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Development of carboxylic acid replacements in indole-*N*-acetamide inhibitors of hepatitis C virus NS5B polymerase

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Abstract—Allosteric inhibition of the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase enzyme has recently emerged as a viable strategy toward blocking replication of viral RNA in cell-based systems. We report here 2 series of indole-*N*-acetamides, bearing physicochemically diverse carboxylic acid replacements, which show potent affinity for the NS5B enzyme with reduced potential for formation of glucuronide conjugates. Preliminary optimization of these series furnished compounds that are potent in the blockade of subgenomic HCV RNA replication in HUH-7 cells.

HCV is a major human pathogen associated with chronic hepatitis and liver disease, cirrhosis, hepato-cellular carcinoma, and liver failure.[1] Worldwide, there are an estimated 170 million chronic carriers,[2] whilst in the US alone, 4 million have antibodies to HCV, indicating an on-going or previous infection with the virus. For over 10 years, frontline therapies have been based around interferon-α, commonly dosing in conjunction with ribavirin. Despite progress with such therapies (e.g., introduction of pegylated interferon),[3] sustained viral response (SVR) rates are still typically poor, particularly for genotype-1 infections that predominate in Europe, Japan, and the U.S.[4] In addition, therapy is often accompanied by significant adverse side effects[5]—consequently, there is a pressing need for new and broadly effective therapeutics to combat HCV.[3,6]

HCV is a small, enveloped, single stranded positive RNA virus in the Flaviviridae family. The genome is approximately 10,000 nucleotides and encodes a single polyprotein of about 3000 amino acids. This polyprotein comprises the structural (C, E1, and E2) and non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins that are required for replication and packaging of viral genomic RNA. NS5B is the viral RNA-dependent RNA polymerase (RdRp). The RdRp activity of NS5B is essential for viral replication[7] and has no functional equivalent in uninfected mammalian cells—thus making the NS5B protein an attractive target for drug discovery.[8]

The NS5B RdRp comprises the palm, fingers, and thumb subdomains common to nucleotide polymerizing enzymes.[9] Inhibition of NS5B can be achieved through interaction at the active site, for which both nucleoside ligands[10,11] and non-nucleoside inhibitors have been described.[11–15] Alternatively, several allosteric inhibitor binding sites on NS5B have been identified distal to the catalytic center.[11,16] Recent reports from our laboratories documented the development of *N*-acetamido-indole-6-carboxylates, such as **1**, as potent inhibitors interacting at one such allosteric site that lies close to a conserved amino acid, proline 495, on the surface of the thumb domain.[17–19] An issue with compounds from this class, however, was that glucuronide conjugates of the carboxylic acid were frequently observed as major circulating metabolites (Fig. 1).

To moderate the formation of glucuronide conjugates in the systemic circulation, viable alternatives to the C6-carboxylate were sought. There are a number of moieties documented in the literature as mimetics for carboxylic acids, with diverse physical attributes and spanning a multi-log unit range of pK_a values. In this

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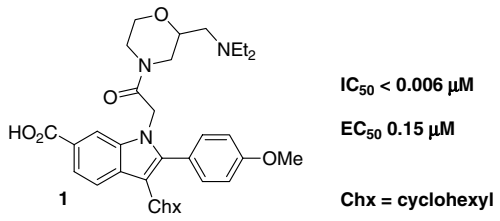


Figure 1. Potent *N*-acetamido-indole-6-carboxylic acid inhibitor of HCV NS5B.

paper, we describe the discovery and initial optimization of 2 such structural classes (oxadiazolones and acyl sulfonamides/sulfonyl ureas) chosen for their disparate pK_a's and physical characteristics—leading to indole *N*-acetamides with reduced potential regarding formation of glucuronide conjugates, that retain intrinsic affinity for the NS5B enzyme and potency as inhibitors of HCV replication in a surrogate cell-based assay.

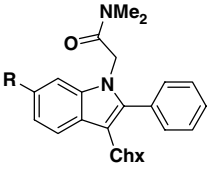
The compounds described herein were assessed for activity (IC_{50}) against the purified ΔC55 NS5B enzyme in the presence of heterogeneous template RNA. Inhibition of replication of subgenomic HCV RNA was measured in HUH-7 cells using a modification of the procedure of Bartenschlager.[20] Unless otherwise stated, cell-based data (EC_{50}) were measured in the presence of 10% fetal calf serum. Pharmacokinetic studies in rats were performed with $n = 3$, and the following dosing parameters were used: iv 3 mg/kg (60% DMSO + 20% PEG400 + 20% H_2O), po 3 mg/kg (PEG400).

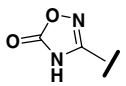
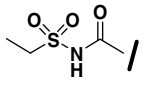
The indoles reported in Tables 1–4 were prepared from the methyl 2-bromo-3-cyclohexylindole-6-carboxylate precursor[17] **2**, as outlined in Scheme 1. Thus, either BBr_3 or hydrolytic cleavage of the methyl ester, formation of the primary amide and dehydration to the nitrile, followed by alkylation with NaH/*tert*-butyl bromoacetate afforded intermediate **3**. Preparation of the amide oxime and a one-pot acylation/cyclization protocol using carbonyldiimidazole generated the oxadiazolone. Pd-mediated cross-coupling with the appropriate arylboronic acid then installed the aromatic functionality at C2 in **4**. Unmasking of the *tert*-butyl ester and TBTU-promoted amide bond formation yielded the final compounds **5**. Clearly, this strategy is well suited to modifications to the acetamide dimension in the final step, however, the synthesis could be readily tailored to fit final diversification steps at alternative positions around the indole. Hence, for example, manipulation at C6 could be performed equally well after diversification at C2 and in the *N*1-acetamide moieties, simply by moving steps in the synthetic sequence. Initial Suzuki coupling on **2** followed by the 3-step alkylation, deprotection, amide bond formation sequence, and final ester cleavage afforded C6 carboxylic acid **6**. The carboxylate moiety could then either be manipulated as before to set up the oxadiazolone ring, or subjected to EDAC mediated coupling with sulfonamides or sulfamides to yield the corresponding acylsulfonamides or sulfonylureas **7**.

As illustrated in Scheme 2, taking bromo derivative **8** through the analogous synthetic sequence allows functionalization at C2 as the final step.

In the field of angiotensin II receptor antagonists, heterocycles such as oxadiazolones have been reported as replacements for carboxylic acid moieties.[21] Although 2 tautomeric forms can be envisaged, studies suggest that generally the non-aromatic oxadiazolone form is

Table 1. HCV NS5B polymerase enzyme inhibition (IC_{50}), cell-based efficacy (EC_{50}), and in vivo rat pharmacokinetic properties for diverse C6 acid moieties



Compound	R	IC_{50}/EC_{50}[a] (μM)	F_{rat} (%)[b,c]/ $t_{1/2}$ (h)[d]	Cl (ml/min/kg)[e]/ AUC (μM h)[f]
9		0.086/**1.5**	45/5	32/2
10		0.068/**4.4**	20/13	12/2
11		0.046/**2.1**	24/2	51/1

[a] IC_{50}/EC_{50} values are means from at least 2 experiments.
[b] Compounds were dosed as trifluoroacetate salts. po/iv 3 mg/kg body weight; $n = 3$. Vehicle iv 60% DMSO/20% PEG400/20% H_2O, po PEG400.
[c] Oral bioavailability.
[d] Terminal phase plasma halflife following iv administration.
[e] Plasma clearance.
[f] After oral dosing.