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

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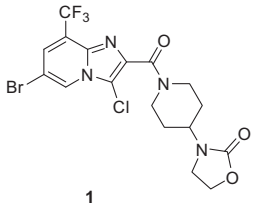
ABSTRACT

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.

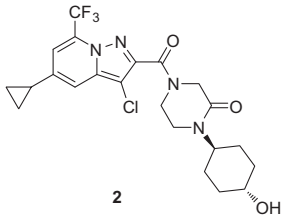
Hepatitis C is a blood-borne disease of the liver transmitted by the Hepatitis C Virus (HCV) and is a leading cause of chronic liver disease and liver transplantations.[1,2] The World Health Organization (WHO) estimates that about 150 million people are chronically infected with HCV, and more than 350,000 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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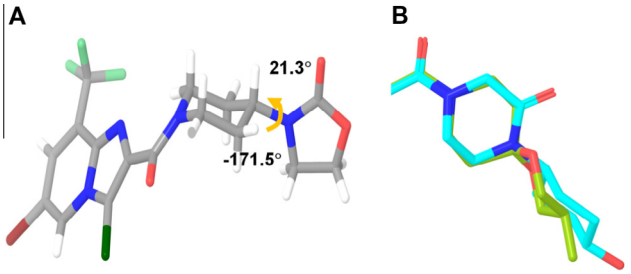
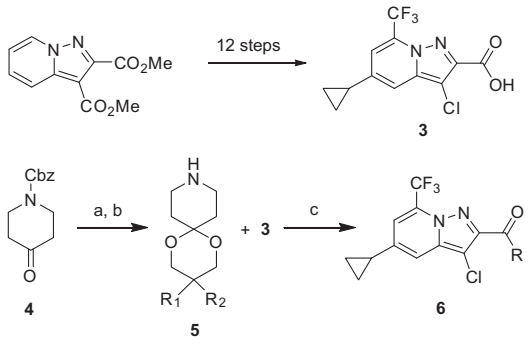


Figure 1. (A) X-ray crystal structure of compound **1**. (B). Overlay of models for compounds **2** (cyan) and **6e** (green).

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

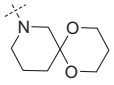
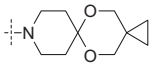
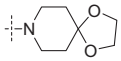
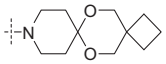
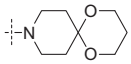
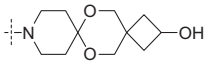
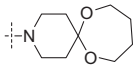
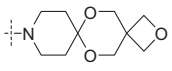
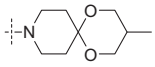
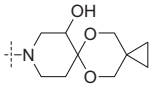
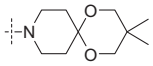
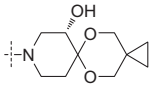
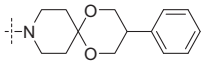
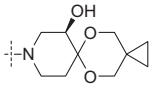
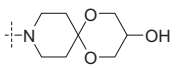
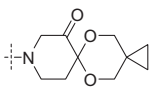
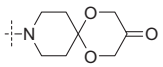


Scheme 1. General synthetic scheme for spiro analogs. Reagents and conditions: (a) diol (1 equiv), *p*TsOH (0.05 equiv), toluene, 135–140 °C, Dean–Stark apparatus; (b) H_2 (1 atm), 10% Pd/C Degussa type, MeOH; (c) Method A: amine (1–1.5 equiv), T3P (1-propanephosphoric acid anhydride, 50% wt solution 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.