Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Structure−Activity Relationship (SAR) Optimization of 6-(Indol-2-yl)pyridine-3-sulfonamides: Identification of Potent, Selective, and Orally Bioavailable Small Molecules Targeting Hepatitis C (HCV) NS4B

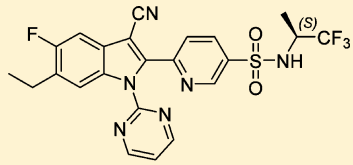
Nanjing Zhang,[†,*] Xiaoyan Zhang,[†] Jin Zhu,[†] Anthony Turpoff,[†] Guangming Chen,[†] Christie Morrill,[†] Song Huang,[†] William Lennox,[†] Ramesh Kakarla,[†] Ronggang Liu,[†] Chunshi Li,[†] Hongyu Ren,[†] Neil Almstead,[†] Srikanth Venkatraman,[‡] F. George Njoroge,[‡] Zhengxian Gu,[†] Valerie Clausen,[†] Jason Graci,[†] Stephen P. Jung,[†] Yingcong Zheng,[†] Joseph M. Colacino,[†] Fred Lahser,[‡] Josephine Sheedy,[†] Anna Mollin,[†] Marla Weetall,[†] Amin Nomeir,[‡] and Gary M. Karp[†]

[†]PTC Therapeutics, Inc., 100 Corporate Court, South Plainfield, New Jersey 07080, United States

[‡]Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, United States

Supporting Information

ABSTRACT: A novel, potent, and orally bioavailable inhibitor of hepatitis C RNA replication targeting NS4B, compound **4t** (PTC725), has been identified through chemical optimization of the 6-(indol-2-yl)pyridine-3-sulfonamide **2** to improve DMPK and safety properties. The focus of the SAR investigations has been to identify the optimal combination of substituents at the indole N-1, C-5, and C-6 positions and the sulfonamide group to limit the potential for in vivo oxidative metabolism and to achieve an acceptable pharmacokinetic profile. Compound **4t** has excellent potency against the HCV 1b replicon, with an EC_{50} = 2 nM and a selectivity index of >5000 with respect to cellular GAPDH. Compound **4t** has an overall favorable pharmacokinetic profile with oral bioavailability values of 62%, 78%, and 18% in rats, dogs, and monkeys, respectively, as well as favorable tissue distribution properties with a liver to plasma exposure ratio of 25 in rats.



4t
EC_{50} (1b) = 2 nM

INTRODUCTION

Hepatitis C virus (HCV) infects an estimated 150 million people worldwide, with about 4 million afflicted in the United States.[1] HCV infections typically progress to a chronic state, often resulting in fibrosis, cirrhosis, and hepatocellular carcinoma, and is the leading cause for liver transplantation in the U.S.[2] There are at least six HCV genotypes, of which genotype 1 is the most prevalent in the U.S. and has been particularly difficult to treat.[3] The new standard of care for patients infected with genotype 1a or 1b is a combination of the recently approved HCV protease inhibitor telaprevir or boceprevir with pegylated interferon and the antiviral agent ribavirin, increasing the sustained virologic response (SVR) to about 70%.[4] However, the high mutation rate of HCV and the rapid emergence of drug resistance necessitate the continued discovery and development of drugs with novel mechanisms of action.[5]

The HCV genome is a positive sense single-stranded RNA of approximately 9.6 kb that encodes a polyprotein precursor which is processed to individual structural (C, E1, E2, and p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins by host and viral proteases.[6] The recent development of direct-acting antiviral agents[7] has been mainly focused on the viral proteins NS3,[8] NS5A,[9] and the RNA-dependent RNA polymerase NS5B.[10] The nonstructural protein NS4B,[11] a 27 kDa integral membrane protein which plays an essential role in HCV replication, however, has remained relatively unexploited as a target for chemotherapeutic intervention.[12] Recently, imidazopyridines targeting HCV NS4B have been disclosed.[13]

We recently reported on two novel series of inhibitors of HCV RNA replication, the *N*-(4′-(indol-2-yl)phenyl)-sulfonamides[14] exemplified by **1** and the 6-(indol-2-yl)-pyridine-3-sulfonamides[15] exemplified by **2** (Figure 1). Optimization in the 6-(indol-2-yl)pyridine-3-sulfonamide series led to highly potent and selective inhibitors of HCV RNA replication that target HCV NS4B, of which compound **2** showed highly potent activity (EC_{50} = 4 nM) against the HCV 1b replicon[16] as well as excellent pharmacokinetic properties in rats. However, safety evaluation in rats identified two potential liabilities: extensive *N*-dealkylation of the sulfonamide moiety, presumably generating 1,3-difluoroacetone,[17] and significant metabolism of the indole ring, leading to the formation of several glutathione (GSH) adducts. GSH adducts are indicative of the formation of reactive intermediates which might be associated with toxicity.[18] Furthermore, generation of these

Special Issue: HCV Therapies

Received: October 18, 2013

   dx.doi.org/10.1021/jm401621g | *J. Med. Chem.* XXXX, XXX, XXX−XXX

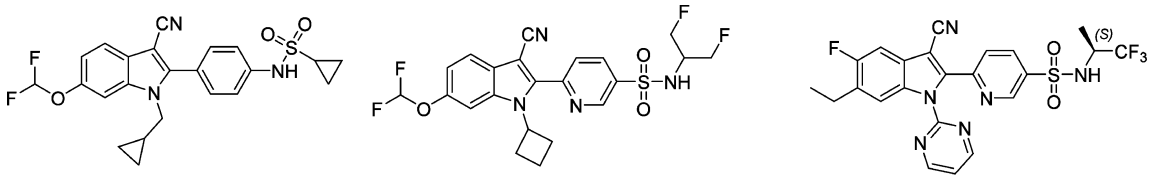
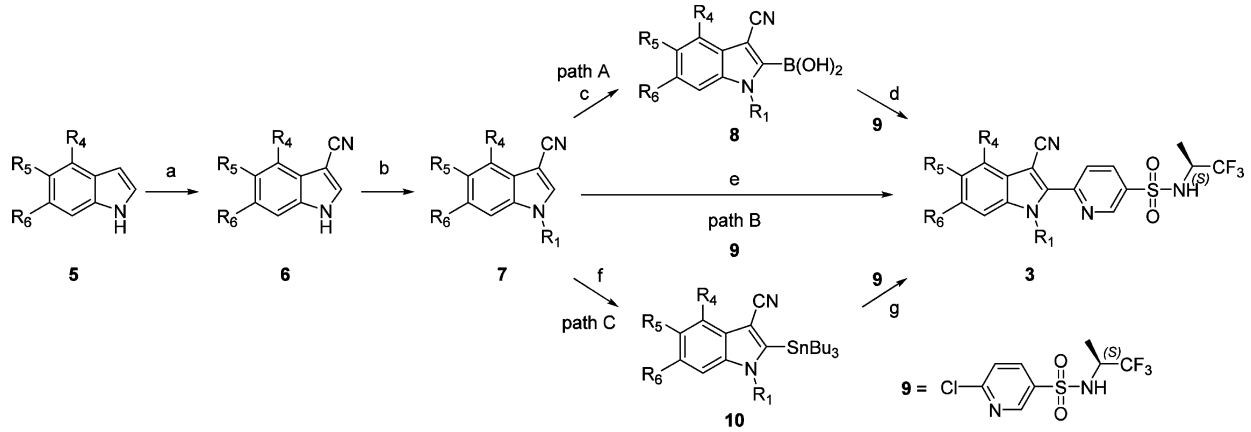


Figure 1. Novel HCV inhibitors and their activity against the HCV 1b replicon.

Scheme 1[a]



[a]Conditions: (a) chlorosulfonyl isocyanate, DMF, −78 to −10 °C; (b) R_1X, Cs_2CO_3, DMF, 100 °C; (c) $B(Oi\text{-}Pr)_3$, LDA, THF, −78 °C; (d) **9**, $PdCl_2dppf$, aq K_2CO_3, acetonitrile, 60 °C; (e) $B(Oi\text{-}Pr)_3$, LDA, THF, −78 °C then **9**, $PdCl_2dppf$, aq K_2CO_3, DMF, 80 °C; (f) LDA, Bu_3SnI, THF, −78 to 0 °C; (g) **9**, $Pd(PPh_3)_4$, CuI, DMF, 100 °C.

adducts may result in further GSH depletion in HCV patients, who are already depleted in GSH as a result of their disease and are thus especially susceptible to other drug-mediated liver injuries.[19]

Herein, we describe our work to optimize the in vivo DMPK and safety profile of this novel class of 6-(indol-2-yl)pyridine-3-sulfonamides, which led to the identification of **4t** (PTC725),[20] a potent, selective, and orally bioavailable compound targeting HCV NS4B currently under preclinical development.

CHEMISTRY

The general synthesis of compounds **3** is outlined in Scheme 1. The starting indoles **5** with appropriate substitutions at the 4-, 5-, and 6- positions were either commercially available or prepared via multistep synthesis from commercial starting materials (see Supporting Information). Cyanation at the indole 3-position with chlorosulfonyl isocyanate provided intermediates **6**, which were alkylated with appropriate alkyl halides at the indole N-1 position to give compounds **7**. The principal strategy to prepare the final targets **3** was to couple **7** with 2-chloropyridin-5-yl sulfonamide **9** prepared from 2-chloropyridin-5-yl sulfonyl chloride and (*S*)-1,1,1-trifluoromethylisopropylamine. Depending on the substituents on the indole ring, three coupling paths were developed. Path A utilizes a boronic acid intermediate **8**, which was subjected to Suzuki coupling with **9** to furnish the target compounds **3**. In cases where the boronic acid intermediate **8** was not sufficiently stable or could not be easily isolated, a one-pot process (path B) was then used in which compounds **7** were converted into a boronate complex intermediate and, without isolation, subsequently subjected to Suzuki coupling with sulfonamide **9**. When the Suzuki coupling strategies in both paths A and B were unsuccessful, a Stille coupling strategy was utilized as depicted in path C in which a stannane intermediate **10** was isolated and used in subsequent Stille coupling reactions.

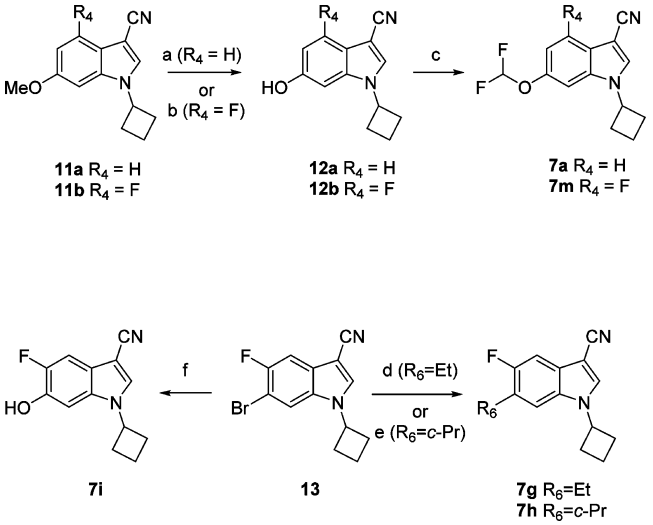
Several intermediates **7** were prepared via functional group transformation. As depicted in Scheme 2, 6-methoxyindoles **11a** and **11b**, readily prepared from the corresponding indoles **5** through the sequence in Scheme 1, were demethylated to give the 6-hydroxyindoles **12a** and **12b** and further transformed to **7a** and **7m** by freonation with chlorodifluoromethane. The 6-bromoindole **13** was readily converted to the 6-ethylindole **7g** and 6-cyclopropylindole **7h** through Negishi or Suzuki coupling reactions. Palladium catalyzed borylation of **13** followed by oxidation with potassium peroxymonosulfate provided the 6-hydroxyindole **7i**.

Additional final compounds **3** were also accessed through direct derivatization and functional group transformations (Scheme 3). Compound **3a**, prepared by the sequence shown in Scheme 1, was brominated at the indole 5-position to give **14**. Bromine−lithium exchange of **14** with *n*-BuLi followed by treatment with triisopropyl borate and subsequent oxidation with potassium peroxymonosulfate provided the 5-OH analogue **3c**. Negishi coupling of **14** with the corresponding zinc reagent provided the 5-Me analogue **3d** and 5-Et analogue **3e**.

Starting from the 6-OH analogue **3i**, methylation at the 6-OH group provided the 6-OMe analogue **3k**. The 6-triflate compound **15** was obtained by treatment of **3i** with triflic anhydride, which was subsequently converted to the 6-SMe analogue **3l** through a palladium catalyzed coupling reaction with sodium thiomethoxide.

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2[a]



[a]Conditions: (a) BBr_3, CH_2Cl_2, −20 °C; (b) EtSH, $AlCl_3$, CH_2Cl_2, 0 °C; (c) CHF_2Cl, NaOH, *i*-PrOH, 75 °C; (d) Et_2Zn, $PdCl_2dppf$, dioxane, 100 °C; (e) *c*-$PrB(OH)_2$, $Pd_2(dba)_3$, *t*-Bu_3PHBF_4, KF, THF, 30 °C; (f) $PdCl_2dppf$, B_2Pin_2, KOAc, dioxane, 80 °C then potassium peroxymonosulfate, acetone.

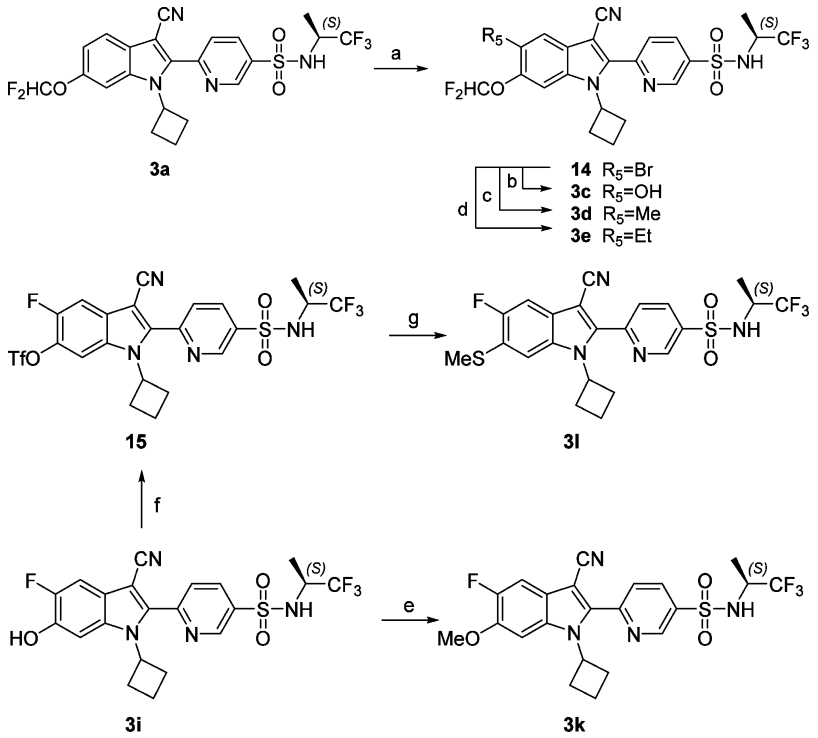
The general synthesis of *N*-aryl analogues **4** is outlined in Scheme 4. Protection of indoles **5** with Boc_2O provided intermediates **16**. LDA treatment of a mixture of **16** and triisopropyl borate at −78 °C followed by acidic aqueous workup furnished boronic acids **17**, which was coupled with the sulfonamide **9** to provide the 6-(indol-2-yl)pyridine-3-sulfonamides **18**. Base induced cleavage of the Boc group followed by cyanation of **19** at the indole 3-position with chlorosulfonyl isocyanate provided intermediates **20**. Copper catalyzed *N*-arylation of intermediates **20** at the indole N-1 position with various commercially available aryl halides provided the final targets **4**.

All synthesized compounds were evaluated for activity against the genotype 1b HCV replicon and selectivity with respect to cellular GAPDH mRNA in replicon-containing Huh-7 cells, as described previously.[15] Briefly, quantitative RT-PCR was performed to quantify the amount of intracellular HCV RNA and the concentration of a compound inhibiting HCV RNA replication by 50% (EC_{50}) is indicated. In parallel, endogenous GAPDH mRNA was quantified as a control to determine selectivity. Pharmacokinetic properties of highly potent compounds were evaluated in rats to assess the potential of the compounds for development as orally administered anti-HCV drugs. Compounds were lyophilized from acetonitrile/water (1:1) and administered PO to male Sprague−Dawley rats at a dose of 10 mg/kg as a suspension of 0.4% hydroxypropyl methylcellulose. Both the area under the plasma concentration−time curve from time zero to 6 h after administration (AUC_{0-6h}) and plasma concentration multiples at 6 h over the replicon EC_{50} were evaluated. Compounds with the best combination of potency and oral exposure in rats were selected for further evaluation.

RESULTS AND DISCUSSION

SAR and Pharmacokinetic Studies. The goal of our optimization effort was to identify compounds with activity against the HCV replicon comparable to that of the lead

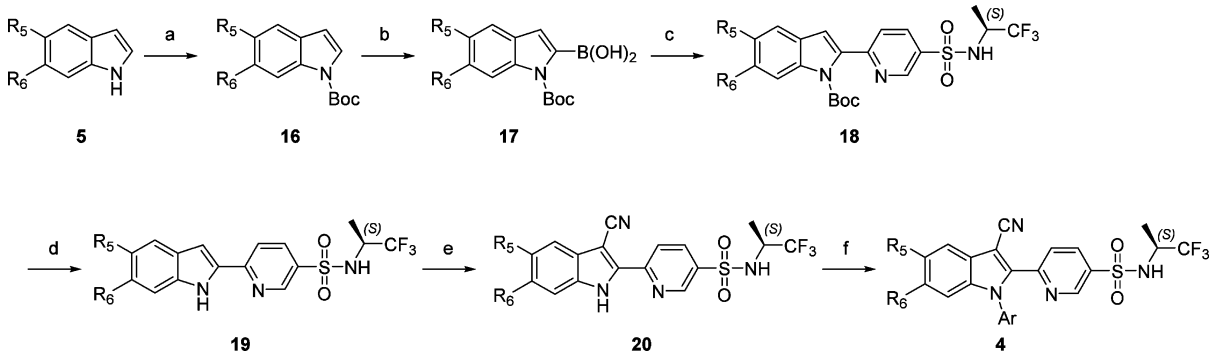
Scheme 3[a]



[a]Conditions: (a) Br_2, HOAc, 95 °C; (b) MeLi, *n*-BuLi, $B(Oi\text{-}Pr)_3$, THF, then potassium peroxymonosulfate, −78 °C to rt; (c) MeZnCl, $PdCl_2dppf$, THF, 40 °C; (d) Et_2Zn, $PdCl_2dppf$, dioxane, 100 °C; (e) MeI, K_2CO_3, 40 °C; (f) Tf_2O, pyridine, CH_2Cl_2, 0 °C to rt; (g) NaSMe, $Pd_2(dba)_3$, xantphos, dioxane, 100 °C.

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4[a]



[a]Conditions: (a) Boc_2O, DMAP, CH_2Cl_2; (b) $B(Oi\text{-}Pr)_3$, LDA, THF, −78 °C to rt; (c) **9**, $Pd_2(dba)_3$, *t*-Bu_3PHBF_4, KF, THF, 60 °C; (d) pyrrolidine, CH_3CN, 50 °C; (e) chlorosulfonyl isocyanate, DMF/CH_3CN (1:1), 0 °C; (f) ArX, CuI, K_2CO_3, DMF, 110 °C.

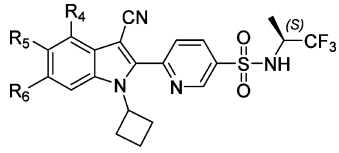
compound **2** but having improved in vivo DMPK and safety profiles. During the identification of **2**, we demonstrated that the metabolic stability of the 6-(indol-2-yl)pyridine-3-sulfonamide series could be significantly improved by incorporating fluorine(s) or other electron-withdrawing groups into the amine moiety of the sulfonamide or by utilizing amines lacking an α-proton such as *tert*-butyl type amines.[15] In particular, the *N*-(*S*)-1,1,1-trifluoroisopropyl sulfonamide group in **3a** provided similar activity against the replicon but with improved microsome stability compared to that of the *N*-1,3-difluoroisopropyl sulfonamide **2**[15] and, very importantly, lacking known liabilities associated with the potential *N*-dealkylated metabolite(s)[21] derived from **2**. Thus it became the group of choice for further optimization.

In the process of metabolite characterization of [^{3}H]-**2**, we identified several GSH adducts formed through metabolic activation of the indole in vivo, presumably through transient epoxide formation on the electron rich indole phenyl ring (data not shown). However, these GSH conjugates could not be identified in vitro in either rat hepatocytes or human or rat liver microsomes or S9 fortified with NADPH and GSH, indicating in this particular case there was no predictive in vitro system to evaluate metabolism via the GSH pathway. Consequently, our strategy was to introduce substituents to the indole phenyl ring that would eliminate two adjacent unsubstituted carbons with the goal of reducing metabolic oxidative arene oxide formation and thus block or reduce GSH adduct formation of these transiently produced reactive intermediates.

Starting from compound **3a**, we evaluated a number of substituents on the indole phenyl ring, particularly at the 4- and 5- positions in conjunction with 6-substituents, which eliminate the presence of two contiguous unsubstituted carbons on the indole phenyl ring. First, a variety of substituents of different size and lipophilicity were investigated at the indole 5-position in combination with 6-$OCHF_2$ substitution. As shown in Table 1, the size of the substituent has a significant impact on replicon activity. The 5-F substitution was well tolerated, with compound **3b** providing the best activity (EC_{50} = 7 nM), slightly less potent than the parent compound **3a**. Other small substituents, e.g., 5-Me (**3d**) and 5-OH (**3c**), led to a 54- and 32-fold decrease in activity, respectively. The larger 5-Et substituent (**3e**) resulted in a >600-fold decrease in activity compared to that of **3a**.

Having identified the F- group as the optimal substituent at the indole 5-position, we next re-evaluated substitutions at the indole 6-position. Consistent with our previous findings,[15]

Table 1. SAR of Substitution on the Indole Core



3

compd	R_4	R_5	R_6	replicon 1b EC_{50} (nM)	GAPDH IC_{50} (nM)
3a	H	H	CHF_2O	2	9600
3b	H	F	CHF_2O	7	9100
3c	H	OH	CHF_2O	63	>10000
3d	H	Me	CHF_2O	108	10000
3e	H	Et	CHF_2O	1400	>10000
3f	H	F	Me	7	>10000
3g	H	F	Et	4	>10000
3h	H	F	*c*-Pr	28	>10000
3i	H	F	HO	410	>10000
3j	H	F	Cl	460	>10000
3k	H	F	MeO	74	>10000
3l	H	F	SMe	35	>10000
3m	F	H	CHF_2O	18	>10000
3n	F	H	Me	153	>10000

small lipophilic groups were preferred. In addition to the 6-$OCHF_2$ (**3b**), small alkyl groups such as 6-Me (**3f**) and 6-Et (**3g**) showed single-digit nanomolar activity, with **3g** as the most potent (EC_{50} = 4 nM). Decreased activity was observed for *c*-Pr (**3h**), OMe (**3k**), and SMe (**3l**), with replicon EC_{50}s in the range of 28−74 nM. A further decrease in activity was observed for the 6-Cl (**3j**) and 6-OH (**3i**) analogues, with EC_{50}s of 460 and 410 nM, respectively. The 6-OMe (**3k**), though of similar size but differing in electronic properties and lipophilicity from the $OCHF_2$ group (**3b**), decreased activity by more than 10-fold.

Compounds substituted at the indole C-4 and C-6 positions also do not present two contiguous H-bearing carbon atoms and thus would also be expected to have a reduced potential for GSH adduct formation. At the indole 4-position, selected fluoro substitution was also evaluated. In combination with a 6-$OCHF_2$ group, compound **3m** was 2-fold less active (EC_{50} = 18 nM) than **3b** and 9-fold less active than **3a**. However, when combined with a 6-Me group (**3n**), the activity was reduced by more than 20-fold (**3n** vs **3f**).

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

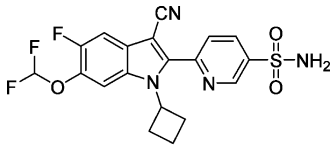
Table 2. Pharmacokinetic Properties of Selected Compounds

compd	replicon 1b EC_{50} (nM)	rat PK (PO)[a] AUC_{0-6h} (nM·h)	plasma fold over EC_{50} at 6 h	liver/plasma[b] ratio
3b	7	13400	277	11.4
3f	7	1100	24	29.1
3g	4	300	18	30.6

[a]The compounds were lyophilized from acetonitrile/water (1:1) and dosed PO as an oral suspension at 10 mg/kg in 0.4% hydroxypropyl methylcellulose to male Sprague–Dawley rats (n = 2). [b]Determined at 6 h.

The potent 5,6-disubstituted analogues **3b**, **3f**, and **3g** were assessed for oral exposure in rats (Table 2). After oral administration at 10 mg/kg, compound **3b** bearing 5-F, 6-$OCHF_2$ substitution showed excellent plasma exposure (13400 nM·h) and high plasma concentration multiples over the replicon EC_{50} (277-fold) at 6 h. This is far superior to both compound **3f** with 5-F, 6-Me substitution (1100 nM·h), and **3g** with 5-F, 6-Et substitution (300 nM·h) which have plasma exposure ratios at 6 h < 25-fold their respective EC_{50}s. In addition, because the liver is the primary target organ of HCV, it is desirable for HCV drugs to partition efficiently into the liver. In this regard, these compounds had very favorable liver to plasma ratios (10–30-fold).

Compound **3b** was further evaluated based on its excellent activity against the replicon and high oral exposure in rats. In vivo metabolite characterization of [^{3}H]-**3b** was evaluated in bile duct cannulated rats to assess the potential for in vivo GSH conjugation. After a 5 mg/kg dose, a single GSH adduct accounting for 2.5% of the dose was observed. This compared favorably with the metabolism of [^{3}H]-**2**, in which several GSH adducts accounting for 17–20% of the dose were observed, indicating that the approach to reduce the level of GSH adducts by incorporating 5-F substitution worked as expected. The metabolite **3o** derived from the oxidative N-dealkylation of the sulfonamide group in **3b** (Figure 2) was present in significant quantities in the bile. In plasma, **3o** was the major metabolite. Importantly, no known toxicities have been reported for 1,1,1-trifluoroacetone,[21] the presumed metabolite formed upon oxidative N-dealkylation of **3b**.



3o
EC_{50} = 330 nM
6-(indol-2-yl)pyridine-3-sulfonamide

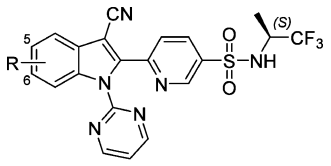
Figure 2. The major plasma metabolite of **3b** in rats.

Comprehensive PK studies were also conducted in rats, dogs, and monkeys. Compound **3b** had excellent exposure in all three species, with AUC_{0-inf} values of 24790–59900 nM·h when dosed orally at 10 mg/kg in rats and 5 mg/kg in dogs and monkeys. Compound **3b** had oral bioavailability values of 49%, 91%, and 35% in rats, dogs, and monkeys, respectively. However, the IV clearance of **3b** is low in rats (3.0 mL/min/kg) and monkeys (2.2 mL/min/kg) and particularly low in dogs (0.3 mL/min/kg). The IV half-lives for rats, monkeys, and dogs are 11, 22, and 149 h, respectively. The low clearance and long half-life in large animal species was of some concern.

We turned our attention to the subset of compounds with 5-F, 6-alkyl substitution such as **3f** and **3g**, which demonstrated excellent replicon activity and whereby the 6-alkyl group could potentially function as an additional metabolic site to increase clearance. However, as shown in Table 2, the oral exposures of **3f** and **3g** were modest and required further improvement.

In the course of optimization of compound **3a**, one direction we have taken was to replace the indole N-alkyl groups with heteroaryl groups in order to expand the chemical space and improve potency and DMPK properties. Starting from compound **3a**, we replaced the indole N-cyclobutyl substituent with a pyrimidine ring (**4a**) whereby the potency and selectivity were retained and the oral exposure in rats (AUC_{0-6h} = 20120 nM·h) was significantly improved (Table 3). Moreover, when the 6-$OCHF_2$ group was replaced by a 6-methyl substituent, the N-2′-pyrimidine analogue **4b** retained excellent potency (EC_{50} = 3 nM). Thus we decided to pursue analogues containing both 5-F, 6-alkyl substitution and N-aryl moieties. We were gratified to find that compound **4d**, obtained from introduction of a F group to the 5-position of **4b**, showed only slightly reduced activity (EC_{50} = 6 nM) but had excellent oral exposure

Table 3. SAR of R Substitution on Replicon Activity and Rat PK



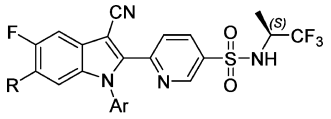
4

compd	R	replicon 1b EC_{50} (nM)	GAPDH IC_{50} (nM)	Rat PK (PO)[a] AUC_{0-6h} (nM·h)
4a	6-$OCHF_2$	7	>10000	20120
4b	6-Me	3	>10000	nd[b]
4c	5-F, 6-$OCHF_2$	16	>10000	3809
4d	5-F, 6-Me	6	>10000	8910

[a]Compounds were lyophilized from acetonitrile/water (1:1) and administered to male Sprague–Dawley rats (n = 2) PO at a dose of 10 mg/kg as a suspension in 0.4% hydroxypropyl methylcellulose. [b]Not determined.

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX–XXX

Table 4. Replicon Activity and Rat PK of 5-F-6-Alkyl-*N*-arylindoles



4

Compd	R	Ar	Replicon 1b[a] EC_{50} (nM)	Rat PK (PO)[b] $AUC_{0\text{-}6h}$ (nM·h)	Plasma (6h)/ EC_{50}[b]
4d	Me		6	8910	213
4e	Me		18	4516	49
4f	Me		11	6387[c]	65[c]
4g	Me		11	2596[c]	24[c]
4h	Me		9	885	9
4i	Me		13	3259[c]	22[c]
4j	Me		3	1763[c]	96[c]
4k	Me		20	10270	35
4l	Me		7	6273	38
4m	Me		2	480[c]	29[c]
4n	Me		32	n.d.[d]	n.d.[d]
4o	Me		4	4084[c]	187[c]
4p	Me		8	3454	42
4q	Me		25	n.d.[d]	n.d.[d]
4r	Me		3	8537	194
4s	Me		400	n.d.[d]	n.d.[d]
4t	Et		2	8164	342
4u	Et		5	3851	69
4v	Et		9	6383	47
4w	Et		4	345[c]	14[c]
4x	Et		2	2745[c]	347[c]
4y	Et		3	8689	136
4z	Et		23	7239	44
4aa	Et		13	1785	12
4bb	*c*-Pr		6	2044	75
4cc	*c*-Pr		16	n.d.[d]	n.d.[d]
4dd	*c*-Pr		12	1701	20

[a]All compounds had GAPDH mRNA IC_{50}s >10000 nM, the highest concentration tested. [b]Compounds were lyophilized from acetonitrile/water (1:1) and administered to male Sprague–Dawley rats (n = 2) PO at a dose of 10 mg/kg as a suspension in 0.4% hydroxypropyl methylcellulose. [c]Compounds were lyophilized from acetonitrile/water (1:1) and dosed PO at 10 mg/kg as a suspension in 0.5% hydroxypropyl methylcellulose and 0.1% Tween 80 to male Sprague–Dawley rats (n = 3). [d]Not determined.

(AUC_{0-6h} = 8910 μM·h). The PK of the *N*-2′-pyrimidinyl analogue **4d** compared very favorably with the *N*-cyclobutyl analogue **3f**. Interestingly, when a F group was introduced to the 5-position of compound **4a**, the oral exposure of the resulting analogue **4c** decreased significantly while the potency was slightly reduced as well.

We then undertook a comprehensive investigation of *N*-aryl analogues in the 5-F, 6-alkyl series. Starting from the readily

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX–XXX

available building block 5-F, 6-Me indole, we initially explored a series of 5-F, 6-Me indole analogues substituted with a wide variety of heteroaryl rings attached to the indole nitrogen (**4d**–**4s**). As shown in Table 4, replacement of the 2′-pyrimidinyl ring with a variety of heteroaryl rings provided a series of compounds (**4e**–**4m**) with activity against the replicon at low nanomolar concentrations ($EC_{50} \leq 20$ nM). The investigated heteroaryls include electron deficient rings such as pyridines (**4e**–**4g**), pyrazines (**4h**), and pyridazines (**4i**), and electron rich moieties such as thiazoles (**4j**–**4l**) and thiophenes (**4m**), indicating that a diverse set of *N*-heteroaryls can be tolerated and can potentially be used as "handles" to modulate PK or other properties. The activity of the *N*-pyridyl derivatives **4e**–**4g** was not affected by the attachment position of the pyridine ring to the indole nitrogen. The 2′- (**4e**), 3′- (**4f**), and 4′-pyridyl (**4g**) analogues had similar potency, with EC_{50} values ranging from 11 to 18 nM. A greater difference in activity was observed for compounds substituted with a thiazole ring. Activity decreased in the order of 2′-(**4j**), 5′-(**4l**), and 4′-thiazolyl (**4k**), with replicon EC_{50} values of 3, 7, and 20 nM, respectively. The 4′-thiazole analogue **4k** had the highest oral exposure (AUC_{0-6h} = 10270 nM·h). However, when the plasma concentration at 6 h was normalized to potency, the 2′-thiazole **4j** had the highest concentration multiples over replicon EC_{50} (96-fold). The 2′-thienyl analogue **4m** was very potent, with a replicon EC_{50} = 2 nM but had only modest oral exposure. Overall, the 2′-pyrimidinyl analogue **4d** provided an optimal balance of replicon potency (EC_{50} = 6 nM) and oral exposure (AUC_{0-6h} = 8910 nM·h), with plasma concentration multiples over the replicon EC_{50} of more than 200-fold at 6 h.

Next, we examined the effect of substituents on the heteroaryl rings attached to the indole N-1. Introducing a fluorine atom to the 2′-pyrimidinyl ring at the distal (5′) position (**4o**) was well tolerated (EC_{50} = 4 nM). Replacing the 5′-F with 5′-Me (**4n**) resulted in an 8-fold reduction in activity. Introducing a fluorine atom to the 2′-pyridyl ring at the distal 5′- position (**4p**) led to a slight improvement in activity (EC_{50} = 8 nM) compared to that of the parent **4e**. Replacing the 5′-F substituent on the 2′-pyridyl ring with 5′-Me (**4q**) reduced activity (EC_{50} = 25 nM). Addition of a fluorine atom at the 3′-position of the 2′-pyridyl ring (**4r**) conferred improved activity (EC_{50} = 3 nM) compared to that of the parent 2′-pyridyl analogue **4e** (EC_{50} = 18 nM), while a larger substituent, e.g., 3′-Me (**4s**), reduced the activity significantly (EC_{50} = 400 nM), suggesting that the torsion angle between the indole and the *N*-aryl ring may play an important role in replicon activity. In addition to conferring potent activity against the replicon, the small, electronegative fluorine atom in **4o**, **4p**, and **4r** also imparts good oral exposures, with AUC values >3000 nM·h and high plasma concentration multiples, particularly for **4o** and **4r** with 187- and 194-fold over their respective replicon EC_{50}s at 6 h.

Having investigated various indole *N*-heteroaryl rings in the 5-fluoro-6-methyl series, we next examined a limited set of 5-fluoro-6-ethyl indole analogues with selected heteroaryl groups attached to the indole nitrogen (**4t**–**4aa**). Replacement of the 5-F, 6-Me substitution (**4b**) with 5-F, 6-Et (**4t**) afforded one of the most potent analogues in this series. Compound **4t** (EC_{50} = 2 nM) is 3-fold more potent than **4b** while maintaining similar oral exposure in rats (AUC_{0-6h} = 8164 nM·h). At 6 h, the plasma concentration is more than 340-fold over the replicon EC_{50}. Replacement of the 2′-pyrimidinyl ring in **4t** with other heteroaryl rings (**4u**–**4x**) generally provided analogues with equal or improved activity compared to that of the corresponding 5-fluoro-6-methyl analogues. Introducing a fluorine atom at the 5′-positon of the 2′-pyrimidine (**4y**) retained the replicon activity compared to **4t**, whereas substituting with a methyl group at the same position (**4z**) resulted in decreased activity, a similar SAR trend as observed in the 5-fluoro-6-methyl indole series. The 5′-F-2′-pyridinyl indole **4aa** showed decreased activity compared to that of the parent 2′-pyridyl compound **4u** in contrast to the analogous modification in the 5-fluoro-6-methyl series (**4p** and **4e**), where introduction of the fluorine led to a small increase in activity.

A limited exploration of the 5-fluoro-6-*c*-propyl indoles (**4bb**–**4dd**) was also conducted. Although the 5-F, 6-*c*-Pr indole **4bb** containing a 2′-pyrimidine moiety was equipotent to the corresponding 5-F, 6-Me analogue **4d**, the oral exposure was reduced significantly. The 2′-pyridine analogues **4cc** and **4dd** had reduced activity compared to that of **4bb**.

Several compounds with an optimal balance between activity against the replicon (EC_{50} < 10 nM) and good oral exposure in rats (AUC_{0-6h} > 3000 nM·h) with plasma concentration multiples over the replicon EC_{50} at 6 h of more than 50-fold were identified (Table 4). These compounds were selected for further pharmaceutical profiling, including evaluation of their potential for drug–drug interactions as these drugs will likely be coadministered with other medications. Such evaluation included characterizing the compounds for their ability to inhibit or induce the major drug metabolizing enzymes. The inhibition of cytochrome P450 enzymes (CYPs) was evaluated by determining the activity of the compounds against CYPs 2D6, 3A4, and 2C9 in human liver microsomes. The results revealed essentially no inhibition, with IC_{50} values >10 μM, the highest concentration tested (Table 5).

Table 5. Profile of Selected Compounds

compd	CYP inhibition (3A4, 2D6, 2C9) IC_{50} (μM)	CYP 3A4 PXR (% activation, 10 μM)	human hepatocyte CYP3A4 induction[a] (% activation)	
			activity	mRNA expression
4d	>10	36	7% (3 μM)	39.8% (3 μM)
			3% (10 μM)	41.5% (10 μM)
4o	>10	96	nd[b]	nd[b]
4r	>10	59	nd[b]	nd[b]
4t	>10	37	6% (3 μM)	9.8% (3 μM)
			9% (5 μM)	15.4% (5 μM)
4u	>10	54	nd[b]	nd[b]
4y	>10	71	nd[b]	nd[b]

[a]Percent induction relative to rifampicin, ref 22. [b]Not determined.

The CYP induction was initially evaluated at 10 μM in a PXR (pregnane X receptor) reporter gene assay. PXR is a nuclear receptor that when activated increases the transcription and therefore the synthesis and activity of several drug metabolizing enzymes and transporters including CYP 3A4, an important phase I oxidative enzyme responsible for the metabolism of many drugs.[22] Therefore, compounds with significant PXR activation have the potential to induce drug–drug interactions. Compounds that showed a strong signal (>40% of positive control rifampicin) were deprioritized. Compounds with a borderline signal (30–40%) were further evaluated for induction in cryopreserved cultured human hepatocytes in which the activity and mRNA expression levels of CYP3A4

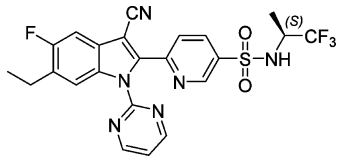
dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX–XXX

were evaluated following a 3-day incubation with test compound at several concentrations.

Four of the six selected compounds were found to be transactivators of PXR. The fluoro-substituted *N*-2′-pyrimidinyl indole analogues **4o** and **4y** and the *N*-(2′-pyridinyl) indoles **4r** and **4u** showed significant PXR induction (>50% induction relative to rifampicin), while the PXR induction for the 2′-pyrimidinyl analogues **4d** and **4t** showed a borderline signal (35−40%). In a follow-up study using cultured cryopreserved human hepatocytes, both compounds **4d** and **4t** showed <10% induction of CYP 3A4 at the highest concentration tested. However, compound **4d** was found to increase CYP3A4 mRNA expression levels by ∼40% with respect to rifampicin, whereas compound **4t** showed much lower activation (∼10%) at the same tested concentration (3 μM). Therefore, compound **4t** was advanced for further evaluation.

Profile of the Lead Compound. The pharmacokinetic profiles of compound **4t** in rats, dogs, and monkeys were evaluated and are summarized in Table 6.[20] When dosed orally in rats at 10 mg/kg, **4t** had an area under the concentration−time curve from time zero extrapolated to infinity ($AUC_{0-\infty}$) of 20000 nM·h, with an oral bioavailability of 62% and achieved a plasma concentration of 325-fold over the replicon EC_{50} at 8 h. When dosed orally to dogs at 5 mg/kg, **4t** had an $AUC_{0-\infty}$ of 29000 nM·h, with an oral bioavailability of 78% and a plasma concentration of 460-fold over the replicon EC_{50} at 8 h. At an oral dose of 5 mg/kg in monkeys, compound **4t** had moderate exposure with an $AUC_{0-\infty}$ of 3100 nM·h and 18% bioavailability. Compound **4t** possesses a moderate terminal half-life (IV) in all species, ranging from 7.5 to 9 h. The systemic clearance (CL) is higher in rats and monkeys (∼10−11 mL/min/kg) than in dogs (4.6 mL/min/kg), which compares favorably to the earlier lead **3b**.

Table 6. Pharmacokinetic Profiles of 4t Following Single Dose Administration to Rats, Dogs, and Monkeys[a]



4t

parameters	rat[b]	dog[c]	monkey[d]
$AUC_{0-\infty}$ (PO, nM·h)	20000	29000	3100
C_{max} (PO, nM)	2400	4000	540
CL (IV) (mL/min/kg)	10.1	4.6	11.0
$T_{1/2}$ (IV) (h)	7.5	8.9	8.0
Vdss, (L/kg)	3.9	2.1	3.8
F (%)	62	78	18
plasma/(8 h)/EC_{50}	325	460	50
liver/plasma/brain ratio	25/1/0.22	nd[e]	nd[e]

[a]Selected data taken from ref 20. [b]Compound **4t** was lyophilized from acetonitrile/water (1/1) and administered PO to fed male Sprague−Dawley rats (n = 3) at a dose of 10 mg/kg as a suspension in 0.4% hydroxypropyl methylcellulose. It was administered IV at a dose of 6 mg/kg as a solution in *N*-methyl pyrrolidinone/PEG300/propylene glycol (10:40:50). [c]Administered to fed beagle dogs (n = 3) PO at a dose of 5 mg/kg as a suspension in 0.4% hydroxypropyl methylcellulose. It was administered IV at a dose of 2.5 mg/kg as a solution in *N*-methyl pyrrolidinone/PEG300/propylene glycol (5:50:45). [d]Administered to fed cynomolgous monkeys (n = 3) PO at a dose of 5 mg/kg as a suspension in 0.4% hydroxypropyl methylcellulose. It was administered IV at a dose of 2.5 mg/kg as a solution in *N*-methyl pyrrolidinone/PEG300/propylene glycol (5:40:55). [e]Not determined.

Because HCV primarily targets the liver, it is desirable that drugs have distribution properties that favor exposure in that organ. We measured the concentration of **4t** in plasma, liver, and brain at multiple time points over 48 h following a 10 mg/kg oral dose in rats. The mean drug concentration in liver was 25-fold higher than in plasma (based on AUC values). For comparison, the concentration in brain was ∼5-fold lower than in plasma, indicating limited brain uptake thereby reducing the risk of central nervous system toxicity.

Metabolite formation of [^{3}H]-**4t** was evaluated in bile duct cannulated rats, dogs, and monkeys to assess the metabolic profile and potential for in vivo GSH conjugation. Compound **4t** was metabolized in rats, dogs, and monkeys. Similar to **3b**, a single GSH conjugate accounting for 2% of the dose was observed in rat bile. No GSH adducts were identified in dog or monkey bile. Three primary metabolites of **4t** were detected in rat plasma (Figure 3): **4ee**, the metabolite derived from the oxidative *N*-dealkylation of the sulfonamide group, **4ff**, the metabolite derived from hydroxylation of the ethyl group, and **4gg**, the metabolite derived from both *N*-dealkylation and hydroxylation. The structures of these metabolites were confirmed by independent synthesis. The observation of two hydroxylated metabolites **4ff** and **4gg** validated our approach of using the 6-alkyl group as a metabolic handle for increased clearance. Among the three metabolites, only the hydroxylated metabolite **4ff** retains modest replicon activity. The metabolites **4ee** and **4gg**, resulting from sulfonamide *N*-dealkylation, had dramatically reduced replicon activity. These metabolites were rapidly cleared from plasma and were found in varying amounts in rats, dogs, and monkeys. In addition, compounds **4ee**−**4gg** were observed in rat, dog, and human cryopreserved hepatocytes, indicating that no human specific metabolites of **4t** were identified.

De novo selection of replicon resistance against **4t** resulted in the identification of mutations encoding three amino acid substitutions in the HCV nonstructural protein 4B (NS4B) region, F98L, F98C, and V105M.[20] The NS4B substitutions were separately engineered into the Con1 HCV replicon and each conferred ≥60-fold resistance to **4t**. A mutation encoding the amino acid substitution H94R in NS4B was also identified, conferring 16-fold resistance. Previous results from our group and others identified similar mutations in NS4B using other anti-HCV compounds,[13,20] indicating that **4t** targets HCV NS4B.

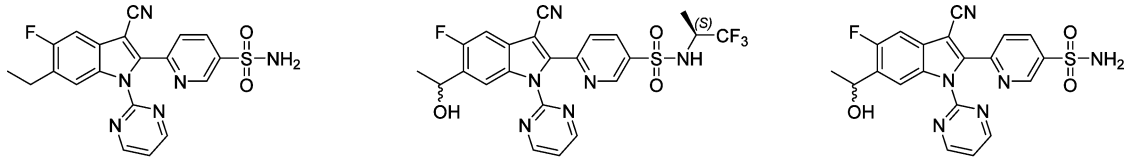
In addition to the activity against the genotype 1b replicon, **4t** also inhibited the replication of the genotype 1a H77S full-length genome with an EC_{50} of 7 nM.[20,23] Further, **4d** had only modest activity against genotype 2a infectious JFH-1 virus in Huh-7 cells with an EC_{50} ∼2200 nM. However, JFH-1 naturally contains a leucine at position 98 of NS4B that we identified as conferring resistance to **4t** in genotype 1b. Thus, genotype 2 would be expected to be less susceptible to this compound.[20]

With a favorable overall profile, **4t** was evaluated in a non-GLP 14-day rat safety study and found to be well-tolerated at doses up to 2000 mg/kg/day.

CONCLUSION

Starting from lead compound **2**, we conducted extensive SAR development to improve the DMPK properties and safety

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX



4ee
EC_{50} (1b) > 10,000 nM

4ff
EC_{50} (1b) = 68 nM

4gg
EC_{50} (1b) > 10,000 nM

Figure 3. Primary metabolites of **4t** in rats and their activity against the HCV 1b replicon.

profile and identified a novel series of *N*-heteroaryl-6-(indol-2-yl)pyridine-3-sulfonamides. Chemical optimization was focused primarily on identifying the optimal heteroaryl moieties at the indole N-1 position and defining the optimal substitution pattern on the indole benzenoid ring to limit the potential for in vivo oxidative metabolism including the formation of GSH adducts. The SAR investigation culminated in identifying compounds with an optimal balance of replicon potency and favorable PK properties including acceptable drug clearance. Several compounds were evaluated for their potential to induce drug−drug interactions, and compound **4t** was selected for further evaluation. Compound **4t** exhibits potent activity in the cell-based HCV 1b replicon (EC_{50} = 2 nM), with more than 5000-fold selectivity with respect to cellular GAPDH mRNA. Compound **4t** has a favorable pharmacokinetic profile in rats, dogs, and monkeys and has favorable tissue distribution, with a liver to plasma exposure ratio of 25 in rats. It has an excellent safety profile, being well tolerated at doses up to 2000 mg/kg/day in a 14-day pilot rat safety study. The overall profile of **4t** makes it a good candidate for preclinical development.

EXPERIMENTAL SECTION

Chemistry. Unless otherwise noted, commercial reagents and solvents were used without further purification. Air or moisture sensitive reactions were performed under either a nitrogen or argon atmosphere. Flash chromatography was performed using silica gel with standard techniques or with silica gel cartridges on an ISCO Combiflash chromatography instrument. NMR spectra were recorded at 500 MHz for ^{1}H on a Bruker NMR spectrometer. The chemical shifts are given in ppm referenced to the deuterated solvent signal. Coupling constants (J) are recorded in hertz (Hz). LC-MS analyses were performed on a Waters Acquity UPLC/MS system with an analytical C18 column, and compounds were detected by UV at 254 nm. All final compounds with reported biological data were determined to be >95% pure based on LC/MS and NMR unless otherwise noted.

*General Procedure for the Cyanation of Indole **5** to Indole-3-carbonitrile **6** (GP-A).* Into a solution of indole **5** (219 mmol, 1.0 equiv) in DMF (100 mL) cooled in a dry ice−acetone bath was added dropwise chlorosulfonyl isocyanate (22.8 g, 262 mmol, 1.2 equiv) while keeping the temperature below −20 °C. After the addition, the temperature was allowed to slowly rise to −10 °C. The mixture was stirred at that temperature for 1.5 h before being poured into ice−water. The mixture was stirred and filtered. The solid was washed with water, dried, and collected to give the corresponding indole-3-carbonitrile **6**. The following compounds were prepared following this procedure:

*6-Difluoromethoxy-5-fluoro-1H-indole-3-carbonitrile (**6b**).* Yield 100%. MS m/z 227.0 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.4 (br s, 1H), 8.36 (d, J = 3.0 Hz, 1H), 7.67 (d, J = 10.5 Hz, 1H), 7.53 (d, J = 6.5 Hz, 1H), 7.27 (t, J = 75 Hz, 1H).

*5-Fluoro-6-methyl-1H-indole-3-carbonitrile (**6f**).* Yield 100%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45−8.68 (br s, 1H), 7.69 (d, J = 3.15 Hz, 1H), 7.39 (d, J = 9.46 Hz, 1H), 7.26 (d, J = 6.32 Hz, 1H), 2.40 (d, J = 1.89 Hz, 3H).

*6-Bromo-5-fluoro-1H-indole-3-carbonitrile (**13**).* Yield 100%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.39 (br s, 1H), 8.36 (d, J = 2.84 Hz, 1H), 7.88 (d, J = 5.99 Hz, 1H), 7.66 (d, J = 8.83 Hz, 1H).

*4-Fluoro-6-methyl-1H-indole-3-carbonitrile (**6n**).* Yield 82%. MS m/z 174.5 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.33 (br s, 1H), 8.23 (d, J = 3.0 Hz, 1H), 7.18 (s, 1H), 6.88 (d, J = 11.5 Hz, 1H), 2.42 (s, 3H).

*General Procedure for the N-Alkylation of Indole-3-carbonitrile **7** (GP-B).* To a solution of indole-3-carbonitrile **6** (92 mmol, 1.0 equiv) in DMF (20 mL) was added cesium carbonate (76 g, 233 mmol, 2.5 equiv) and the corresponding alkyl halide (112 mmol, 1.2 equiv). The mixture was stirred at 100 °C overnight, then cooled to room temperature and poured into ice water (500 mL). The precipitate was collected by filtration, washed with water and ethyl ether, and dried in a vacuum oven at 50 °C to give the corresponding *N*-alkyl-indole-3-carbonitrile **7**.

*1-Cyclobutyl-6-methoxy-1H-indole-3-carbonitrile (**11a**).* This compound was prepared from 6-methoxy-1*H*-indole by following the general procedures GP-A and GP-B (86% yield over 2 steps). MS m/z 227.1 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.13 (s, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.16 (d, J = 2.0 Hz, 1H), 6.96 (dd, J = 8.5, 2.0 Hz, 1H), 5.07 (pentet, J = 8.5 Hz, 1H), 4.15 (s, 3H), 2.65 (m, 2H), 2.52 (m, 2H), 1.98 (m, 2H).

*1-Cyclobutyl-6-hydroxy-1H-indole-3-carbonitrile (**12a**).* To a solution of **11a** (100 g, 442 mmol) in CH_2Cl_2 (700 mL) at −20 °C was added BBr_3 (300 g, 1.2 mol) dropwise over 2.5 h. The mixture was then poured into ice water (2 L), neutralized to pH 7 with 5 N aqueous sodium hydroxide, and stirred overnight at room temperature. The remaining organic solvent was evaporated, and the resulting suspension was filtered. The solid was washed with water (2 × 200 mL), then 1:1 hexane/ethyl ether (2 × 125 mL), and was dried in a stream of nitrogen to give the product **12** as a beige powder (90.0 g, 96% yield). MS m/z 213.1 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.41 (s, 1H), 8.05 (s, 1H), 7.48 (d, J = 8.5 Hz, 1H), 6.99 (d, J = 2.0 Hz, 1H), 6.90 (dd, J = 8.5, 2.0 Hz, 1H), 4.96 (pentet, J = 8.5 Hz, 1H), 2.64 (m, 2H), 2.52 (m, 2H), 1.98 (m, 2H).

*1-Cyclobutyl-6-(difluoromethoxy)-1H-indole-3-carbonitrile (**7a**).* To a solution of **12a** (31.7 g, 149.5 mmol) in 160 mL of 2-propanol at 75 °C was bubbled $CClF_2H$ gas via a needle while four portions of 20% aqueous NaOH were added via an addition funnel at 30 min intervals (40 mL × 4). After 2 h of total reaction time, the mixture was cooled to room temperature and the organic layer was separated and concentrated. The aqueous layer was extracted with ethyl acetate, and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in 4:1 hexane/CH_2Cl_2 (800 mL), stirred with basic alumina (80 g, pH 9.5), and then filtered and concentrated to yield compound **7a** as a white solid (28.3 g, 72% yield). MS m/z 263.1 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.18 (d, J = 2.0 Hz, 1H), 7.12 (dd, J = 8.5, 2.0 Hz, 1H), 6.55 (t, J = 75 Hz, 1H), 4.82 (pentet, J = 8.0 Hz, 1H), 2.66 (m, 2H), 2.47 (m, 2H), 2.01 (m, 2H).

*1-Cyclobutyl-6-(difluoromethoxy)-5-fluoro-1H-indole-3-carbonitrile (**7b**).* This compound was prepared from **6b** by following the general procedure GP-B (77% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.52 (d, J = 9.77 Hz, 1H), 7.26 (d, J = 6.30 Hz, 1H), 6.58 (t, J = 73.5 Hz, 1H), 4.77−4.85 (m, 1H), 2.62−2.70 (m, 2H), 2.39−2.49 (m, 2H), 1.98−2.07 (m, 2H).

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

*1-Cyclobutyl-5-fluoro-6-methyl-1H-indole-3-carbonitrile (**7f**).* This compound was prepared from **6f** by following the general procedure GP-B (92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.35 (d, J = 9.77 Hz, 1H), 7.17 (d, J = 6.31 Hz, 1H), 4.76−4.85 (m, 1H), 2.64 (dt, J = 3.63, 5.60 Hz, 2H), 2.37−2.48 (m, 5H), 1.96−2.04 (m, 2H).

*6-Bromo-1-cyclobutyl-5-fluoro-1H-indole-3-carbonitrile (**13**).* This compound was prepared from 6-bromo-5-fluoro-1*H*-indole by following the general procedures GP-A and GP-B (90% yield over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.60 (d, J = 5.36 Hz, 1H), 7.50 (d, J = 8.51 Hz, 1H), 4.81 (s, 1H), 2.61−2.72 (m, 2H), 2.35−2.52 (m, 2H), 1.96−2.10 (m, 2H)

*1-Cyclobutyl-6-ethyl-5-fluoro-1H-indole-3-carbonitrile (**7g**).* To a solution of **13** (1.3 g, 4.4 mmol) in dioxane (4 mL) under a nitrogen atmosphere was added $PdCl_2$dppf−dichloromethane complex (0.07 g, 0.09 mmol), followed by addition of a solution of Et_2Zn in heptane (8.0 mL, 1.0 M, 8.0 mmol) at room temperature. The mixture was heated to 100 °C and stirred for 1 h and then cooled down to 0 °C, into which saturated sodium bicarbonate (∼25 mL) was added dropwise. The mixture was then treated with water and extracted with dichloromethane. The dichloromethane layer was dried over sodium sulfate and evaporated. The resulting oil was passed through a silica plug and eluted with 10% ethyl acetate in hexane. The eluent was evaporated to give the title compound as a white solid (1.0 g, 89%). MS m/z 243.3 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.37 (d, J = 9.50 Hz, 1H), 7.17 (d, J = 6.00 Hz, 1H), 4.82−4.85 (m, 1H), 2.79 (q, J = 8.00 Hz, 2H), 2.63−2.67 (m, 2H), 2.42−2.46 (m, 2H), 2.00−2.03 (m, 2H), 1.29 (t, J = 8.00 Hz, 3H).

*1-Cyclobutyl-6-cyclopropyl-5-fluoro-1H-indole-3-carbonitrile (**7h**).* To a nitrogen flushed flask was added sequentiallyl 6-bromo-1-cyclobutyl-1*H*-indole-3-carbonitrile **13** (23 g, 83.6 mmol), cyclopropylboronic acid (11.35 g, 125 mmol), $Pd_2(dba)_3$ (1.91 g, 2.1 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (1.46 g, 5.0 mmol), potassium fluoride (29 g, 24.6 mmol), and THF (500 mL). The mixture was stirred overnight at 30 °C. The mixture was then filtered through Celite, concentrated, and purified by silica gel flash chromatography to yield a product containing 1% 3-cyano-1-*N*-cyclobutylindole, which was suspended in boiling hexanes (500 mL) for 10 min and filtered. The purified solid was collected and dried to give the title product as a white solid (78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.33 (d, J = 9.77 Hz, 1H), 6.92 (d, J = 5.99 Hz, 1H), 4.75−4.85 (m, 1H), 2.57−2.68 (m, 2H), 2.40 (dquin, J = 2.68, 9.58 Hz, 2H), 2.13−2.21 (m, 1H), 1.95−2.04 (m, 2H), 1.00−1.06 (m, 2H), 0.70−0.76 (m, 2H).

*1-Cyclobutyl-5-fluoro-6-hydroxy-1H-indole-3-carbonitrile (**7i**).* A mixture of **13** (29.9 g, 102 mmol), bis(pinacolato)diboron (33.7 g, 133 mmol), $PdCl_2$dppf−CH_2Cl_2 complex (4.16 g, 5.1 mmol), and potassium acetate (30 g, 306 mmol) in dioxane (300 mL) was stirred at 80 °C overnight. The mixture was then cooled to room temperature, treated with ethyl acetate (200 mL), and filtered through a silica Celite pad. The filtrate was concentrated, dissolved in acetone (300 mL), and cooled to 0 °C, into which was added a slurry of oxone (125.5 g, 204 mmol) in water (300 mL). The mixture was stirred at room temperature for 15 min, diluted with ethyl acetate, and separated. The aqueous layer was extracted with ethyl acetate. The organic extractions were combined and washed with satd aqueous $NaHSO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified on silica gel to provide **7i** as a light-gray solid (19.2 g, 82%). MS m/z 229.1 $[M - H]^-$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.11 (s, 1H), 7.36 (d, J = 10.72 Hz, 1H), 7.18 (d, J = 7.25 Hz, 1H), 4.90−5.05 (m, 1H), 2.80 (s, 1H), 2.58−2.68 (m, 2H), 2.45−2.58 (m, 2H), 1.90−2.02 (m, 2H).

*6-Chloro-1-cyclobutyl-5-fluoro-1H-indole-3-carbonitrile (**7j**).* This compound was prepared from **5j** by following the general procedures GP-A and GP-B (92% yield over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.50 (d, J = 8.51 Hz, 1H), 7.44 (d, J = 5.67 Hz, 1H), 4.76−4.85 (m, 1H), 2.66 (d, J = 3.47 Hz, 2H), 2.39−2.51 (m, 2H), 1.98−2.08 (m, 2H).

*1-Cyclobutyl-4-fluoro-6-methoxy-1H-indole-3-carbonitrile (**11b**).* This compound was prepared from 4-fluoro-6-methoxy-1*H*-indole by following the general procedures GP-A and GP-B (100% yield over 2 steps). MS m/z 245.3 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H), 6.58−6.68 (m, 2H), 4.70−4.82 (m, 1H), 3.87 (s, 3H), 2.57−2.71 (m, 2H), 2.43 (dt, J = 2.52, 9.62 Hz, 2H), 1.95−2.07 (m, 2H).

*1-Cyclobutyl-4-fluoro-6-hydroxy-1H-indole-3-carbonitrile (**12b**).* To a cooled (0 °C) solution of **11b** (0.6 g, 2.5 mmol) in dichloromethane (20 mL) was added ethanethiol (2.0 mL), followed by the addition of aluminum chloride (1.0 g, 7.5 mmol). The reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by addition of water, and the resulting solids were filtered and washed with water, then dried to provide the title compound as a white solid (0.55 g, 95% yield). MS m/z 231.3 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.65 (s, 1H), 7.94 (s, 1H), 6.69 (d, J = 1.58 Hz, 1H), 6.47 (dd, J = 1.89, 11.98 Hz, 1H), 4.72−4.87 (m, 1H), 2.29−2.54 (m, 4H), 1.75−1.87 (m, 2H).

*1-Cyclobutyl-6-(difluoromethoxy)-4-fluoro-1H-indole-3-carbonitrile (**7m**).* This compound was prepared from **12b** in a similar manner to that described for **7a** (62% yield). MS m/z 281.1 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 1H), 6.89 (d, J = 1.58 Hz, 1H), 6.74 (dd, J = 1.50, 10.0 Hz, 1H), 6.45 (t, J = 70.0 Hz, 1H), 4.68−4.75 (m, 1H), 2.55−2.59 (m, 2H), 2.38−2.33 (m, 2H), 1.92−1.96 (m, 2H).

*1-Cyclobutyl-4-fluoro-6-methyl-1H-indole-3-carbonitrile (**7n**).* This compound was prepared from **6n** following the general procedure GP-B (88% yield). MS m/z 229.7 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.74 (s, 1H), 7.32 (d, J = 11.5 Hz, 1H), 5.52 (pentet, J = 8.5 Hz, 1H), 3.11 (m, 2H), 3.02 (m, 2H), 2.93 (s, 3H), 2.43 (m, 2H).

*General Procedure for the Coupling of **7** with Sulfonamide **9** to Prepare **3**. Path A (GP-C1).* (i) Boronic acid preparation: To a solution of *N*-alkyl-indole-3-carbonitrile **7** (8.5 mmol) and triisopropyl borate (15.2 mmol) in THF (35 mL) at −78 °C was added LDA (1.5 M in cyclohexane, 7.3 mL, 11 mmol). The mixture was stirred at −78 °C for 30 min, then quenched with ice water (60 mL) and stirred for 15 min without cooling. The mixture was extracted with 1/1 hexane/ethyl acetate (20 mL). The aqueous layer was acidified with 2 N aqueous HCl to pH 5 and then extracted with CH_2Cl_2 (60 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to give the corresponding boronic acid **8**. The purity was estimated by UPLC, and the crude boronic acid was used without further purification. (ii) Suzuki coupling: A mixture of the boronic acid **8** prepared as above (1.15 mmol), 6-chloro-pyridine-3-sulfonamide **9** (0.17 g, 0.63 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (20 mg, 0.069 mmol), $Pd_2(dba)_3$ (32 mg, 0.035 mmol), and potassium fluoride (0.4 g, 6.9 mmol) in THF (4.0 mL) was stirred at 40 °C overnight under an Ar atmosphere. The solvent was then evaporated, and the residue was purified by silica gel flash chromatography to provide the corresponding target compound **3**.

Path B (GP-C2). To a solution of *N*-alkyl-indole-3-carbonitrile **7** (0.75 mmol) and triisopropyl borate (1.0 mmol) in THF (2.0 mL) at −78 °C was added dropwise a solution of LDA (1.5 M in cyclohexane, 0.6 mL, 0.9 mmol). After stirring at this temperature for 30 min, acetic acid (50 μL, 0.9 mmol) was added. The mixture was then warmed to room temperature, and the solvent was removed under a stream of nitrogen. To the residue was added 6-chloro-pyridine-3-sulfonamide **9** (0.6 mmol), $PdCl_2$dppf−dichloromethane complex (25 mg, 0.03 mmol), 2 M aqueous K_2CO_3 (1.0 mL, 2.0 mmol), and acetonitrile (2.0 mL). The mixture was stirred for 15 h at room temperature, then diluted with H_2O and extracted into EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. Purification by silica gel chromatography provided the corresponding target compound **3**.

Path C (GP-C3). (i) Stannane preparation: To a solution of *N*-alkyl-indole-3-carbonitrile **7** (2.4 mmol), tri-*n*-butylstannyl iodide (860 μL, 3.1 mmol) in THF (7.0 mL) at −78 °C was added dropwise a solution of LDA (1.5 M in cyclohexane, 2.6 mL, 3.8 mmol). The mixture was allowed to warm to room temperature and then stirred for an additional 3 h. The mixture was then concentrated, and the crude residue was purified by silica gel chromatography to yield the corresponding 2-(tributylstannyl)-indole-3-carbonitrile **10** as a clear oil (90−100% yield). (ii) A mixture of the indole-2-stannane **10** (0.4

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

mmol), 6-chloro-pyridine-3-sulfonamide **9** (0.4 mmol), $Pd(PPh_3)_4$ (45 mg, 0.039 mmol), and CuI (18 mg, 0.094 mmol) in dioxane (0.8 mL) was heated at 100 °C for 1 h. The reaction mixture was then diluted with EtOAc and filtered through Celite. The filtrate was concentrated under vacuum and purified by silica gel chromatography to provide the corresponding target compound **3**.

*(S)-6-(3-Cyano-1-cyclobutyl-6-(difluoromethoxy)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3a**).* This compound was prepared from **7a** and **9** by following the general procedure GP-C3 (43% yield); mp 159−162 °C. MS *m/z* 515.4 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.23 (d, *J* = 2.0 Hz, 1H), 8.34 (dd, *J* = 8.0, 2.5 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.49 (s, 1H), 7.17 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.59 (t, *J* = 76 Hz, 1H), 5.35 (pentet, *J* = 8.5 Hz, 1H), 4.98 (br s, 1H), 4.17 (m, 1H), 2.57 (pentet, *J* = 10.0 Hz, 2H), 2.43 (m, 2H), 1.93 (m, 2H), 1.47 (d, *J* = 7.0 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-(difluoromethoxy)-5-fluoro-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3b**).* This compound was prepared from **7b** and **9** following the general procedure GP-C1 (56% yield); mp 172−175 °C. MS *m/z* 533.2 $[M + H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 9.21 (dd, *J* = 0.90, 2.50 Hz, 1H), 9.01 (br s, 1H), 8.49 (dd, *J* = 2.21, 8.20 Hz, 1H), 8.12 (dd, *J* = 0.79, 8.35 Hz, 1H), 7.80(d, *J* = 9.80 Hz, 1H), 7.78 (d, *J* = 6.70 Hz, 1H), 7.34(t, *J* = 73.2 Hz, 1H), 5.17−5.26 (m, 1H), 4.28−4.40 (m, 1H), 2.32 (m, 4H), 1.73 (m, 2H), 1.11 (d, *J* = 6.94 Hz, 3H).

*(S)-6-(5-Bromo-3-cyano-1-cyclobutyl-6-(difluoromethoxy)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**14**).* A mixture of **3a** (2.2 g, 4.3 mmol) and bromine (0.72 g, 4.5 mmol) in acetic acid (20 mL) was stirred at 95 °C for 6 h and then poured into ice−water. The mixture was neutralized with NaOH to pH ∼6 and filtered. The resulting solid was washed with water, dried, and then purified by silica gel flash chromatography with ethyl acetate in hexanes (5 to 25% gradient) to give **14** (0.92 g, 36%). MS *m/z* 592.9/594.9 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.22 (dd, *J* = 0.79, 2.36 Hz, 1H), 8.35 (dd, *J* = 2.52, 8.20 Hz, 1H), 8.07 (s, 1H), 8.02 (dd, *J* = 0.79, 8.35 Hz, 1H), 7.60 (s, 1H), 6.59 (t, *J* = 73.5 Hz, 1H), 5.28−5.37 (m, 1H), 4.94 (d, *J* = 9.46 Hz, 1H), 4.12−4.21 (m, 1H), 2.37−2.55 (m, 4H), 1.84−1.95 (m, 2H), 1.47 (d, *J* = 6.94 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-(difluoromethoxy)-5-hydroxy-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3c**).* To a solution of **14** (0.3 g, 0.5 mmol) in THF (2.0 mL) at −78 °C was added a solution of MeLi in THF (1.5 M × 0.33 mL, 0.5 mmol) followed by a solution of *n*-BuLi in hexane (1.6 M × 0.38 mL, 0.6 mmol) after 5 min. $B(Oi\text{-}Pr)_3$ was added, and the temperature was allowed to rise to room temperature. The reaction was then quenched with saturated ammonium chloride and extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate, and evaporated. The residue was purified by silica gel flash chromatography with ethyl acetate in dichloromethane (0 to 30% gradient) to provide **3c** (28 mg, 10%). MS *m/z* 530.9 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.21 (dd, *J* = 0.60, 2.21 Hz, 1H), 8.33 (dd, *J* = 2.21, 8.20 Hz, 1H), 7.98 (dd, *J* = 0.60, 8.20 Hz, 1H), 7.44 (s, 1H), 7.31 (s, 1H), 6.63 (t, *J* = 73.8 Hz, 1H), 6.38 (br s, 1H), 5.70 (d, *J* = 9.40 Hz, 1H), 5.23−5.30 (m, 1H), 4.11−4.18 (m, 1H), 2.44−2.52 (m, 2H), 2.35−2.43 (m, 2H), 1.79−1.91 (m, 2H), 1.42 (d, *J* = 6.94 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-(difluoromethoxy)-5-methyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3d**).* Into a mixture of **14** (0.3 g, 0.54 mmol) and $PdCl_2$dppf (40 mg, 0.054 mmol) in THF (2.0 mL) under a nitrogen atmosphere was added a solution of MeZnCl (2.0 M × 0.6 mL, 1.2 mmol). The mixture was then stirred at 40 °C for 20 h. The reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic layers were combined, washed with brine, dried, and purified by reverse phase HPLC to give **3d** (66 mg, 23%). MS *m/z* 529.2 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.21 (dd, *J* = 0.63, 2.52 Hz, 1H), 8.33 (dd, *J* = 2.52, 8.20 Hz, 1H), 8.02 (dd, *J* = 0.90, 8.20 Hz, 1H), 7.64 (s, 1H), 7.45 (s, 1H), 6.57 (t, *J* = 73.8, 1H), 5.29−5.37 (m, 1H), 5.12 (d, *J* = 9.46 Hz, 1H), 4.11−4.20 (m, 1H), 2.49−2.60 (m, 2H), 2.37−2.46 (m, 5H), 1.82−1.95 (m, 2H), 1.45 (d, *J* = 6.94 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-(difluoromethoxy)-5-ethyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3e**).* To a mixture of **14** (0.15 g, 0.27 mmol) and $PdCl_2$dppf (10 mg, 0.013 mmol) in dioxane (2.0 mL) under a nitrogen atmosphere was added a solution of diethylzinc in heptane (1.0 M × 1.0 mL, 1.0 mmol). The mixture was stirred at 100 °C for 3 h, then cooled and carefully quenched with methanol. The mixture was diluted with ethyl acetate and then washed with 2N HCl and brine. The organic layer was dried over sodium sulfate and evaporated, and the residue was purified by reverse phase HPLC to give **3e** (85 mg, 58%). MS *m/z* 543.0 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.21 (d, *J* = 2.21 Hz, 1H), 8.33 (dd, *J* = 2.05, 8.04 Hz, 1H), 8.02 (d, *J* = 8.20 Hz, 1H), 7.66 (s, 1H), 7.45 (s, 1H), 6.56 (t, *J* = 73.5 Hz, 1H), 5.33 (t, *J* = 8.67 Hz, 1H), 5.09 (d, *J* = 9.46 Hz, 1H), 4.15 (td, *J* = 6.98, 9.38 Hz, 1H), 2.82 (q, *J* = 7.57 Hz, 2H), 2.49−2.60 (m, 2H), 2.37−2.46 (m, 2H), 1.83−1.96 (m, 2H), 1.45 (d, *J* = 6.94 Hz, 3H), 1.28−1.33 (m, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-5-fluoro-6-methyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3f**).* This compound was prepared from **7f** and **9** by following the general procedure GP-C1 (68% yield); mp 218−219 °C. MS *m/z* 481.2 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.21 (br s, 1H), 8.32 (dd, *J* = 2.21, 8.20 Hz, 1H), 8.01 (d, *J* = 8.20 Hz, 1H), 7.49 (d, *J* = 6.31 Hz, 1H), 7.41 (d, *J* = 9.14 Hz, 1H), 5.28−5.37 (m, 1H), 5.04 (d, *J* = 9.14 Hz, 1H), 4.14−4.20 (m, 1H), 2.54−2.65 (m, 2H), 2.46 (d, *J* = 1.89 Hz, 3H), 2.36−2.45 (m, 2H), 1.83−1.97 (m, 2H), 1.45 (d, *J* = 6.94 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-ethyl-5-fluoro-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3g**).* This compound was prepared from **7g** and **9** by following the general procedure GP-C1 (58% yield); mp 205−206 °C. MS *m/z* 495.2 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.13 (d, *J* = 1.89 Hz, 1H), 8.19−8.30 (m, 1H), 7.93 (dd, *J* = 0.63, 8.20 Hz, 1H), 7.38−7.45 (m, 1H), 7.34 (d, *J* = 9.46 Hz, 1H), 5.18−5.32 (m, 1H), 4.81−4.90 (m, 1H), 3.99−4.15 (m, 1H), 2.76 (q, *J* = 7.60 Hz, 2H), 2.43−2.58 (m, 2H), 2.29−2.39 (m, 2H), 1.75−1.90 (m, 2H), 1.38 (d, *J* = 7.25 Hz, 3H), 1.25 (t, *J* = 7.60 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-cyclopropyl-5-fluoro-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3h**).* This compound was prepared from **7h** and **9** by following the general procedure GP-C1 (34 mg, 31%). MS *m/z* 507.4 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.20 (d, *J* = 1.90 Hz, 1H), 8.32 (dd, *J* = 2.50, 8.20 Hz, 1H), 8.00 (d, *J* = 8.20 Hz, 1H), 7.41 (d, *J* = 9.80 Hz, 1H), 7.23 (d, *J* = 6.00 Hz, 1H), 5.27−5.35 (m, 1H), 5.08 (d, *J* = 9.50 Hz, 1H), 4.12−4.19 (m, 1H), 2.46−2.58 (m, 2H), 2.36−2.45 (m, 2H), 2.19−2.25 (m, 1H), 1.84−1.93 (m, 2H), 1.45 (d, *J* = 6.94 Hz, 3H), 1.06−1.12 (m, 2H), 0.75−0.80 (m, 2H).

*(S)-6-(3-Cyano-1-cyclobutyl-5-fluoro-6-hydroxy-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3i**).* This compound was prepared from **7i** and **9** by following the general procedure GP-C2 (97% yield); mp 194−196 °C. MS *m/z* 483.3 $[M + H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 9.10 (d, *J* = 1.5 Hz, 1H), 8.92 (br s, 1H), 8.38 (dd, *J* = 2.52, 8.20 Hz, 1H), 7.98 (dd, *J* = 0.63, 8.20 Hz, 1H), 7.43 (d, *J* = 10.72 Hz, 1H), 7.27 (d, *J* = 7.25 Hz, 1H), 5.13 (t, *J* = 8.51 Hz, 1H), 4.27 (br s, 1H), 2.27−2.38 (m, 2H), 2.27−2.16 (m, 2H), 1.63−1.77 (m, 2H), 1.03 (d, *J* = 6.62 Hz, 3H).

*(S)-6-(6-Chloro-3-cyano-1-cyclobutyl-5-fluoro-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3j**).* This compound was prepared from **7j** and **9** by following the general procedure GP-C1 (52%). MS *m/z* 501.0/503.0 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.22 (dd, *J* = 0.70, 8.20 Hz, 1H), 8.34 (dd, *J* = 2.20, 8.20 Hz, 1H), 8.02 (dd, *J* = 1.00, 8.20 Hz, 1H), 7.74 (d, *J* = 6.00 Hz, 1H), 7.56 (d, *J* = 8.20 Hz, 1H), 5.28−5.36 (m, 1H), 4.93 (d, *J* = 9.40 Hz, 1H), 4.14−4.20 (m, 1H), 2.49−2.59 (m, 2H), 2.39−2.46 (m, 2H), 1.85−1.97 (m, 2H), 1.47 (d, *J* = 6.94 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-5-fluoro-6-methoxy-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3k**).* A mixture of **3i** (0.2 g, 0.41 mmol), MeI (65 mg, 0.46 mmol), and aqueous NaOH (6.25 M × 0.13 mL, 0.82 mmol) in THF was stirred at 40 °C overnight. The mixture was purified by reverse phase HPLC to provide **3k** (3.0 mg, 1%). MS *m/z* 497.4 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 9.16 (dd, *J* = 0.79, 2.36 Hz, 1H), 8.23−8.32 (dd, *J* = 2.5, 8.2 Hz, 1H), 7.97−8.05 (d, *J* = 8.2 Hz, 1H), 7.49 (d, *J* = 9.77 Hz, 1H), 7.34 (d, *J* = 7.25 Hz, 1H), 5.27−5.39 (m, 1H), 4.71−4.84 (m, 1H),

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

2.94 (d, J = 0.63 Hz, 3H), 2.55−2.68 (m, 2H), 2.33−2.48 (m, 2H), 1.82−1.97 (m, 3H), 1.40 (d, J = 7.25 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-5-fluoro-6-(methylthio)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3l**).* To a solution of **3i** (1.1 g, 2.5 mmol) and pyridine (1.0 mL, 12.5 mmol) in CH_2Cl_2 (25 mL) was added triflic anhydride (0.5 mL, 3.0 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 2 h, then treated with ether (50 mL), washed with 1N HCl and brine, and then dried and evaporated to give the crude triflate **15**, which was used in the next step without further purification.

A mixture of the triflate **15** obtained above (90 mg, 0.16 mmol), sodium thiomethoxide (13 mg, 0.18 mmol), $Pd_2(dba)_3$ (3.7 mg, 0.004 mmol), xantphos (4.6 mg, 0.008 mmol), and DIPEA (41 mg, 0.32 mmol) in dioxane (2.0 mL) was stirred at 100 °C overnight under an Ar atmosphere. The mixture was then cooled, treated with water, and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and evaporated. The residue was purified by silica gel chromatography to provide **3l** (88 mg, 100%); mp 201−203 °C. MS m/z 513.6 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (dd, J = 0.6, 2.2 Hz, 1H), 8.33 (dd, J = 2.2, 8.2 Hz, 1H), 8.01 (dd, J = 0.9, 8.2 Hz, 1H), 7.58 (d, J = 6.0 Hz, 1H), 7.46 (d, J = 9.2 Hz, 1H), 5.39−5.31 (m, 1H), 5.26 (d, J = 9.4 Hz, 1H), 4.18−4.13 (m, 1H), 2.56 (m, 5H), 2.38−2.49 (m, 2H), 1.86−1.95 (m, 2H), 1.44 (d, J = 6.9 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-6-(difluoromethoxy)-4-fluoro-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3m**).* This compound was prepared from **7m** and **9** following the general procedure GP-C1 (27% yield); mp 210−212 °C. MS m/z 533.1 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 9.01 (d, J = 1.58 Hz, 1H), 8.13 (dd, J = 2.52, 8.20 Hz, 1H), 7.83 (dd, J = 0.95, 8.20 Hz, 1H), 6.67 (d, J = 8.20 Hz, 1H), 6.36 (t, J = 70.0 Hz, 1H), 5.01−5.16 (m, 1H), 4.65−4.77 (m, 1H), 3.87−4.07 (m, 1H), 2.08−2.36 (m, 5H), 1.64−1.73 (m, 2H), 1.25 (d, J = 6.94 Hz, 3H).

*(S)-6-(3-Cyano-1-cyclobutyl-4-fluoro-6-methyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**3n**).* This compound was prepared from **7n** and **9** by following the general procedure GP-C3 (50% yield); mp 205−210 °C. MS m/z 481.4 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.34 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.26 (s, 1H), 6.84 (d, J = 9.0 Hz, 1H), 5.29 (pentet, J = 8.5 Hz, 1H), 5.04 (d, J = 9.5 Hz, 1H), 4.16 (m, 1H), 2.60 (m, 2H), 2.53 (s, 3H), 2.40 (m, 2H), 1.89 (m, 2H), 1.45 (d, J = 7.0 Hz, 3H).

*General Procedure for the Preparation of **16**.* To a solution of **5** (1.25 g, 5.3 mmol) in CH_2Cl_2 (10 mL) were added di-*tert*-butyl dicarbonate (1.5 g, 7.0 mmol) and DMAP (13 mg, 0.1 mmol). The reaction mixture was kept stirring for 2 h at room temperature and then partitioned between CH_2Cl_2 and water. The organic layer was washed with water and brine and dried over Na_2SO_4 and evaporated. The resulting white solid was usually pure enough to be used in the next step without further purification.

*General Procedure for the Preparation of **17**.* Into a solution of **16** (3.5 mmol) and tri-isopropyl borate (1.2 mL, 5.25 mmol) in THF (7.0 mL) at −78 °C was added LDA (1.5 M in cyclohexane, 2.8 mL, 4.2 mmol) under nitrogen. The mixture was stirred at −78 °C for 15 min and then for 2 h at 0 °C. The reaction was quenched by slow addition of acetic acid (0.24 mL, 4.2 mmol), followed by the removal of solvents by rotary evaporation. The resulting boronic acid **17** was used in the next step without further purification.

*General Procedure for the Preparation of **18**.* Into a solution of the boronic acid **17** prepared as above, sulfonamide **9** (0.91 g, 3.15 mmol), and $PdCl_2$dppf (0.23 g, 0.31 mmol) in acetonitrile (21 mL) was added a solution of potassium carbonate (2.0 M, 7.0 mL, 14 mmol). The mixture was stirred at 50 °C overnight, cooled to room temperature, and then quenched by addition of HCl (1 N) to pH ∼5. The mixture was then extracted with ethyl acetate. The combined organics were dried over Na_2SO_4 and concentrated to give a crude product which was usually used in the next step without further purification.

*General Procedure for the Preparation of **19**.* A mixture of **18** prepared as above and pyrrolidine (1.5 mL, 18 mmol) in acetonitrile (15 mL) was stirred at 50 °C for 2 h. The solvents were then evaporated, and the residue was partitioned between ethyl acetate and HCl (1N). The aqueous phase was further extracted with ethyl acetate. The combined organics were washed with 1N HCl and brine and then dried over Na_2SO_4 and concentrated to give a crude product which was purified by silica gel flash chromatography using ethyl acetate in hexanes as eluent to give the desired product **19**.

*General Procedure for the Preparation of **20**.* To a solution of **19** (0.53 mmol) in a mixture of DMF (0.5 mL) and acetonitrile (0.5 mL) was added chlorosulfonyl isocyanate dropwise at −50 °C. The reaction mixture was kept stirring at 0 °C for 0.5 h and then quenched with ice, poured into water, and then stirred at room temperature for an additional 0.5 h. The resulting solid was collected by filtration, washed sequentially with water, hexane, and CH_2Cl_2, and air-dried to give **19**, which was usually pure enough to be used in the next step without further purification.

*General Procedure for the Preparation of **4**.* A mixture of **20** (0.5 mmol), aryl halide (1.0 mmol), potassium carbonate (1.5 mmol), and copper(I) iodide (20 mg, 0.1 mmol) were mixed with DMF (2.5 mL). The system was evacuated and replaced with an argon atmosphere. The reaction mixture was stirred at 110 °C overnight, then cooled to 0 °C, diluted with ethyl acetate, and neutralized with 1N HCl. The ethyl acetate layer was washed with water and brine, dried over Na_2SO_4, and filtered through silica gel pad and evaporated. The resulting residue was purified by silica gel flash chromatography using ethyl acetate in hexanes as eluent to give the desired product **4**.

The following compounds were prepared according to the reaction sequence outlined in Scheme 4 and the general procedure described as above.

*(S)-6-(3-Cyano-6-(difluoromethoxy)-1-(pyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4a**).* Melting point 186−189 °C. MS m/z 539.3 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (d, J = 2.2 Hz, 1H), 8.68 (d, J = 4.6 Hz, 2H), 8.31 (dd, J = 2.2, 8.2 Hz, 1H), 8.11 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 2.2 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.30 (t, J = 4.5 Hz, 1H), 7.26 (d, J = 1.9 Hz, 1H), 6.59 (t, J = 73.8 Hz, 1H), 4.84 (d, J = 9.5 Hz, 1H), 4.06−4.12 (m, 1H), 1.44 (d, J = 6.9 Hz, 3H).

*(S)-6-(3-Cyano-6-methyl-1-(pyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4b**).* Melting point 195−198 °C. MS m/z 487.2 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.80 (d, J = 2.52 Hz, 1H), 8.69 (d, J = 4.7 Hz, 2H), 8.28 (dd, J = 2.36, 8.35 Hz, 1H), 8.12 (dd, J = 0.79, 8.35 Hz, 1H), 7.90 (d, J = 0.63 Hz, 1H), 7.75 (d, J = 8.20 Hz, 1H), 7.28 (t, J = 4.7 Hz, 1H), 7.27 (d, J = 8.2 Hz, 1H), 5.08 (d, J = 9.4 Hz, 1H), 4.02−4.10 (m, 1H), 2.53 (s, 3H), 1.41 (d, J = 7.25 Hz, 3H).

*(S)-6-(3-Cyano-6-(difluoromethoxy)-5-fluoro-1-(pyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4c**).* MS m/z 556.9 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.80 (br s, 1H), 8.69 (d, J = 4.7 Hz, 2H), 8.28 (dd, J = 2.5, 8.5 Hz, 1H), 8.13 (d, J = 6.6 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.65 (d, J = 9.1 Hz, 1H), 7.32 (t, J = 4.7 Hz, 1H), 6.63 (t, J = 73.4 Hz, 1H), 4.54−4.63 (m, 1H), 1.39 (d, J = 7.25 Hz, 3H).

*(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(pyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4d**).* MS m/z 505.1 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.81 (dd, J = 0.6, 2.5 Hz, 1H), 8.68 (d, J = 4.7 Hz, 2H), 8.29 (dd, J = 2.5, 8.5 Hz, 1H), 8.11 (dd, J = 0.6, 8.5 Hz, 1H), 7.96 (d, J = 6.3 Hz, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.29 (t, J = 4.7 Hz, 1H), 4.89 (d, J = 9.1 Hz, 1H), 4.06−4.11 (m, 1H), 2.44 (d, J = 1.5 Hz, 3H), 1.42 (d, J = 7.0 Hz, 3H).

*(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(pyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4e**).* Melting point 160−164 °C. MS m/z 504.8 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.88 (dd, J = 0.9, 2.2 Hz, 1H), 8.49−8.52 (m, 1H), 8.39 (dd, J = 2.6, 8.2 Hz, 1H), 8.02 (dt, J = 1.9, 7.7 Hz, 1H), 7.97 (dd, J = 0.8, 8.3 Hz, 1H), 7.66 (br s, 1H), 7.48−7.53 (m, 4H), 4.22−4.32 (m, 1H), 2.38 (d, J = 1.6 Hz, 3H), 1.25 (d, J = 6.9 Hz, 3H).

*(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(pyridin-3-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4f**).* Melting point 217−221 °C. MS m/z 503.5 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (dd, J = 0.79, 2.36 Hz, 2H), 8.67−8.73 (m, 1H), 8.61 (d, J = 2.21 Hz, 1H), 8.35 (dd, J = 2.36, 8.35 Hz, 1H), 7.91−7.96

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

(m, 1H), 7.88 (dd, J = 0.79, 8.35 Hz, 1H), 7.65 (d, J = 9.14 Hz, 1H), 7.56−7.61 (m, 1H), 7.28 (d, J = 5.99 Hz, 1H), 4.19−4.32 (m, 1H), 2.34 (d, J = 1.58 Hz, 3H), 1.02 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(pyridin-4-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4g)***. Melting point 250−255 °C. MS m/z 504.5 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.91−8.94 (m, 1H), 8.74 (d, J = 5.36 Hz, 2H), 8.41 (dd, J = 2.36, 8.35 Hz, 1H), 8.01 (dd, J = 0.79, 8.35 Hz, 1H), 7.64−7.70 (m, 1H), 7.54 (d, J = 9.14 Hz, 1H), 7.39−7.45 (m, 3H), 4.23−4.34 (m, 1H), 2.39 (dd, J = 0.63, 2.21 Hz, 3H), 1.25 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(pyrazin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4h)***. Melting point 220−223 °C. MS m/z 505.4 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (dd, J = 0.79, 2.05 Hz, 1H), 8.67 (d, J = 2.52 Hz, 1H), 8.65 (d, J = 0.95 Hz, 1H), 8.51 (dd, J = 1.26, 2.52 Hz, 1H), 8.23−8.30 (m, 2H), 7.52 (d, J = 8.83 Hz, 1H), 7.27 (d, J = 5.40 Hz, 1H), 4.96 (d, J = 9.46 Hz, 1H), 4.01−4.10 (m, 1H), 2.41 (d, J = 1.89 Hz, 3H), 1.41 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(pyridazin-3-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4i)***. Melting point 245−250 °C. MS m/z 504.5 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.36 (dd, J = 2.21, 4.10 Hz, 1H), 8.89 (br s, 1H), 8.76−8.80 (m, 1H), 8.41 (dd, J = 2.36, 8.35 Hz, 1H), 8.10−8.15 (m, 1H), 7.94−8.00 (m, 2H), 7.69 (d, J = 9.14 Hz, 1H), 7.52 (d, J = 5.99 Hz, 1H), 4.17−4.32 (m, 1H), 2.36 (d, J = 1.58 Hz, 3H), 1.03 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(thiazol-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4j)***. Melting point 203−208 °C. MS m/z 509.5 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.99 (dd, J = 0.79, 2.36 Hz, 1H), 8.46 (dd, J = 2.21, 8.20 Hz, 1H), 8.09 (dd, J = 0.79, 8.35 Hz, 1H), 7.79−7.83 (m, 2H), 7.70 (br s, 1H), 7.63 (d, J = 6.31 Hz, 1H), 7.54 (d, J = 9.14 Hz, 1H), 4.26−4.36 (m, 1H), 2.42 (d, J = 2.21 Hz, 3H), 1.26 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(thiazol-4-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4k)***. Melting point 184−189 °C. MS m/z 509.5 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.17 (d, J = 2.2 Hz, 1H), 8.96 (dd, J = 0.9, 2.2 Hz, 1H), 8.88−8.94 (m, 1H), 8.33 (dd, J = 2.2, 8.5 Hz, 1H), 8.02 (d, J = 2.21 Hz, 1H), 7.75 (dd, J = 0.79, 8.35 Hz, 1H), 7.63 (d, J = 9.46 Hz, 1H), 7.42 (d, J = 5.3 Hz, 1H), 4.19−4.34 (m, 1H), 2.37 (d, J = 1.58 Hz, 3H), 1.05 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(thiazol-5-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4l)***. Melting point 234−238 °C. MS m/z 509.5 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.25 (d, J = 0.95 Hz, 1H), 9.01 (dd, J = 0.9, 2.2 Hz, 1H), 8.94 (d, J = 8.6 Hz, 1H), 8.37 (dd, J = 2.2, 8.2 Hz, 1H), 8.13 (d, J = 0.63 Hz, 1H), 7.91 (dd, J = 1.0, 8.2 Hz, 1H), 7.64 (d, J = 9.1 Hz, 1H), 7.35 (d, J = 6.0 Hz, 1H), 4.23−4.34 (m, 1H), 2.37 (d, J = 1.58 Hz, 3H), 1.04 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(thiophen-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4m)***. MS m/z 508.5 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.05 (dd, J = 0.95, 2.52 Hz, 1H), 8.92 (d, J = 8.5 Hz, 1H), 8.31 (dd, J = 2.36, 8.35 Hz, 1H), 7.75 (dd, J = 0.79, 8.35 Hz, 1H), 7.69 (dd, J = 1.42, 5.52 Hz, 1H), 7.62 (d, J = 9.46 Hz, 1H), 7.35 (dd, J = 1.58, 3.78 Hz, 1H), 7.32 (d, J = 5.99 Hz, 1H), 7.15 (dd, J = 3.78, 5.67 Hz, 1H), 4.23−4.34 (m, 1H), 2.36 (d, J = 1.58 Hz, 3H), 1.04 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(5-methylpyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4n)***. Melting point 179−184 °C. MS m/z 518.5 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.87 (dd, J = 0.79, 2.36 Hz, 1H), 8.63 (d, J = 0.95 Hz, 2H), 8.44 (dd, J = 2.36, 8.35 Hz, 1H), 8.09 (dd, J = 0.79, 8.35 Hz, 1H), 8.02 (d, J = 7.20 Hz, 1H), 7.68 (d, J = 9.14 Hz, 1H), 7.47 (d, J = 9.14 Hz, 1H), 4.23−4.35 (m, 1H), 2.40 (d, J = 1.95 Hz, 3H), 2.38 (s, 3H), 1.27 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-1-(5-fluoropyrimidin-2-yl)-6-methyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4o)***. Melting point 201−203 °C. MS m/z 507.1 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.87 (dd, J = 0.95, 2.52 Hz, 1H), 8.81 (s, 2H), 8.47 (dd, J = 2.6, 8.6 Hz, 1H), 8.17 (dd, J = 1.0, 8.2 Hz, 1H), 7.94−7.99 (m, 1H), 7.70−7.76 (m, 1H), 7.48 (d, J = 9.14 Hz, 1H), 4.23−4.34 (m, 1H), 2.40 (d, J = 1.89 Hz, 3H), 1.27 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-1-(5-fluoropyridin-2-yl)-6-methyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4p)***. Melting point 239−241 °C. MS m/z 512.8 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (dd, J = 1.0, 2.5 Hz, 1H), 8.34 (d, J = 2.5 Hz, 1H), 8.24 (dd, J = 2.5, 8.2 Hz, 1H), 8.11 (dd, J = 0.9, 8.5 Hz, 1H), 7.58−7.63 (m, 1H), 7.50 (d, J = 8.9 Hz, 1H), 7.31−7.34 (m, 1H), 7.21 (d, J = 6.3 Hz, 1H), 4.86 (d, J = 9.5 Hz, 1H), 4.04−4.10 (m, 1H), 2.39 (d, J = 1.5 Hz, 3H), 1.42 (d, J = 6.9 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(5-methylpyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4q)***. Melting point 250−254 °C. MS m/z 517.5 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.90 (dd, J = 1.0, 2.2 Hz, 1H), 8.37 (dd, J = 3.5, 8.2 Hz, 1H), 8.33−8.35 (m, 1H), 7.92 (dd, J = 0.9, 8.5 Hz, 1H), 7.81−7.85 (m, 1H), 7.58−7.66 (br s, 1H), 7.50 (d, J = 9.5 Hz, 1H), 7.49 (d, J = 5.9 Hz, 1H), 7.40 (d, J = 8.51 Hz, 1H), 4.24−4.32 (m, 1H), 2.42 (s, 3H), 2.38 (d, J = 2.2 Hz, 3H), 1.25 (d, J = 6.94 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-1-(3-fluoropyridin-2-yl)-6-methyl-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4r)***. Melting point 193−198 °C. MS m/z 521.5 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.78 (dd, J = 0.9, 2.2 Hz, 1H), 8.46 (dd, J = 2.5, 8.5 Hz, 1H), 8.41 (td, J = 0.9, 4.7 Hz, 1H), 8.24 (dd, J = 0.9, 8.5 Hz, 1H), 7.88−7.94 (m, 1H), 7.68−7.72 (m, 1H), 7.64 (br s, 1H), 7.56 (d, J = 9.2 Hz, 1H), 7.37 (d, J = 6.0 Hz, 1H), 4.22−4.32 (m, 1H), 2.38 (d, J = 1.89 Hz, 3H), 1.23 (d, J = 7.25 Hz, 3H).

(S)-6-(3-Cyano-5-fluoro-6-methyl-1-(3-methylpyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4s)***. Melting point 219−221 °C. MS m/z 518.2 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (d, J = 1.9 Hz, 1H), 8.38 (br s, 1H), 8.17 (dd, J = 2.2, 8.5 Hz, 1H), 8.12 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 7.2 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 7.38−7.42 (m, 1H), 6.78 (d, J = 6.0 Hz, 1H), 4.88 (d, J = 9.4 Hz, 1H), 3.99−4.06 (m, 1H), 2.35 (d, J = 2.2 Hz, 3H), 2.04 (d, J = 3.15 Hz, 3H), 1.38 (d, J = 7.0 Hz, 3H).

(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(pyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4t)***. Melting point 195−198 °C. MS m/z 519.1 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.81 (dd, J = 0.9, 2.2 Hz, 1H), 8.69 (d, J = 4.73 Hz, 2H), 8.29 (dd, J = 2.5, 8.2 Hz, 1H), 8.11 (dd, J = 0.95, 8.51 Hz, 1H), 7.97 (d, J = 6.3 Hz, 1H), 7.48 (d, J = 9.46 Hz, 1H), 7.30 (t, J = 4.89 Hz, 1H), 4.90 (d, J = 9.8 Hz, 1H), 4.04−4.12 (m, 1H), 2.83 (q, J = 7.5 Hz, 2H), 1.42 (d, J = 6.94 Hz, 3H), 1.30 (t, J = 7.57 Hz, 3H).

(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(pyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4u)***. Melting point 205−208 °C. MS m/z 517.8 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79−8.81 (m, 1H), 8.50−8.52 (m, 1H), 8.21 (dd, J = 2.5, 8.5 Hz, 1H), 8.02 (dd, J = 1.0, 8.5 Hz, 1H), 7.89 (dt, J = 1.9, 7.6 Hz, 1H), 7.50 (d, J = 9.5 Hz, 1H), 7.41 (ddd, J = 1.0, 4.8, 7.3 Hz, 1H), 7.28−7.32 (m, 2H), 4.85 (d, J = 9.4 Hz, 1H), 4.01−4.09 (m, 1H), 2.78 (t, J = 7.6 Hz, 2H), 1.41 (d, J = 6.9 Hz, 3H), 1.25 (t, J = 7.6 Hz, 3H).

(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(pyridin-4-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4v)***. Melting point 209−215 °C. MS m/z 518.3 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.94 (dd, J = 0.79, 2.36 Hz, 1H), 8.78 (br s, 2H), 8.42 (dd, J = 2.6, 8.2 Hz, 1H), 8.02 (dd, J = 1.0, 8.5 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 9.46 Hz, 1H), 7.48 (br s, 2H), 7.43 (d, J = 5.99 Hz, 1H), 4.24−4.34 (m, 1H), 2.80 (q, J = 7.5 Hz, 2H), 1.25 (d, J = 7.2 Hz, 3H), 1.23 (t, J = 7.5 Hz, 3H).

(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(pyrazin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4w)***. Melting point 225−229 °C. MS m/z 519.0 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.82 (dd, J = 0.79, 2.36 Hz, 1H), 8.79 (d, J = 1.89 Hz, 1H), 8.75 (d, J = 2.6 Hz, 1H), 8.62 (dd, J = 1.42, 2.68 Hz, 1H), 8.48 (dd, J = 2.6, 8.6 Hz, 1H), 8.24 (dd, J = 0.9, 8.5 Hz, 1H), 7.64−7.69 (br s, 1H), 7.60 (d, J = 5.67 Hz, 1H), 7.57 (d, J = 9.46 Hz, 1H), 4.25−4.32 (m, 1H), 2.81 (q, J = 7.5 Hz, 2H), 1.21−1.27 (m, 6H).

(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(thiazol-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide ***(4x)***. Melting point 187−191 °C. MS m/z 523.5 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.99 (dd, J = 0.79, 2.36 Hz, 1H), 8.46 (dd, J = 2.5, 8.2 Hz, 1H), 8.09 (dd, J = 1.0, 8.2 Hz, 1H), 7.80 (s, 2H), 7.70 (br s, 1H),

dx.doi.org/10.1021/jm401621g | J. Med. Chem. XXXX, XXX, XXX−XXX

7.64 (d, J = 5.67 Hz, 1H), 7.54 (d, J = 9.46 Hz, 1H), 4.26–4.36 (m, 1H), 2.81 (q, J = 7.57 Hz, 2H), 1.26 (d, J = 7.0 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H).

*(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(5-fluoropyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4y**).* MS m/z 537.0 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.87 (dd, J = 0.9, 2.5 Hz, 1H), 8.83 (s, 2H), 8.48 (dd, J = 2.5, 8.2 Hz, 1H), 8.19 (dd, J = 0.9, 8.2 Hz, 1H), 8.01 (d, J = 6.3 Hz, 1H), 7.64–7.69 (m, 1H), 7.54 (d, J = 9.77 Hz, 1H), 4.26–4.33 (m, 1H), 2.82 (q, J = 7.5 Hz, 2H), 1.24–1.28 (m, 6H).

*(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(5-methylpyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4z**).* Melting point 201–206 °C. MS m/z 533.2 $[M + H]^+$. ^{1}H NMR (500 MHz, acetone-d_6) δ 8.87 (dd, J = 0.63, 2.21 Hz, 1H), 8.65 (d, J = 0.63 Hz, 2H), 8.45 (dd, J = 2.21, 8.20 Hz, 1H), 8.09–8.12 (m, 1H), 8.07 (d, J = 6.62 Hz, 1H), 7.62–7.69 (br s, 1H), 7.52 (d, J = 9.46 Hz, 1H), 4.25–4.34 (m, 1H), 2.82 (q, J = 7.57 Hz, 2H), 2.39 (s, 3H), 1.24–1.30 (m, 6H).

*(S)-6-(3-Cyano-6-ethyl-5-fluoro-1-(5-fluoropyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4aa**).* Melting point 210–212 °C. MS m/z 535.8 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (dd, J = 1.0, 2.5 Hz, 1H), 8.35 (d, J = 2.5 Hz, 1H), 8.24 (dd, J = 2.5, 8.2 Hz, 1H), 8.10 (dd, J = 0.9, 8.2 Hz, 1H), 7.59–7.63 (m, 1H), 7.50 (d, J = 9.4 Hz, 1H), 7.32–7.35 (m, 1H), 7.20 (d, J = 5.4 Hz, 1H), 4.83 (d, J = 9.5 Hz, 1H), 4.04–4.10 (m, 1H), 2.78 (1, J = 7.5 Hz, 2H), 1.42 (d, J = 7.0 Hz, 3H), 1.25 (t, J = 7.5 Hz, 3H).

*(S)-6-(3-Cyano-6-cyclopropyl-5-fluoro-1-(pyrimidin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4bb**).* Melting point 236–240 °C. MS m/z 531.2 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (dd, J = 0.9, 2.2 Hz, 1H), 8.68 (d, J = 4.7 Hz, 2H), 8.28 (dd, J = 2.6, 8.5 Hz, 1H), 8.10 (dd, J = 0.9, 8.5 Hz, 1H), 7.71 (d, J = 6.0 Hz, 1H), 7.47 (d, J = 9.1 Hz, 1H), 7.29 (t, J = 4.7 Hz, 1H), 4.88 (d, J = 9.4 Hz, 1H), 4.04–4.12 (m, 1H), 2.19–2.25 (m, 1H), 1.42 (d, J = 6.94 Hz, 3H), 1.02–1.09 (m, 2H), 0.76–0.81 (m, 2H).

*(S)-6-(3-Cyano-6-cyclopropyl-5-fluoro-1-(pyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4cc**).* Melting point 220–223 °C. MS m/z 530.1 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (dd, J = 1.0, 2.5 Hz, 1H), 8.49–8.52 (m, 1H), 8.20 (dd, J = 2.5, 8.5 Hz, 1H), 8.00 (dd, J = 0.9, 8.5 Hz, 1H), 7.88 (dt, J = 1.9, 7.6 Hz, 1H), 7.49 (d, J = 9.4 Hz, 1H), 7.38–7.42 (ddd, J = 0.9, 4.7, 7.5 Hz, 1H), 7.24–7.27 (m, 1H), 7.02 (d, J = 5.9 Hz, 1H), 4.85 (d, J = 9.7 Hz, 1H), 4.00–4.09 (m, 1H), 2.14–2.21 (m, 1H), 1.40 (d, J = 7.25 Hz, 3H), 0.99–1.04 (m, 2H), 0.68–0.72 (m, 2H).

*(S)-6-(3-Cyano-6-cyclopropyl-5-fluoro-1-(5-fluoropyridin-2-yl)-1H-indol-2-yl)-N-(1,1,1-trifluoropropan-2-yl)pyridine-3-sulfonamide (**4dd**).* Melting point 240–242 °C. MS m/z 548.2 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (d, J = 2.5 Hz, 1H), 8.34 (d, J = 2.84 Hz, 1H), 8.23 (dd, J = 2.5, 8.5 Hz, 1H), 8.09 (dd, J = 0.9, 8.2 Hz, 1H), 7.58–7.63 (m, 1H), 7.50 (d, J = 9.46 Hz, 1H), 7.30 (dd, J = 3.5, 8.8 Hz, 1H), 6.94 (d, J = 5.7 Hz, 1H), 4.84 (d, J = 9.8 Hz, 1H), 4.03–4.10 (m, 1H), 2.15–2.22 (m, 1H), 1.42 (d, J = 6.94 Hz, 3H), 0.99–1.06 (m, 2H), 0.66–0.72 (m, 2H).

Biological Assays. *HCV Replicon Assay.* Huh-7 cells harboring a genotype 1b HCV bicistronic replicon (Con1)[16] were plated at 5000 cells/well in 96-well plates. Compounds were added to the plates with a final concentration of 0.5% DMSO, and plates were incubated at 37 °C. At the end of compound treatment, the cells were lysed with Cell-to-cDNA lysis buffer (Ambion, Austin, TX). RT-PCR was carried out using TaqMan One-Step RT-PCR Master Mix reagents kit (Applied Biosystems) with HCV primers (sense S66 [ACGCAGAAAGCGTCTAGCCAT] and antisense A165 [TACTCACCGGTTCCGCAGA]) and probe (5′-6FAM-TCCTGGAGGCTGCACGACACTCAT-3′ TAMRA) at 100 μM. The effect of the compound on the amount of the housekeeping gene GAPDH (glyceraldehyde 3-phosphate dehydrogenase) mRNA as a control was determined by using Predeveloped TaqMan Assay reagents (Applied Biosystems, Foster City, CA). Both HCV replicon and GAPDH RNAs were amplified in the same well using ABI 7900HT (Applied Biosystems). EC_{50} values are reported as the average of at least two independent determinations.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Syntheses for indoles **5b** and **5n** and sulfonamides **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1-9089129238. E-mail: nzhang@ptcbio.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Takashi Komatsu, Shirley Yeh, and Jane Yang (PTC Therapeutics, Inc.) for generating data reported in this manuscript. We also thank Drs. Alan Bass, John Piwinski, and Malcolm MacCoss (Merck Research Laboratories) for their helpful discussions during the course of this work.

■ ABBREVIATIONS USED

B_2Pin_2, bis(pinacolato)diboron; GSH, glutathione; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NS, nonstructural; NS4B, nonstructural protein 4B; RT-PCR, real-time polymerase chain reaction

■ REFERENCES

(1) (a) *Hepatitis C Fact Sheet No. 164*; World Health Organization: Geneva, July 2012; www.who.int. (b) Alter, M. J.; Kruszon-Moran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N. Engl. J. Med.* **1999**, *341*, 556–562.

(2) Shepard, C. W.; Finelli, L.; Alter, M. J. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* **2005**, *5*, 558–567.

(3) Zein, N. N. Clinical significance of hepatitis C virus genotypes. *Clin. Microbiol. Rev.* **2000**, *13*, 223–235.

(4) Sheridan, C. New Merck and Vertex drugs raise standard of care in hepatitis C. *Nature Biotechnol.* **2011**, *29*, 553–554.

(5) Rong, L.; Dahari, H.; Ribeiro, R. M.; Perelson, A. S. Rapid emergence of protease inhibitor resistance in hepatitis C virus. *Sci. Transl. Med.* **2010**, *2*, 30ra32.

(6) (a) Kato, N. Genome of human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. *Microb. Comp. Genomics* **2000**, *5*, 129–151. (b) Simmonds, P.; Bukh, J.; Combet, C.; Deleage, G.; Enomoto, N.; Feinstone, S.; Halfon, P.; Inchauspe, G.; Kuiken, C.; Maertens, G.; Mizokami, M.; Murphy, D. G.; Okamoto, H.; Pawlotsky, J. M.; Penin, F.; Sablon, E.; Shin, I. T.; Stuyver, L. J.; Thiel, H. J.; Viazov, S.; Weiner, A. J.; Widell, A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* **2005**, *42*, 962–973.

(7) Kiser, J. J.; Flexner, C. Direct-acting antiviral agents for hepatitis C virus infection. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 427–449.

(8) (a) Chen, K. X.; Njoroge, F. G. A review of HCV protease inhibitors. *Curr. Opin. Invest. Drugs* **2009**, *10*, 821–837. (b) Marks, K. M.; Jacobson, I. M. The first wave: HCV NS3 protease inhibitors telaprevir and boceprevir. *Antiviral Ther.* **2012**, *17*, 1119–1131.

(9) (a) Gao, M.; Nettles, R. E.; Belema, M.; Snyder, L. B.; Nguyen, V. N.; Fridell, R. A.; Serrano-Wu, M. H.; Langley, D. R.; Sun, J.-H.; O'Boyle, D. R., II.; Lemm, J. A.; Wang, C.; Knipe, J. O.; Chien, C.; Colonno, R. J.; Grasela, D. M.; Meanwell, N. A.; Hamann, L. G. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* **2010**, *465*, 96–100. (b) Belda, O.; Targett-Adams, P. Small molecule inhibitors of the hepatitis C virus-encoded NS5A protein. *Virus Res.* **2012**, *170*, 1–14. (c) Suk-Fong, L.

A. HCV NS5A inhibitors in development. *Clin. Liver Dis.* **2013**, *17*, 111−121.
(10) (a) Watkins, W. J.; Ray, A. S.; Chong, L. S. HCV NS5B polymerase inhibitors. *Curr. Opin. Drug Discovery Dev.* **2010**, *13*, 441−465. (b) Sofia, M. J.; Chang, W.; Furman, P. A.; Ralph T. Mosley, R. T.; Bruce S. Ross, B. S. Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase. *J. Med. Chem.* **2012**, *55*, 2481−2531.
(11) (a) Sklan, E. H.; Glenn, J. S. HCV NS4B: From obscurity to central stage. In *Hepatitis C Viruses: Genomes and Molecular Biology*; Tan, S. L., Ed.; Horizon Bioscience: Norfolk, UK, 2006; Chapter 8. (b) Einav, S.; Sobol, H. D.; Gehrig, E.; Glenn, J. S. The hepatitis C virus (HCV) NS4B RNA binding inhibitor clemizole is highly synergistic with HCV protease inhibitors. *J. Infect. Dis.* **2010**, *202*, 65−74.
(12) (a) Dvory-Sobol, H.; Pang, P. S.; Glenn, J. S. The future of HCV therapy: NS4B as an antiviral target. *Viruses* **2010**, *2*, 2481−2492. (b) Gouttenoire, J.; Penin, F.; Moradpour, D. Hepatitis C virus nonstructural protein 4B: a journey into unexplored territory. *Rev. Med. Virol.* **2010**, *20*, 117−129. (c) Rai, R.; Deval, J. New opportunities in anti-hepatitis C virus drug discovery: Targeting NS4B. *Antiviral Res.* **2011**, *90*, 93−101.
(13) (a) Shotwell, J. B.; Baskaran, S.; Chong, P.; Creech, K. L.; Crosby, R. M.; Dickson, H.; Fang, J.; Garrido, D.; Mathis, A.; Maung, J.; Parks, D. J.; Pouliot, J. J.; Price, D. J.; Rai, R.; Seal, J. W., III; Schmitz, U.; Tai, V. W. F.; Thomson, M.; Xie, M.; Xiong, Z. Z.; Peat, A. J. Imidazo[1,2-*a*]pyridines that directly interact with hepatitis C NS4B: Initial preclinical characterization. *ACS Med. Chem. Lett.* **2012**, *3*, 565−569. (b) Miller, J. F.; Chong, P. Y.; Shotwell, J. B.; Catalano, J. G.; Tai, V. W.-F.; Fang, J. M.; Banka, A. L.; Roberts, C. D.; Youngman, M. K.; Zhang, H.; Xiong, Z.; Mathis, A.; Pouliot, J. J.; Hamatake, R. K.; Price, D. J.; Seal, J. W.; Stroup, L. L.; Creech, K. L.; Carballo, L. H.; Todd, D.; Spaltenstein, A.; Furst, S. M.; Hong, Z.; Peat, A. J. Hepatitis C replication inhibitors that target the viral NS4B protein. *J. Med. Chem.* **2013**, DOI: 10.1021/jm400125h.
(14) Chen, G.; Ren, H.; Turpoff, A.; Arefolov, A.; Wilde, R.; Takasugi, J.; Khan, A.; Almstead, N.; Gu, Z.; Komatsu, T.; Freund, C.; Breslin, J.; Colacino, J.; Hedrick, J.; Weetall, M.; Karp, G. M. Discovery of *N*-(4′-(indol-2-yl)phenyl) sulfonamides as novel inhibitors of HCV replication. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3942−3946.
(15) Zhang, X.; Zhang, N.; Chen, G.; Turpoff, A.; Ren, H.; Takasugi, J.; Morrill, C.; Zhu, J.; Li, C.; Lennox, W.; Paget, S.; Liu, Y.; Almstead, N.; Njoroge, F. G.; Gu, Z.; Komatsu, T.; Clausen, V.; Espiritu, C.; Graci, J.; Colacino, J.; Lahser, F.; Risher, N.; Weetall, M.; Nomeir, A.; Karp, G. M. Discovery of novel HCV inhibitors: synthesis and biological activity of 6-(indol-2-yl)pyridine-3-sulfonamides targeting hepatitis C virus NS4B. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3947−3953.
(16) Lohmann, V.; Körner, F.; Koch, J.-O.; Herian, U.; Theilmann, L.; Bartenschlager, R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* **1999**, *285*, 110−113.
(17) Biological activity of 1,3-difluoroacetone, see: (a) Menon, K. I.; Feldwick, M. G.; Noakes, P. S.; Mead, R. J. The mode of toxic action of the pesticide Gliftor: the metabolism of 1,3-difluoroacetone to (−)-erythro-fluorocitrate. *J. Biochem. Mol. Toxicol.* **2001**, *15*, 47−54. (b) Feldwick, M. G.; Noakes, P. S.; Prause, U.; Mead, R. J.; Kostyniak, P. J. The biochemical toxicology of 1,3-difluoro-2-propanol, the major ingredient of the pesticide gliftor: the potential of 4-methylpyrazole as an antidote. *J. Biochem. Mol. Toxicol.* **1998**, *12*, 41−52.
(18) Toxicology of glutathione adducts, see: (a) Walsh, J. S.; Miwa, G. T. Bioactivation of drugs: risk and drug design. *Annu. Rev. Pharmacol. Toxicol.* **2011**, *51*, 145−167. (b) Park, B. K.; Laverty, H.; Srivastava, A.; Antoine, D. J.; Naisbitt, D.; Williams, D. P. Drug bioactivation and protein adduct formation in the pathogenesis of drug-induced toxicity. *Chem. Biol. Interact.* **2011**, *192*, 30−36.
(19) HCV patients may have depleted glutathione levels, see: Barbaro, G.; di Lorenzo, G.; Soldini, M.; Parrotto, S.; Bellomo, G.; Belloni, G.; Grisorio, B.; Barbarini, G. Hepatic glutathione deficiency in chronic hepatitis C: quantitative evaluation in patients who are HIV positive and HIV negative and correlations with plasmatic and lymphocytic concentrations and with the activity of the liver disease. *Am. J. Gastroenterol.* **1996**, *91*, 2569−2573.
(20) Gu, Z.; Graci, J. D.; Lahser, F. C.; Breslin, J. J.; Jung, S. P.; Crona, J. H; McMonagle, P.; Xia, E.; Liu, S.; Karp, G.; Zhu, J.; Huang, S.; Nomeir, A.; Weetall, M.; Almstead, N. G.; Peltz, S. W.; Tong, X.; Ralston, R.; Colacino, J. M. Identification of PTC725, an orally bioavailable small molecule that selectively targets the hepatitis C virus NS4B protein. *Antimicrob. Agents Chemother.* **2013**, *57*, 3250−3261.
(21) Biological activity of 1,1,1-trifluoroacetone, see: (a) Allen, K. N.; Abeles, R. H. Inhibition kinetics of acetylcholinesterase with fluoromethyl ketones. *Biochemistry* **1989**, *28*, 8466−8473. (b) Allen, K. N.; Abeles, R. H. Inhibition of pig liver esterase by trifluoromethyl ketones: modulators of the catalytic reaction alter inhibition kinetics. *Biochemistry* **1989**, *28*, 135−140. (c) Hammock, B. D.; Wing, K. D.; McLaughlin, J.; Lovell, V. M.; Sparks, T. C. Trifluoromethylketones as possible transition state analog inhibitors of juvenile hormone esterase. *Pestic. Biochem. Physiol.* **1982**, *17*, 76−88. (d) Brodbeck, U.; Schweikert, K.; Gentinetta, R.; Rottenberg, M. Fluorinated aldehydes and ketones acting as quasi-substrate inhibitors of acetylcholinesterase. *Biochim. Biophys. Acta* **1979**, *567*, 357−369.
(22) (a) Moore, J. T.; Kliewer, S. A. Use of the nuclear receptor PXR to predict drug interactions. *Toxicology* **2000**, *153*, 1−10. (b) Gao, Y.-D.; Olson, S. H.; Balkovec, J. M.; Zhu, Y.; Royo, I.; Yabut, J.; Evers, R.; Tan, E. Y.; Tang, W.; Hartley, D. P.; Mosley, R. T. Attenuating pregnane X receptor (PXR) activation: a molecular modeling approach. *Xenobiotica* **2007**, *37* (2), 124−138.
(23) Yi, M.; Villanueva, R. A.; Thomas, D. L.; Wakita, T.; Lemon, S. M. Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured human hepatoma cells. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 2310−2315.