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Discovery of novel HCV inhibitors: Synthesis and biological activity of 6-(indol-2-yl)pyridine-3-sulfonamides targeting hepatitis C virus NS4B

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ABSTRACT

A novel series of 6-(indol-2-yl)pyridine-3-sulfonamides was prepared and evaluated for their ability to inhibit HCV RNA replication in the HCV replicon cell culture assay. Preliminary optimization of this series furnished compounds with low nanomolar potency against the HCV genotype 1b replicon. Among these, compound **8c** has identified as a potent HCV replicon inhibitor (EC_{50} = 4 nM) with a selectivity index with respect to cellular GAPDH of more than 2500. Further, compound **8c** had a good pharmacokinetic profile in rats with an IV half-life of 6 h and oral bioavailability (F) of 62%. Selection of HCV replicon resistance identified an amino acid substitution in HCV NS4B that confers resistance to these compounds. These compounds hold promise as a new chemotype with anti-HCV activity mediated through an underexploited viral target.

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including fibrosis, cirrhosis and liver cancer.[1] Globally, up to 3% of the world's population is chronically infected with HCV and 3–4 million people are newly infected each year.[2] There is no vaccine available to prevent hepatitis C. In the past decade, the standard of care for the treatment of chronic hepatitis C has been based exclusively on the combination of pegylated interferon and ribavirin, a combination which is ineffective in genotype 1 patients (<50% sustained virologic response), contraindicated in some patients, or associated with severe side effects.[3] A new era of therapy for HCV infection has emerged with the approval of the first direct acting antiviral agents, the HCV protease inhibitors Incivek™ (telaprevir) and Victrelis™ (boceprevir). These agents prevent HCV replication by inhibiting the activity of the HCV protease NS3/4A.[4] Results from clinical trials have demonstrated that boceprevir or telaprevir in combination with pegylated interferon and ribavirin significantly improve sustained virologic response rates, both in treatment-naïve patients and in prior relapsers and nonresponders.[5] However, the ideal treatment regimen for HCV infection, in which interferon is spared or eliminated, has not yet been realized, and the drawbacks of current HCV therapy necessitate the development of more effective anti-HCV agents.

Besides protease inhibitors, a number of other HCV inhibitors are in preclinical or clinical development targeting viral proteins such as NS5A[6a] and the RNA-dependent RNA polymerase NS5B.[6b,6c] Recently, the HCV NS4B protein has emerged as an unexploited target for direct acting antivirals.[7] NS4B is a 27-kDa integral membrane protein that has a number of described functions but is thought to act primarily as a scaffold for assembly at the host endoplasmic reticulum of viral replication complexes that are required for HCV RNA replication.[8]

We recently described the discovery and chemical optimization of *N*-(4′-(indol-2-yl)phenyl)sulfonamides as novel inhibitors of the HCV 1b replicon.[9] We have identified the sulfonamide moiety attached to the phenyl ring (e.g., compound **1**, Fig. 1) as a preferred pharmacophore leading to submicromolar potency in the replicon cell-based assay. As part of the continuing optimization of this novel series, we reversed the arrangement of the sulfonamide group attached to the phenyl ring in compound **1**, placing the sulfur atom adjacent to the aromatic ring. This approach resulted in the identification of **2a** as a potent HCV replicon inhibitor (EC_{50} = 100 nM). This compound is about fourfold more potent than compound **1**. Although very potent, **2a** has poor aqueous solubility and is highly metabolized in the presence of human liver microsomes. Oral

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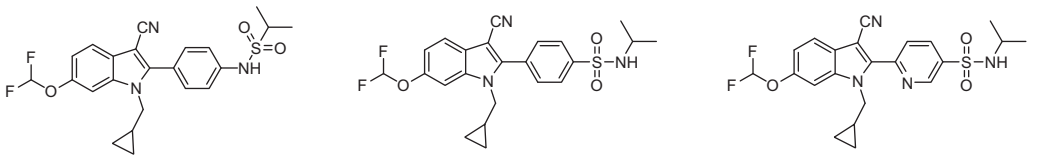


Figure 1. Structures of representative compounds and activity against the HCV 1b replicon.

administration of a single 10 mg/kg dose of **2a** to rats resulted in the formation of a major metabolite corresponding to a loss of 42 mass units suggesting that the major metabolite **2b** was formed by oxidative N-dealkylation of **2a** (Fig. 2). The structure of the metabolite was confirmed by synthesis of the primary sulfonamide **2b**. The metabolite **2b** was found to be significantly less active than the parent (**2a**) with an EC_{50} of 4700 nM in the replicon assay. These results provided us with a starting point to optimize further the antiviral activity as well as metabolic and pharmacokinetic properties of this class of compounds. In response to the limitations of compound **2a**, a small number of arene sulfonamides were investigated by replacing the phenyl ring with nitrogen-containing heteroaryl rings. We found that (indol-2-yl)pyridine **3a** (Fig. 1) provided the most potent activity compared to other arene substitution patterns (compounds **4**–**6** in Table 1). We therefore chose to explore further the effect of substitutions using the 6-(indol-2-yl)pyridine-3-sulfonamide as a core structure. The preparation of these compounds and an initial assessment of their biological properties are the subject of this article.

The general synthetic route to prepare the (indol-2-yl)arene sulfonamides **2**–**8** is outlined in Scheme 1. The target compounds were prepared through a Suzuki coupling strategy by treatment of the boronic acid **9b**, generated from the key intermediate **9a**, with the corresponding aryl halides **10**–**13**. The synthesis of **9a** with various R^1 and R^2 substitutions on the indole ring was described previously.[9]

The synthetic approaches to the intermediate aryl chlorides **10** and **12** and aryl bromides **11** and **13** utilized in the Suzuki coupling described in Scheme 1 are illustrated in Scheme 2. Diazotization of commercially available 5-amino-2-chloropyridine followed by chlorosulfonylation gave the sulfonyl chloride **14b** using a modified procedure reported previously.[10] Condensation of **14a** (commercially available) or **14b** with various amines, in the presence of pyridine at 0 °C afforded the corresponding sulfonamide derivatives **10**. The pyridine building block **11**, in which the sulfonamide group is *ortho* to the pyridine nitrogen, was prepared by converting 2-chloro-5-bromopyridine to the corresponding mercaptopyridine **15**. Compound **15** was then oxidized in situ followed by amination. Sulfonylation of 2-aminopyrimidine gave an intermediate sulfonic acid **16**,[11] which was then converted to the desired 2-chloropyrimidine-5-sulfonamide **12** via sulfonyl chloride **17**. The synthesis of 5-bromopyrimidine-2-sulfonamide **13** entails the conversion of 5-bromo-2-chloropyrimidine to the corresponding mercaptopyrimidine **18**, which was then elaborated to the desired sulfonamide

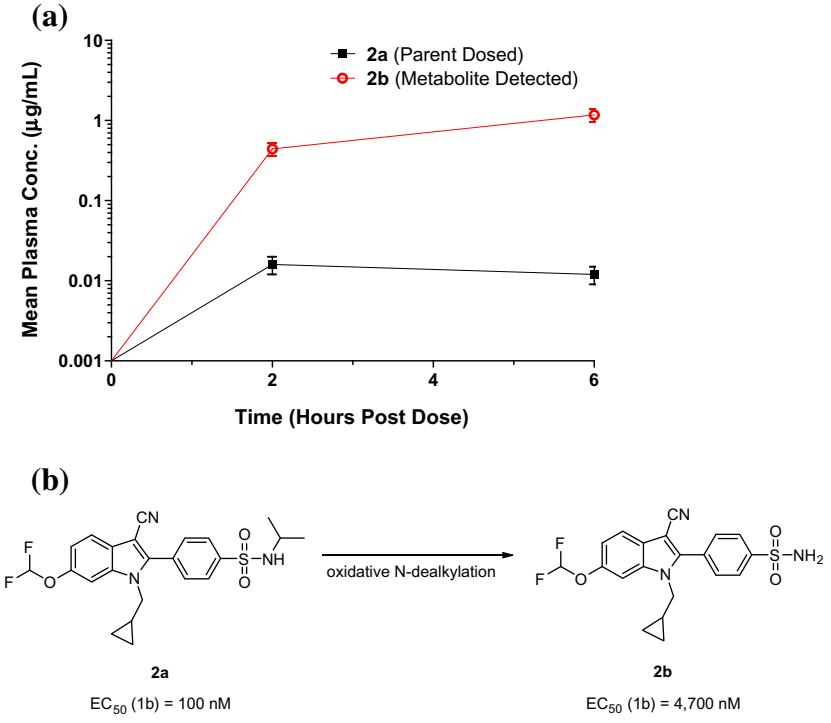
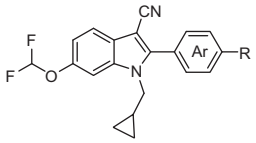
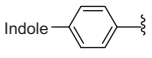
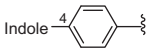
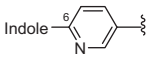
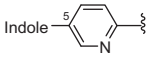
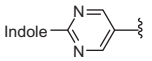
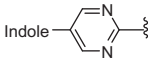


Figure 2. (a) Plasma concentrations of **2a** and metabolite **2b** following administration of a 10 mg/kg oral dose of **2a** in Sprague–Dawley rats ($n = 3$). (b) Major metabolic pathway of **2a** in rats.

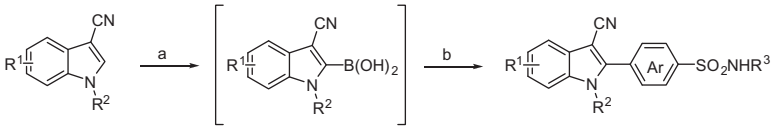
Table 1
Effect of various arene sulfonamides on replicon activity, selectivity and metabolic stability



1-6

Compd	Indole–Ar	R	HCV replicon 1b EC_{50} (nM)[a]	GAPDH IC_{50}[a] (nM)	HLM, CL_{int}[b] (mL/min/kg)
1			390	>10,000	<1
2a			100	5000	204
3a			33	>10,000	51
4			560	>10,000	297
5			240	>10,000	24
6			10,000	>10,000	n.d.[c]

[a] See Ref. 19 for assay conditions.
[b] See Ref. 20 for assay conditions.
[c] Not determined.



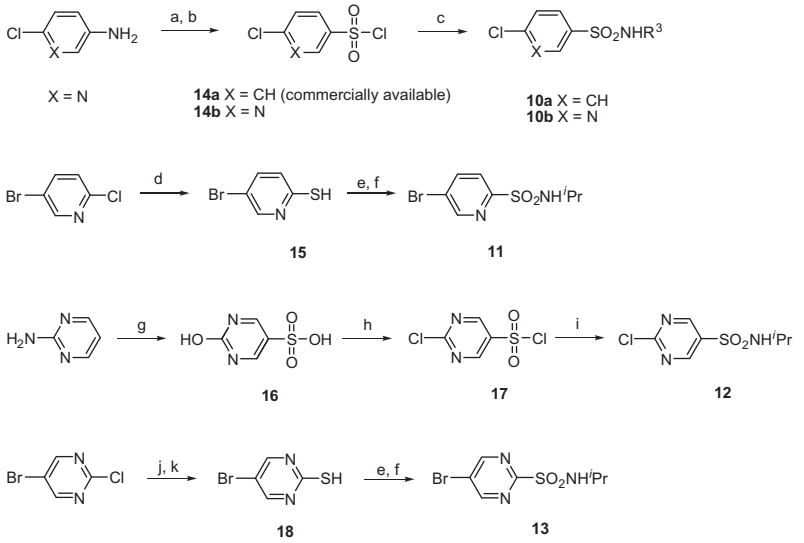
9a **9b** **2-8**

Scheme 1. Reagents and conditions: (a) LDA, THF, $B(OMe)_3$, −78 °C; (b) aryl halides (**10**–**13**), $Pd_2(dba)_3$, $^tBu_3PHBF_4$; KF, THF.

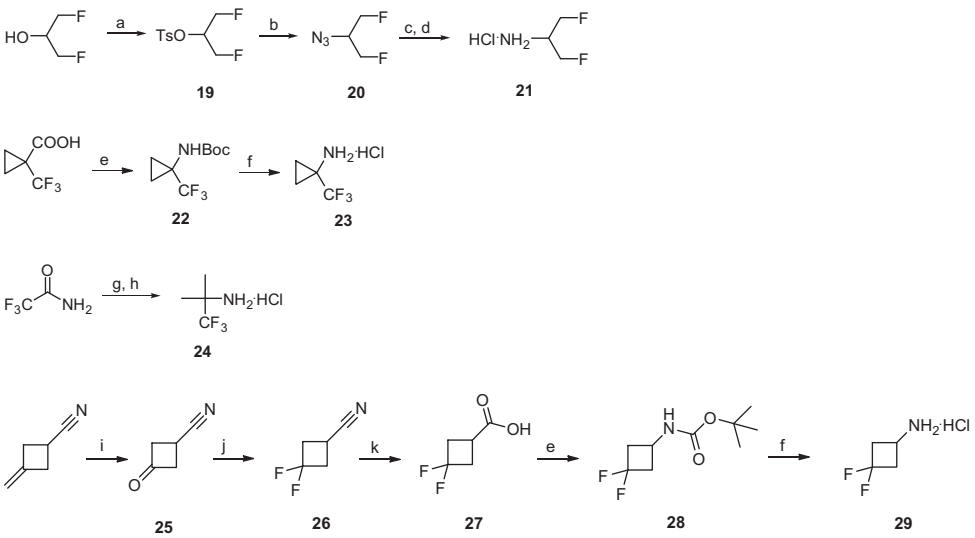
13 under conditions similar to those utilized in the preparation of intermediate **11**.

Amines **21**, **23**, **24**, and **29** were prepared as outlined in Scheme 3. 1,3-Difluoropropan-2-amine **21** was prepared from commercially available 1,3-difluoro-2-propanol in four steps. 1,3-Difluoro-2-propanol was first transformed into tosylate **19** and then treated with sodium azide to produce azide **20**. Reduction of azide **20** through hydrogenation, followed by treatment with HCl provided the desired amine **21** as the hydrochloride salt. 1-(Trifluoromethyl)cyclopropanamine **23** was prepared from the corresponding 1-trifluoromethylcyclopropane-1-carboxylic acid by a modified Curtius rearrangement with diphenyl phosphorazidate (DPPA) and triethylamine in *tert*-butyl alcohol with subsequent acidic cleavage of the Boc moiety.[12] Trifluoro-2-methylpropan-2-amine **24** was prepared by treatment of 2,2,2-trifluoroacetamide with methylcerium chloride, generated in situ from cerium chloride and methyllithium. The product was easily isolated as the hydrochloride salt in high yield.[13] 3-Methylenecyclobutanecarbonitrile was converted into 3,3-difluorocyclobutanecarboxylic acid **27** in three steps according to the procedure reported previously.[14] Compound **27** was then converted to 3,3-difluorocyclobutanamine hydrochloride **29** using the same method as described for the preparation of **23**.

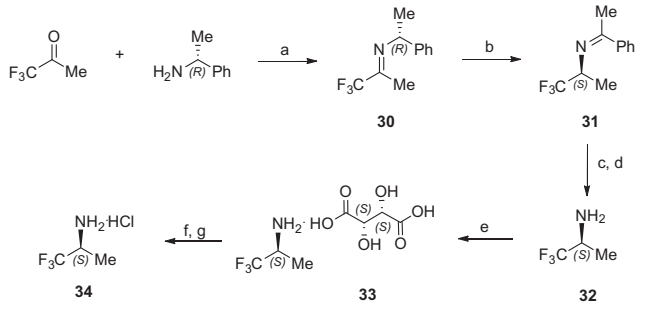
The chiral amine (*S*)-1,1,1-trifluoropropan-2-amine **34** was obtained by asymmetric synthesis from 1,1,1-trifluoropropan-2-one and (*R*)-1-phenylethanamine by a modification of the method of Soloshonok et al.[15] as shown in Scheme 4. The chiral Schiff base **30** was prepared by direct condensation of 1,1,1-trifluoropropan-2-one and (*R*)-1-phenylethanamine. Isomerization of **30** to **31** was achieved in a solution of DBU at 60 °C, followed by hydrolysis under acidic conditions to give the desired chiral amine **32** in moderate yield with 92% enantiomeric excess (ee). The optical purity of **32** was further improved by salt formation of **32** with D-tartaric acid. Crystallization of the diastereomer-rich mixture provided tartaric acid salt **33** with >99% ee, which was then converted into the hydrochloride salt of **34** in high yield with retention of high enantiomeric purity. The (*R*)-enantiomer of **34** was obtained from (*S*)-1-phenylethanamine following the same procedure. The optical purity of **34** and its enantiomer was determined by chiral HPLC analysis of the *N*-(3,5-dinitrobenzoyl) derivative and the absolute configuration was confirmed by comparison with an authentic sample.[16]



Scheme 2. Reagents and conditions: (a) $NaNO_2$, HCl (concd), 0 °C, 82%; (b) $SOCl_2$, H_2O, CuCl, 70%; (c) R^3NH_2, pyridine, 0 °C. (d) NaSH, DMF, 70 °C; (e) NaOCl, HCl; (f) isopropyl amine; (g) H_2SO_4, 180 °C, 48 h; (h) PCl_5, 180 °C, 4 h, 24% for two steps; (i) isopropyl amine, triethylamine, THF, −78 °C, 0.5 h, 94%; (j) thiourea; (k) NaOH (1 N), 85% for two steps.



Scheme 3. Reagents and conditions: (a) TsCl, pyridine, 91%; (b) NaN_3, DMSO, 70 °C, 5 h; (c) H_2, Pd/C (10%), EtOAc, 3 h; (d) HCl, 78% from **19**; (e) DPPA, Et_3N, *t*-BuOH, 80 °C, o/n, 84–97%; (f) HCl (1 N), 110 °C, 2 h, 86%; (g) $CeCl_3$, MeLi, THF, −78 °C to rt; (h) HCl, 75% for two steps; (i) $RuCl_3$, $NaIO_4$, CH_2Cl_2, MeCN, H_2O; 91%; (j) DAST, CH_2Cl_2, 0 °C to rt, 99%; (k) NaOH, MeOH, H_2O, 88%.



Scheme 4. Reagents and conditions: (a) *p*-TsOH (5%), $Si(OEt)_4$ (0.5 equiv), 2-Me-THF, reflux, 16 h; (b) DBU (1.5 equiv), 60 °C, 20 h; (c) HCl (concd), MTBE, rt, 13 h; (d) NaOH (50%), H_2O, reflux, then distillation; 58% for 4 steps, 92% ee; (e) D-tartaric acid, MeOH, reflux, then recrystallization; 90%, 99% ee; (f) NaOH (50%), H_2O, reflux, then distillation, 87%; (g) HCl (6 M), rt, 1 h, 99% , >99% ee.

The compounds described in this study were evaluated for inhibition of HCV subgenomic RNA replication using an Huh-7 derived cell line.[17] The metabolic stability of these compounds in human and rat liver microsomes was also evaluated to predict their in vivo metabolic stability.[18] Initial efforts were focused on evaluating the heteroarenes attached to the 2-position of the indole ring while retaining the *N*-isopropyl sulfonamide group on the heteroarenes (Table 1). Replacement of the phenyl ring (**2a**) with a pyridine ring (**3a**) not only improved potency against the replicon by more than threefold, but also improved selectivity with respect to cellular GAPDH. The isomeric 5-(indol-2-yl)pyridine **4** was 17-fold less potent than the 6-(indol-2-yl)pyridine **3a** in the replicon assay (EC_{50} = 560 vs 33 nM). Adding an additional nitrogen to the arene (leading to pyrimidine **5**) was also detrimental to activity compared to **3a**. Furthermore, 5-(indo-2-yl)pyrimidine **6**, the regioisomer of pyrimidine **5**, had diminished activity against the

replicon, indicating the precise requirement for the substitution pattern of the link between the indole ring and sulfonamide group. Even though all compounds described in Table 1 were substituted with an *N*-isopropyl sulfonamide group, comparison between compounds **3a** and **5** and the lead compound **2a** revealed that an arene modification could result in significant improvement in vitro metabolic stability.

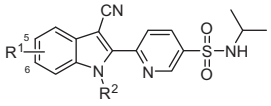
Maintaining the 6-(indol-2-yl)pyridine core structure in **3a**, we next examined variation of the substituents R^1 and R^2 attached to the indole ring (Table 2), based on our earlier studies[9] indicating that substituent modification on the indole could dramatically impact replicon activity. For example, we observed that 6-difluoromethoxy substitution on the indole ring, as compared to other functional groups, generally provided compounds with increased activity against the replicon. Therefore, we initially screened 6-difluoromethoxyindole analogs substituted with simple alkyl groups on the indole nitrogen. Potent inhibition of the replicon (EC_{50} <100 nM) was maintained regardless of the size of the alkyl chain. Furthermore, compounds **3b**–**3f** had a moderate reduction in metabolism as compared to the lead compound **3a**. When the 6-difluoromethoxy group in **3d** (EC_{50} = 12 nM) was replaced by a 6-cyclopropyl group in **7a** (EC_{50} = 21 nM), replicon potency was only slightly reduced. However, the metabolic stability of **7a** was improved compared to that of **3d**. Moving substituents from the 6-position of the indole to the 5-position (**3d** vs **7f**, **7a** vs **7h**, **3g** vs **7g**), typically resulted in a more than 10-fold decrease in activity. Compared to the difluoromethoxy substituted compounds **3d** and **7f**, the trifluoromethoxy analogs **3g** and **7g** have improved metabolic stability but at the expense of reduced replicon activity. Halogen substitution could be tolerated at the 5-position of the indole as evidenced by the 5-F (**7i**) and 5-Cl (**7j**) analogs which had EC_{50} values of 26 and 28 nM, respectively.

SAR studies of the R^1 substituent indicated that substitution with 6-difluoromethoxy and 6-cyclopropyl groups were preferred as they represent a good balance between activity and metabolic stability. We then explored the combination of these R^1 substituents with various sulfonamide groups (R^3), while maintaining the R_2 group as cyclobutyl, to study further improvements to potency and metabolic stability. Results from selected examples are shown in Table 3.

Introducing hydrophilic groups to the terminus of the isopropyl group (e.g., 1,3-dihydroxyisopropyl, **8d**) led to a significant loss of activity (EC_{50} = 940 nM) compared to compound **3d**. However, replacement of the isopropyl group in **3d** with the more lipophilic 1,3-difluoroisopropyl group (sulfonamide **8c**) led to a modest increase in activity (EC_{50} = 4 nM). The same level of potency observed for compound **8c** was also obtained with the 6-cyclopropyl analog **8o** (EC_{50} = 4 nM). Several mono-, di-, and trifluoroalkyl sulfonamides were then investigated. Among these fluoroalkyl substituted analogs, compounds **8h** and **8r**, containing the (*S*)-trifluoroisopropyl sulfonamide group (EC_{50} = 2 and 12 nM, respectively), also provided similar replicon activity compared to compounds **8c** and **8o**. However, the corresponding (*R*)-trifluoroisopropyl enantiomers, **8g** and **8q** were 30- and 10-fold less potent, respectively, than the corresponding (*S*)-enantiomers **8h** and **8r**, indicating a stereochemical bias towards activity. Removal of the methyl group (trifluoroethyl analog **8l**) led to a further sixfold drop in activity compared to that of the (*R*)-trifluoroisopropyl analog **8g**. Complete removal of the sulfonamide alkyl chain (primary sulfonamide **8n**) resulted in a further threefold decrease in activity (EC_{50} = 1300 nM).

Importantly, we also found that structural modifications to the sulfonamide moiety (R^3) have a pronounced effect on metabolic stability (Table 3). Based on our understanding of the metabolic pathway leading to **2a**, we hypothesized that introducing electron-withdrawing groups on the alkyl chain (e.g., fluorine) would suppress oxidative N-dealkylation. As expected, when the isopropyl group in **3d** was replaced by alkyl groups bearing strong electron-withdrawing substituents such as trifluoromethyl (**8g**, **8h**, **8i**, **8j**, **8k** and **8l**) and cyano (**8m**), the metabolic stability was improved. Sulfonamide substituents containing *tert*-butyl type groups, which do not possess an abstractable α-hydrogen on the carbon adjacent to the sulfonamide nitrogen atom, are typically more resistant to oxidative N-dealkylation. The *t*-butyl sulfonamide **8a** was metabolically stable. The most active analogs containing the 1,3-difluoroisopropyl (**8c** and **8o**) or (*S*)-trifluoroisopropyl sulfonamides (**8h**)

Table 2
Effect of R^1 and R^2 groups on activity and metabolic stability of selected compounds

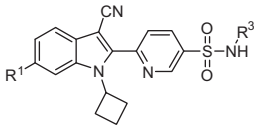


3a-3g, 7a-7j

Compd	R^1	R^2	HCV replicon 1b EC_{50}[a] (nM)	GAPDH IC_{50} (nM)[a]	HLM, CL_{int}[b] (mL/min/kg)
3a	6-$OCHF_2$	*c*-$PrCH_2$	33	>10,000	51
3b	6-$OCHF_2$	*n*-Pr	72	>10,000	40
3c	6-$OCHF_2$	*i*-Pr	61	>10,000	20
3d	6-$OCHF_2$	*c*-Bu	12	9200	35
3e	6-$OCHF_2$	*c*-Pentyl	15	>10,000	23
3f	6-$OCHF_2$	*c*-Hexyl	22	>10,000	16
3g	6-OCF_3	*c*-Bu	67	>10,000	6
7a	6-*c*-Pr	*c*-Bu	21	9600	12
7b	6-*c*-Bu	*c*-Bu	550	>10,000	24
7c	6-CHF_2	*c*-Bu	13	>10,000	48
7d	6-OCH_2CHF_2	*c*-Bu	1900	>10,000	54
7e	6-*i*-PrS	*c*-Bu	1310	>10,000	53
7f	5-$OCHF_2$	*c*-Bu	540	7100	48
7g	5-OCF_3	*c*-Bu	1100	>10,000	1
7h	5-*c*-Pr	*c*-Bu	580	>10,000	<1
7i	5-F	*c*-Bu	26	>10,000	121
7j	5-Cl	*c*-Bu	28	>10,000	50

[a] See Ref. 19 for assay conditions.
[b] See Ref. 20 for assay conditions.

Table 3
Effect of R^1 and R^3 groups on activity and metabolic stability



3d,7a, 8a-8r

Compd	R^1	R^3	HCV replicon 1b EC_{50}[a] (nM)	GAPDH IC_{50}[a] (nM)	HLM, CL_{int}[b] (mL/min/kg)
3d	$-OCHF_2$		12	9200	35
7a	*c*-Pr		21	9600	12
8a	$-OCHF_2$		27	9500	<1
8b	$-OCHF_2$		23	9000	104
8c	$-OCHF_2$		4	>10,000	10
8d	$-OCHF_2$		940	>10,000	<1
8e	$-OCHF_2$		520	5200	274
8f	$-OCHF_2$		2700	9500	38
8g	$-OCHF_2$		67	>10,000	<1
8h	$-OCHF_2$		2	9800	<1
8i	$-OCHF_2$		180	>10,000	<1
8j	$-OCHF_2$		30	>10,000	<1
8k	$-OCHF_2$		55	>10,000	<1
8l	$-OCHF_2$		420	>10,000	8
8m[c]	$-OCHF_2$		220	>10,000	<1
8n	$-OCHF_2$	H	1300	>10,000	<1
8o	*c*-Pr		4	>10,000	22
8p	*c*-Pr		74	>10,000	<1
8q	*c*-Pr		125	>10,000	15
8r	*c*-Pr		12	>10,000	49

[a] See Ref. 19 for assay conditions.
[b] See Ref. 20 for assay conditions.
[c] Racemic.

exhibited a good balance between activity against the replicon and metabolic stability.

Encouraged by the above results, we evaluated the pharmacokinetic properties of selected compounds in the rat after oral administration of a single 10 mg/kg dose in 0.4% hydroxypropyl methylcellulose (Table 4). The *N*-isopropyl sulfonamide analogs **3d** and **7a** had only moderate oral exposure (C_{max} and $AUC_{0-6\ h}$) in rat. By comparison, the 1,3-difluoroisopropyl sulfonamides **8c** and **8o** had a profound improvement in oral exposure. The oral exposure of these compounds correlated well with in vitro metabolism. Further, **8c** had moderate IV clearance of 13 mL/min/kg and oral bioavailability of 62% in the rat (Table 5).

Selected compounds including **8c** were tested against isolated HCV enzymes (HCV polymerase, protease, and helicase) and had no significant inhibition of any of these enzymes at concentrations up to 10,000 nM (data not shown). To investigate further the mechanism of action of these compounds, we selected for resistance by passaging HCV replicon cells in the presence of **8c** at

Table 4
Replicon activity, metabolic stability and rat PK of selected compounds

Compd	Replicon 1b[a] EC_{50} (nM)	Replicon 1b[a] EC_{90} (nM)	GAPDH IC_{50}[a] (nM)[a]	HLM, CL_{int}[b] (mL/min/kg)	RLM, CL_{int}[b] (mL/min/kg)	$AUC_{0-6\ h}$[c] (nM h)	T_{Max}[c] (h)	C_{Max}[c] (μg/mL)
3d	12	60	9200	35	99	579	2	0.053
7a	21	180	9600	12	15	964	2	0.087
8c	4	30	>10,000	10	2	3418	3	0.348
8o	4	40	>10,000	22	<1	6204	2	0.759

[a] See Ref. 19 for assay conditions.
[b] See Ref. 20 for assay conditions.
[c] Compounds were lyophilized from acetonitrile/water (1:1) and administered PO as a suspension at a dose of 10 mg/kg in 0.4% hydroxypropyl methylcellulose to male Sprague–Dawley rats ($n = 2$). Plasma samples were obtained from the rats for analysis at 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h post-dose.

Table 5
In vitro and in vivo Rat DMPK profile for **8c**[a]

C_{max} (PO) (μg/mL)	$AUC_{0-\infty}$ (nM h) PO / IV	$t_{1/2}$ (IV) h	CL (IV) (mL/min/kg)	V_{ss} (L/kg)	F (%)
0.673	15990/12,990	6.0	13	4.7	62

[a] Dosed PO as a suspension at 10 mg/kg in 0.4% hydroxypropyl methylcellulose; administered IV as a solution at a dose of 5 mg/kg in *N*-methyl pyrrolidinone/PEG300/propylene glycol (10:50:40) to male Sprague–Dawley rats.

2X, 20X, and 60X the replicon EC_{90} (60, 600, and 1800 nM, respectively). Resistant replicons were isolated and sequenced, revealing a number of mutations leading to amino acid substitutions in the HCV NS4B protein (data not shown). One of these, a phenylalanine to cysteine substitution at position 98 of NS4B (F98C), was engineered into the wild type replicon. The resulting replicon was sequenced to confirm the presence of the engineered mutation and was shown to be >70-fold less sensitive than the wild type replicon to **8c**. A leucine substitution at this amino acid position (F98L) has been reported to confer resistance to a series of imidazo[1,2-*a*]pyridine HCV inhibitors targeting NS4B.[18]

In summary, we have described the identification and development of a novel series of 6-(indol-2-yl)pyridine-3-sulfonamides that target the HCV NS4B. Preliminary optimization of this series furnished compounds with highly potent and selective activity against the HCV replicon. The sulfonamide group was found to be susceptible to metabolism and modification of both the arene and sulfonamide moieties resulted in the identification of compounds with improved oral exposure, leading to the discovery of **8c**, a highly potent analog with excellent pharmacokinetic properties in the rat. Further SAR analyses and mechanistic studies of this novel class of HCV inhibitors will be reported in a subsequent communication.

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19. EC_{50} values are the average of at least two independent determinations. Huh7 cells harboring a genotype **1b** HCV bicistronic replicon (Con1) were plated at 5000 cells/well in 96 well plates. Compounds were added to the plates with a final DMSO concentration of 0.5% and plates were incubated at 37 °C. Cells were harvested 3 days post dosing and replicon RNA, and GAPDH RNA as an endogenous control for selectivity, were quantified by real time RT-PCR.
20. Compounds were incubated at a concentration of 1 μM with pooled human or Sprague–Dawley liver microsomes (0.5 mg/mL protein concentration) for 0, 10, 20, 30 and 60 min, in the presence of 1 mM EDTA, 3 mM $MgCl_2$, 1.3 mM NADP, 3.3 mM D-glucose 6-phosphate and 1 unit/mL of glucose 6-phosphate dehydrogenase. Incubated samples were centrifuged at 2000 rpm and the supernatants were analyzed by LC/MS.