | 30 | 50 | 66 | 123 |
| $T_{½}$ rat (min)[d] | <15 | 28 | 80 | 132 |
| *c*Log*P*[e] | 2.96 | 2.68 | 2.58 | 2.88 |
| TPSA[f] | 56 | 76 | 67 | 87 |
| $LLE_{calc}$[g] | 4.5 | 5.3 | 5.0 | 4.6 |

[a] NS4B binding assay as measured by displacement of a radioligand.[19]
[b] Kinetic solubility measured at pH 7.4.[35]
[c] Passive Papp is defined as the A → B Papp value in the presence of P-gp inhibitor GF 120918.
[d] Half-life of compounds in human and rat hepatocytes.[18]
[e] *c*Log*P*s are calculated using the Daylight/BioByte v4.3 algorithm, BioByte Corp., Claremont, CA.
[f] Topological polar surface areas (TPSAs) are calculated using Ertl's method.[36]
[g] Lipophilicity ligand efficiency (LLE) is defines as $pIC_{50}$ (or $pK_i$) – *c*log*P* (or log*D*).[37]

**Table 4**
In vivo rat DMPK profile of compounds **6j**, **6p**, **22b** and **27d**

| Compound | 6j | 6p | 22b | 27c |
|---|---|---|---|---|
| IV Clearance[a,b] | 37.8 | 35.7 | 56.5 | 25.7 |
| $V_{dss}$ | 4.5 | 0.8 | 2.1 | 2.0 |
| $C_{max}$[c] | 254 | 1134 | 269 | 344 |
| PO AUC 0–24 | 693 | 2792 | 1059 | 1528 |
| %*F* | 29 | >100 | 72 | 49 |

[a] All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.
[b] Dosed as a solution of DMSO/20%HPβCD (10:90); IV dosed 1 mg/kg; PO dosed 5 mg/kg; clearance (mL/min/kg); $V_{dss}$ (L/kg); $C_{max}$ (ng/mL); PO AUC 0–24 (h•ng/mL).
[c] Concentrations of all 4 compounds taken at 24 h were below level of quantitation.

The unique spirocyclic amines, which were developed through modeling studies, offer new fragments that target different chemical space for future drug discovery.

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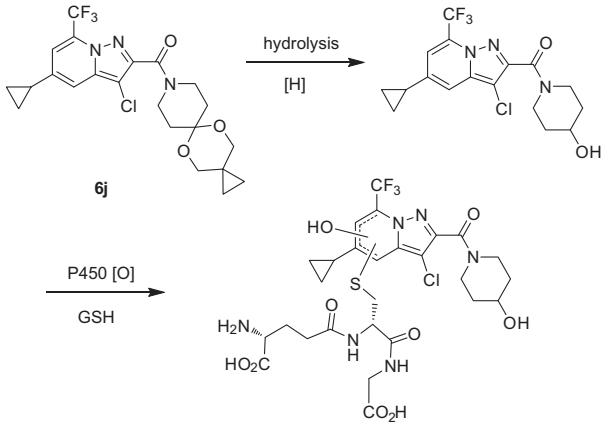
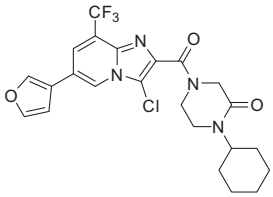
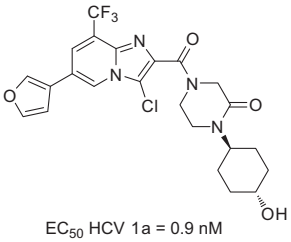
31. The corresponding six membered 1,3-oxazinan-2-one analogs are less active.
32. Detailed description of the HCV replicon assay and NS4B binding assay can be found in Ref. 19. The replicon data represents the average of experimental data with $n = 2$ or more.
33. A proposed mechanism for glutathione conjugation of compound **6j** is drawn below. The addition of hydroxyl group in compound **6p** may have altered the routes of metabolism.




34. We previously[19] showed that addition of hydroxyl group to the cyclohexyl ring improves HCV 1a potency as demonstrated by the following pair of compounds with imidazopyridine core. The corresponding cyclohexyl analog for compound **2** has not been made.



$EC_{50}$ HCV 1a = 11 nM
$EC_{50}$ HCV 1b = 3.5 nM



$EC_{50}$ HCV 1a = 0.9 nM
$EC_{50}$ HCV 1b = 9.7 nM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