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6-(Azaindol-2-yl)pyridine-3-sulfonamides as potent and selective inhibitors targeting hepatitis C virus NS4B

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ABSTRACT

A Structure–activity relationship investigation of various 6-(azaindol-2-yl)pyridine-3-sulfonamides using the HCV replicon cell culture assay led to the identification of a potent series of 7-azaindoles that target the hepatitis C virus NS4B. Compound **2ac**, identified via further optimization of the series, has excellent potency against the HCV 1b replicon with an EC_{50} of 2 nM and a selectivity index of >5000 with respect to cellular GAPDH RNA. Compound **2ac** also has excellent oral plasma exposure levels in rats, dogs and monkeys and has a favorable liver to plasma distribution profile in rats.

Keywords:
HCV inhibitors
Replicon
6-(Azaindol-2-yl)pyridine-3-sulfonamides
Structure–activity relationship
NS4B

Hepatitis C virus (HCV) is an enveloped, single-stranded positive sense 9.4 kb RNA virus belonging to the Hepacivirus genus of the Flaviviridae family. HCV infects an estimated 150 million people worldwide and an estimated 3-4 million people in the United States.[1] Approximately 80% of HCV infections develop into chronic hepatitis that can ultimately lead to liver fibrosis, cirrhosis and hepatocellular carcinoma, and is the leading reason for liver transplantation in the United States.[2] In the U.S. alone, up to 10,000 deaths per year are attributed to hepatitis C related liver disease. In recent years, significant progress has been made in the area of HCV therapy. Three direct acting antiviral agents targeting the HCV protease; Incivek™ (telaprevir)[3], Victrelis™ (boceprevir)[4], and Olysio™ (simeprevir)[5], were granted FDA approval in the last few years. In 2013, the first drug targeting the viral RNA polymerase, Sovaldi™ (sofosbuvir)[6] was granted FDA approval. Harvoni®, a single tablet combination of sofosbuvir and ledipasvir was approved in 2014 for the treatment for patients with genotype 1 chronic hepatitis C. This all-oral, interferon-free therapy was shown to improve the sustained virologic response to >94% in clinical trials. Sofosbuvir has also been approved as part of the first all-oral dual therapy for patients infected with HCV genotype 2 or 3.[6-10] However, there is still a continued medical need in finding agents that act on novel HCV targets, so that in combination with the current or future standard of care, the emergence of resistance and the rebound of viremia after cessation of treatment can be curtailed.[11-13]

In addition to newer compounds targeting the NS3 protease and NS5B RNA polymerase, several compounds targeting other viral proteins, without an obvious enzymatic activity, e.g., NS5A, are in preclinical or clinical development.[14] The non-structural membrane-bound protein NS4B,[15] a 27-kDa integral membrane protein, plays an essential role in HCV replication and has recently emerged as a potential drug target for the treatment of chronic HCV infection.[16] Several novel chemotypes targeting HCV NS4B have been disclosed in recent publications.[17]

Previously,[17c, 17d] we reported the identification of a series of 6-(indol-2-yl)pyridine-3-sulfonamides as novel inhibitors of HCV RNA replication that target HCV NS4B. Compound **1**[17d] (Fig. 1) is highly potent and selective with an EC_{50} of 7 nM against the HCV 1b replicon and a selectivity index of 1300 with respect to its effect on levels of cellular GAPDH RNA. The combination of the 5-F and 6-$OCHF_2$ substituents in the indole phenyl ring of **1**, resulting from chemical optimization of the DMPK and safety profile, provided reduced metabolic oxidative arene oxide formation and a significant decrease in glutathione (GSH) adduct formation. In order to further expand the chemical space and explore novel scaffolds with favorable pharmaceutical properties, we envisioned replacing the indole core with various azaindole moieties resulting in compounds **2** (Fig. 1). The presence of additional nitrogen atoms in the azaindole ring would lead to reduced electronic density thus rendering it less prone to oxidative metabolism. Further, the presence of additional nitrogen atoms in the arene moiety, could, depending on the substitution pattern, eliminate the potential for glutathione adduct formation. Herein, we report on the identification of several novel azaindoles that target HCV NS4B.

We first surveyed various azaindoles by walking the aza N around the six-membered ring. Based on our previous experience in the indole series whereby the combination of *N*-cyclobutyl and (*S*)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide groups generally provided compounds with the highest potency,[17c] we prepared analogs in several azaindole series containing these key moieties in combination with selected substituents on the pyridine ring of the azaindole core. The general synthetic strategies utilized for the syntheses of **2** as outlined in Scheme 1 have been described previously.[17d]

Commercially available 5-methoxy-, 6-methyl-, and 6-chloro-4-azaindoles (**3a–c**) were alkylated at the N-1 position with cyclobutyl bromide in the presence of Cs_2CO_3 in DMF, followed by cyanation at C-3 with chlorosulfonyl isocyanate in a mixture of DMF and acetonitrile providing 1-cyclobutyl-3-cyano-4-azaindoles **5a–c** (Scheme 2). The transformations of **5a–c** to **2a–c** were realized through Stille coupling chemistry as shown in path C in Scheme 1.

The 6-methyl-5-azaindole **3d** was prepared starting from readily available 2,5-dimethylpyridine **9** (Scheme 3). Compound **9** was oxidized to the *N*-oxide **10** by *m*-CPBA, followed by nitration with fuming nitric acid to provide nitropyridine *N*-oxide **11**. Batcho-Leimgruber indole synthesis furnished **12** in 3 steps with a 52% yield. Iron reduction of **12** in acetic acid provided the 6-methyl-5-azaindole intermediate **3d** which was elaborated to **5d** via N-alkylation and cyanation. Compound **5d** was converted to **2d** using the Stille coupling strategy described above (Scheme 1). Utilizing similar synthetic methodology, compound **2e** was prepared from commercially available 6-chloro-5-azaindole.

6-Azaindole analogs **2f** and **2g** were prepared from commercially available 5-chloro- and 5-methyl-6-azaindole, respectively, according to the chemistry described in Scheme 1 via the Stille coupling strategy.

5,7-Diazaindole intermediates **5h** and **5i** were prepared from commercially available 5-bromo-2,4-dichloropyrimidine **13** (Scheme 4). Reaction of **13** with cyclobutylamine provided **14**, which was converted to **15** in methanol in the presence of K_2CO_3. Compound **15** then underwent Sonagashira coupling with trimethylsilylacetylene, followed by desilylation with K_2CO_3 in methanol to furnish **16**. CuI promoted cyclization of **16** to **4h** was accomplished by heating a DMF solution in the presence of Barton base at 140 °C. Demethylation of **4h** with HBr in acetic acid at 100 °C gave a phenol intermediate, which was treated with sodium difluoroacetate in DMF in the presence of Cs_2CO_3 to yield **4i**. Cyanation of **4h** and **4i** with chlorosulfonyl isocyanate in a mixture of DMF and acetonitrile (1:1) provided **5h** and **5i**, respectively. Compounds **2h** and **2i** were then prepared via the Stille coupling strategy utilizing **5h** and **5i** (Scheme 1).

Utilizing the Stille coupling methodology described in Scheme 1, several 7-azaindole analogs were synthesized from commercially available azaindole starting materials. The 5-methoxy-7-azaindole analogs **2j**, **2r**, and **2s** were prepared from readily available 5-methoxy-7-azaindole. The 5-chloro-7-azaindole analogs **2l**, **2x**, and **2y** were prepared starting from 5-chloro-7-azaindole. Compounds **2k**, **2m**, **2n**, **2o**, **2ab**, **2ac**, and **2ad** were prepared from 6-methoxy-7-azaindole, 6-chloro-7-azaindole, 5-methyl-7-azaindole, 6-methyl-7-azaindole, 5-fluoro-7-azaindole, 5-trifluoromethyl-7-azaindole and 7-azaindole, respectively.

Additional 7-azaindole analogs were prepared from the versatile 5-bromo-7-azaindole **17** (Scheme 5). Compound **17** was converted to 5-bromo-1-cyclobutyl-3-cyano-7-azaindole **18** in two steps (Scheme 5). Palladium catalyzed boronate formation followed by Oxone® oxidation provided the 5-hydoxy intermediate **19**. Alkylation of **19** with alkylating reagents furnished 5-alkoxy intermediates **5t–v**. Freonation of **19** gave the 5-difluoromethoxy intermediate **5w**. Negishi coupling of **18** with ethyl- or cyclopropylzinc halides, prepared *in situ* from the corresponding Grignard reagents and $ZnCl_2$, in the presence of catalytic $PdCl_2$(dppf) provided **5z** and **5aa**. The target compounds **2t–v** and **2aa** were synthesized through a Suzuki coupling strategy; compound **2w** was prepared via a Stille coupling strategy (Scheme 1).

These compounds were then evaluated for inhibition of HCV subgenomic RNA replication using an Huh-7 derived cell line.[18] Results for the initial set of analogs indicated that the most potent compounds are derived from the 7-azaindole series (Table 1), particularly those compounds substituted at the 5-position of the 7-azaindole ring. Compounds **2j**, **2l**, and **2n** exhibit replicon EC_{50} values <30 nM and have >330 fold selectivity with respect to their effect on levels of cellular GAPDH RNA. The corresponding 6-substituted 7-azaindole analogs showed decreased activity against the replicon. The activity of the 6-OMe (**2k**) and 6-Cl (**2m**) analogs against the replicon decreased 35- and 41-fold, respectively, compared to the activity of the corresponding 5-subsituted analogs **2j** and **2l**. The 6-Me analog **2o** showed a ~7-fold decrease in activity against the replicon compared to the 5-Me analog **2n**. The 4-azaindole sulfonamides **2a–c** showed moderate to good replicon activity with the 6-Me (**2b**) and 6-Cl (**2c**) analogs exhibiting EC_{50} values of 119 and 94 nM, respectively. The 5-OMe analog (**2a**) was less potent (EC_{50} = 230 nM). Direct comparison of the 4-azaindole and 7-azaindole series shows the significant effect imparted by the azaindole substituent on activity against the replicon. 5-OMe substitution favors the 7-azaindole **2j** over the 4-azaindole **2a** by ~8-fold. In contrast, 6-Cl substitution favors the 4-azaindole **2c** over the 7-azaindole **2m** by ~7-fold. 6-Me substitution results in comparable activity against the replicon between the 4- (**2b**) and 7-azaindole (**2o**) analogs with EC_{50} values of 119 and 87 nM, respectively.

A comparison of methyl and chloro substituted 5-aza and 6-azaindole analogs revealed that the activity of the 5-azaindole analogs against the replicon is about 2–7 fold more potent than that of the 6-azaindole analogs. As an example, the 5-Me-6-azaindole analog **2g** inhibits HCV RNA replication with an EC_{50} of 1,400 nM, compared to the 6-Me-5-azaindole analog **2d**, which has an EC_{50} of 230 nM. The 5,7-diazaindoles **2h** and **2i** showed modest activity against the replicon, with EC_{50} values of 240 and 970 nM, respectively.

Having determined from our initial survey that the 5-substituted 7-azaindole series provided analogs with the most potent activity, we further evaluated this series. Reinvestigation of the azaindole 2-position revealed SAR that was consistent with the indole series reported previously.[17c] Replacement of the 2'-pyridyl ring with either a phenyl or 2'-pyrimidyl group was disadvantageous for potency against the replicon (Table 2). Compared to the 2'-pyridinyl compound **2n**, the replicon EC_{50} values for the 2-phenyl compound **2p** and the 2'-pyrimidyl compound **2q** decreased more than 6-fold and 12-fold, respectively.

Next, we conducted a comprehensive SAR study around the 7-azaindole core by varying R^1 (C-5 position) and R^2 (N-1 position) and the results are summarized in Table 3.

We initially varied R^1 substitution while maintaining R^2 = *c*-Bu. In general, small lipophilic R^1 groups are favored, a finding consistent with that reported for indole series.[17c,d] Trifluoromethyl substitution at C-5 (**2ac**) provided the most potent compound (EC_{50} = 2 nM). Small alkyl groups are also well tolerated at the 5-position. In addition to the 5-Me analog **2n** (EC_{50} = 13 nM), the 5-Et (**2z**), and 5-*c*-Pr (**2aa**) analogs were also very potent with replicon EC_{50} values of 15 and 40 nM, respectively. Halogens such as F and Cl were also well-tolerated. The replicon EC_{50} values for the 5-F analog **2ab** and 5-Cl analog **2l** were 46 and 17 nM, respectively. In comparison,

the unsubstituted analog **2ad** was less active (EC_{50} = 217 nM). In addition to the 5-OMe analog **2j** (EC_{50} = 27 nM), several additional 5-alkoxy analogs were evaluated. Increasing the size of the alkoxy group to 5-OEt (**2t**), 5-O-*n*-Pr (**2u**), and 5-O-*i*-Pr (**2v**) resulted in decreased potency with replicon EC_{50} values of 200, 2150 and 7600 nM, respectively. The 5-difluoromethoxy analog **2w**, however, demonstrated good activity (EC_{50} = 50 nM).

The effect of the R^2 group on activity was evaluated in two series (5-OMe and 5-Cl). In the 5-OMe series, the N-*c*-butyl analog **2j** was 5-fold more potent than the N-*c*-pentyl analog **2s** and 10-fold more potent than the N-*c*-PrMe analog **2r**. In the 5-chloro series, the N-*c*-butyl analog **2l** was nearly 2-fold more potent than either the N-*c*-PrMe **2y** or the N-*c*-pentyl analog **2x**. The SAR trend for the R^2 group in the 7-azaindole series is consistent with the observations in the indole series in which the *c*-Bu group provided the most potent activity.[17c]

Compounds with replicon EC_{50} values less than 100 nM were evaluated in a pharmacokinetic screen for plasma drug levels ($AUC_{0\text{-}6h}$) in the rat after oral administration of a single 10 mg/kg dose in 0.4% hydroxypropyl methylcellulose (Table 3). In general, good to excellent drug exposure levels were observed. Among the nine compounds evaluated, the plasma $AUC_{0\text{-}6h}$ ranged from 2389 nM·h to 10,164 nM·h. For all compounds evaluated, the plasma concentration multiples over the in vitro replicon EC_{50} were more than 20-fold at 6 h. The potent 5-CF_3 analog (**2ac**, EC_{50} = 2 nM) had excellent exposure in the rat ($AUC_{0\text{-}6h}$ = 5833 nM·h) and plasma concentration multiples of >500 over the replicon EC_{50} at 6 h. These compounds also showed very favorable drug distribution between liver and plasma. Among the compounds evaluated, liver to plasma ratios at 6 h after a 10 mg/kg oral dose ranged from 6–44-fold. This is important as the primary target organ for HCV is the liver.

To further assess the potential utility of compounds from this series, **2j**, **2l**, **2n**, **2y**, and **2ac** were evaluated for plasma drug levels in beagle dogs and cynomolgus monkeys after oral administration at doses of 2 and 3 mg/kg, respectively (Table 4). Data for compound **1** are also included in Table 4 for comparison. At these doses, oral exposure ($AUC_{0\text{-}24h}$) was higher in dogs than in monkeys. The 5-CF_3 analog (**2ac**) had the highest exposure in both dogs and monkeys ($AUC_{0\text{-}24h}$ = 8162 and 5440 nM·h, respectively). In contrast, the 5-Me (**2n**) and 5-Cl (**2y**) analogs had lower overall exposures in both species and were 9- and 7-fold lower in dog than in monkeys, respectively. Plasma concentration multiples over the in vitro replicon EC_{50} at 8 h were also determined in these species. The 5-CF_3 analog (**2ac**) had the highest plasma exposure multiples in dogs and monkeys (125- and 105-fold above the replicon EC_{50} at 8h, respectively.) Compared to lead compound **1** (EC_{50} = 7 nM), **2ac** (EC_{50} = 2 nM) showed improved replicon potency and higher plasma exposure multiples over the in vitro replicon EC_{50} in rats, dogs and monkeys.

At concentrations up to 10,000 nM, Compound **2j**, a representative example from the series, did not inhibit the HCV NS3 protease or NS5B polymerase in vitro (data not shown). Selection of resistant HCV genotype 1b replicons was performed by serial passage in the presence of **2j** at concentrations of 1X, 10X, and 30X the in vitro replicon EC_{90} (90, 900, and 2700 nM, respectively).[17h] Resistant replicons were isolated, sequenced and found to have mutations in the coding sequence in the HCV NS4B, in particular resulting in the amino acid substitutions F98L, F98C, and V105M. The F98C substitution was identified as the predominant mutation selected at 30X the replicon EC_{90} (found in 15 of 16 clones sequenced). Replicons engineered to contain each of these mutations alone were found to be 25-fold (F98C) and 17-fold (V105M) resistant to **2j**. The same amino acid substitutions in NS4B were previously found to confer 60–70-fold resistance to 6-(indol-2-yl)pyridine-3-sulfonamides.[17c, 17d]

In summary, we have identified several novel azaindole sulfonamide chemotypes that potently and selectively inhibit the replication of the HCV 1b replicon. SAR investigations of various azaindole cores revealed that 5-substituted 7-azaindole sulfonamides provide compounds with the most potent activity against the replicon and that these compounds target HCV NS4B. These compounds are highly selective with no apparent effect on cellular GAPDH RNA levels. Several compounds have demonstrated excellent drug exposure levels in rats with favorable liver to plasma ratios. These efforts led to the identification of **2ac**, a highly potent analog (EC_{50} = 2 nM) with excellent oral exposure in rats, dogs and monkeys.

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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.

Figure 1: Structures of novel HCV inhibitors

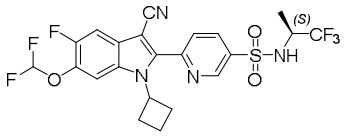
Scheme 1. Reagents and conditions: (a) R_1X, Cs_2CO_3, DMF, rt to 100 °C; (b) chlorosulfonyl isocyanate, CH_3CN/DMF, –20 °C to 50 °C; (c) $B(O^iPr)_3$, LDA, THF, –78 °C; (d) **8**, $PdCl_2$(dppf), aqueous K_2CO_3, CH_3CN, 60 °C; (e) $B(O^iPr)_3$, LDA, THF, –78 °C then **8**, $PdCl_2$(dppf), aqueous K_2CO_3, DMF, 80 °C; (f) LDA, Bu_3SnI, THF, –78 °C to 0 °C; (g) **8**, $Pd(PPh_3)_4$ CuI, dioxane, 100 °C.

Scheme 2. Reagents and conditions: (a) *c*-BuBr, Cs_2CO_3, DMF, 90 °C, 48 h; (g) chlorosulfonyl isocyanate, CH_3CN/DMF, –20 °C to rt.

Scheme 3. Reagents and conditions: (a) *m*-CPBA, $CHCl_3$, 60 °C, overnight, 38%; (b) fuming HNO_3 (90%), conc. H_2SO_4, 100 °C, 2 h, 91%; (c) DMF·DMA, DMF, 95 °C, 1 h; (d) 10% Pd/C, EtOH, rt, 3 h; (e) Fe, AcOH, 5 h, 52% over three steps; (f) *c*-BuBr, Cs_2CO_3, DMF, 90 °C, 48 h; (g) chlorosulfonyl isocyanate, CH_3CN/DMF, –20 °C to rt.

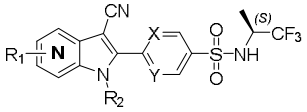
Scheme 4. Reagents and conditions: (a) *c*-$BuNH_2$, DIEA, THF, 0 °C to rt; (b) K_2CO_3, MeOH, 60 °C, 8 h, 100% over two steps; (c) trimethylsilylacetylene, $PdCl_2$(dppf), CuI, Et_3N, THF, 70 °C, 24 h; (d) K_2CO_3, MeOH, rt, 5 h, 79% over two steps; (e) CuI, Barton base, DMF, 140 °C, 3 h, 82%; (f) HBr, AcOH, 100 °C, overnight, 77%; (g) ClF_2CCO_2Na, DMF, Cs_2CO_3, 75 °C, 2 h, 24%; (h) chlorosulfonyl isocyanate, CH_3CN/DMF (1:1), 30–58%.

Scheme 5. Reagents and conditions: (a) *c*-BuBr, Cs_2CO_3, DMF, 60 °C; (b) chlorosulfonyl isocyanate, CH_3CN/DMF (1:1), –20 °C, 80% over two steps; (c) bis(pinacolato)diborane, $PdCl_2$(dppf), KOAc, dioxane, 80 °C, 3h, then Oxone®; (d) RMgBr, $ZnCl_2$, THF, $PdCl_2$(dppf), –78 to 50 °C; (e) alkyl iodide or ClF_2CCO_2Na, DMF, Cs_2CO_3, 50 to 100 °C.



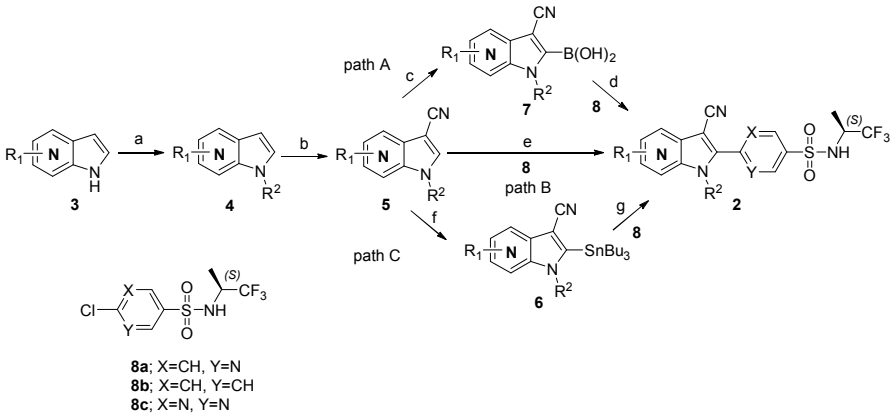
1

EC_{50} (1b) = 7 nM
6-(indol-2-yl)pyridine-3-sulfonamide

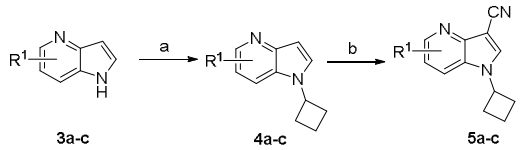


2

Fig. 1

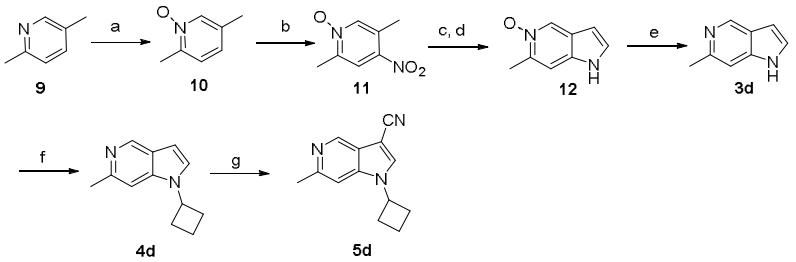


Scheme 1

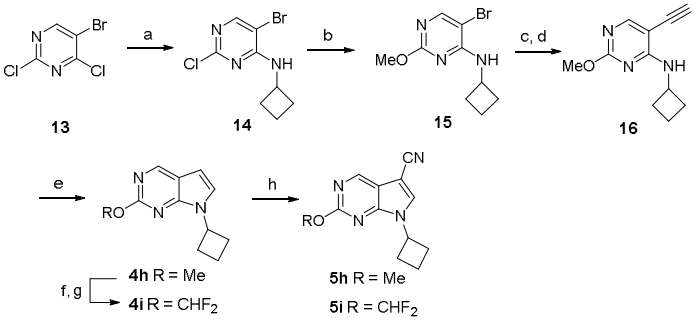


3a-c 4a-c 5a-c

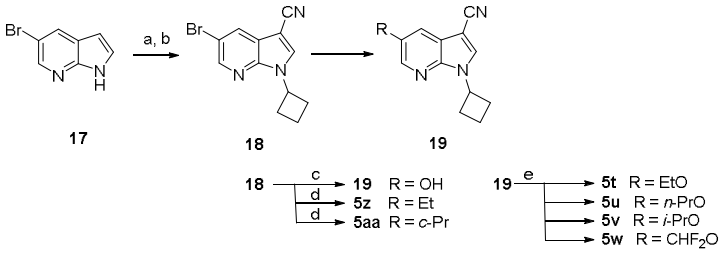
Scheme 2



Scheme 3

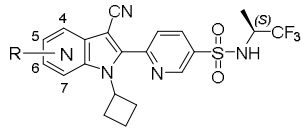


Scheme 4



Scheme 5

Table 1. Activity of selected 6-(azaindol-2-yl)pyridine-3-sulfonamides

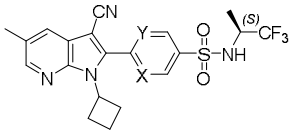


2

Compd	R	Azaindole	HCV replicon 1b EC_{50}[a] (nM)	GAPDH IC_{50}[a] (nM)
2a	5-OMe	4-	230	8900
2b	6-Me	4-	119	>10000
2c	6-Cl	4-	94	2500
2d	6-Me	5-	230	>10000
2e	6-Cl	5-	260	1310
2f	5-Cl	6-	530	>10000
2g	5-Me	6-	1400	>10000
2h	6-OMe	5,7-di	240	>10000
2i	6-$OCHF_2$	5,7-di	970	>10000
2j	5-OMe	7-	27	>9000
2k	6-OMe	7-	960	9700
2l	5-Cl	7-	17	>10000
2m	6-Cl	7-	700	6300
2n	5-Me	7-	13	>10000
2o	6-Me	7-	87	>10000

[a] See Ref. 19 for assay conditions.

Table 2. Effect of various arene sulfonamides on replicon replication

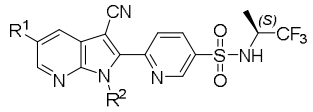


2

Compd	X, Y	HCV replicon 1b EC_{50}[a] (nM)	GAPDH IC_{50}[a] (μM)
2n	N, CH	13	>10000
2p	CH, CH	87	>10000
2q	N, N	160	>10000

[a] See Ref. 19 for assay conditions.

Table 3. Replicon inhibition and rat PK of selected 6-(7'-azaindol-2-yl)pyridine-3-sulfonamides



2

Compd	R^1	R^2	HCV replicon 1b EC_{50}[a] (nM)	GAPDH IC_{50}[a] (nM)	Rat PK (PO)[b] $AUC_{0\text{-}6h}$ (nM·h)	Plasma (6 h) / EC_{50}[d]	Liver / plasma[e] (6 h)
2j	MeO	*c*-Bu	27	>9000	7606	30	14
2r	MeO	*c*-$PrCH_2$	300	7900	n.d.[c]	n.d.[c]	n.d.[c]
2s	MeO	*c*-Pent	150	>10000	n.d.[c]	n.d.[c]	n.d.[c]
2t	EtO	*c*-Bu	200	>10000	n.d.[c]	n.d.[c]	n.d.[c]
2u	*n*-PrO	*c*-Bu	2150	>10000	n.d.[c]	n.d.[c]	n.d.[c]
2v	*i*-PrO	*c*-Bu	7600	>10000	n.d.	n.d.[c]	n.d.[c]
2w	F_2HCO	*c*-Bu	50	>10000	9365	36	7
2l	Cl	*c*-Bu	17	>10000	2389	22	23
2x	Cl	*c*- $PrCH_2$	39	>10000	n.d.[c]	n.d.[c]	n.d.[c]
2y	Cl	*c*- Pent	27	>10000	6844	40	19
2n	Me	*c*-Bu	13	>10000	6925	55	9
2z	Et	*c*-Bu	15	>5160	2628	27	13
2aa	*c*-Pr	*c*-Bu	40	>10000	10164	32	11
2ab	F	*c*-Bu	46	>10000	8779	23	6
2ac	CF_3	*c*-Bu	2	>10000	5833	503	44
2ad	H	*c*-Bu	217	>10000	n.d.[c]	n.d.[c]	n.d.[c]

[a] See Ref. 19 for assay conditions.

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

[c] Not determined.

[d] Ratio of the plasma concentration measured at 6 h post-dose to the replicon EC_{50} value.

[e] Ratio of the liver concentration to the plasma concentration measured at 6 h post-dose.

Table 4. Pharmacokinetic profiles of selected compounds

Compd	HCV replicon 1b EC_{50}[a] (nM)	Rat PK (PO)[b] $AUC_{0\text{-}6h}$ (nM·h)	Dog PK (PO, 2 mpk)[c] $AUC_{0\text{-}24h}$ (nM·h)	Monkey PK (PO, 3 mpk)[d] $AUC_{0\text{-}24h}$ (nM·h)	Plasma concentration (6 h or 8 h) / EC_{50}		
					Rat[e] (6 h)	Dog[f] (8 h)	Monkey[f] (8 h)
1	7	13412	6858	7568	277	33	46
2j	27	7606	4177	2277	30	6	4
2l	17	2389	4081	1035	22	12	2
2n	13	6925	5670	641	52	14	1
2y	27	6844	2067	290	40	2	0.3
2ac	2	5833	8162	5440	506	125	105

[a] See Ref. 19 for assay conditions.

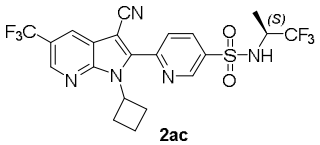
[b] Compounds were lyophilized from acetonitrile/water (1:1) and administered as a suspension in 0.4% hydroxypropyl methylcellulose to male Sprague Dawley rats (n = 2) PO at a dose of 10 mg/kg. Plasma samples were obtained from the rats for LC-MS/MS analysis at 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h post-dose.

[c] Compounds were lyophilized from acetonitrile / water (1:1) and administered as a suspension in 0.4% hydroxypropyl methylcellulose to male beagle dogs (n = 2) PO at a dose of 2 mg/kg. Plasma samples were obtained from the dogs for LC-MS/MS analysis at 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h post-dose.

[d] Compounds were lyophilized from acetonitrile/water (1:1) and administered as a suspension in 0.4% hydroxypropyl methylcellulose to male cynomolgus monkeys (n = 2) PO at a dose of 3 mg/kg. Plasma samples were obtained from the cynomolgus monkeys for LC-MS/MS analysis at 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h post-dose.

[e] Ratio of the plasma concentration measured at 6 h post-dose to the replicon EC_{50} value.

[f] Ratio of the plasma concentration measured at 8 h post-dose to the replicon EC_{50} value.



2ac

EC_{50} (1b) = 2 nM

6-(7-azaindol-2-yl)pyridine-3-sulfonamide

Graphical abstract