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Structure–Activity Relationship (SAR) Development and Discovery of Potent Indole-Based Inhibitors of the Hepatitis C Virus (HCV) NS5B Polymerase

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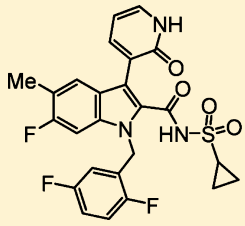
ABSTRACT: Starting with the indole-based C-3 pyridone lead HCV polymerase inhibitor **2**, extensive SAR studies were performed at different positions of the indole core. The best C-5 groups were found to be compact and nonpolar moieties and that the C-6 attachments were not affecting potency. Limited *N*-1 benzyl-type substituent studies indicated that the best substitutions were fluoro or methyl groups at 2′ or 5′ positions of the benzyl group. To improve pharmacokinetic (PK) properties, acylsulfonamides were incorporated as acid isosteres at the C-2 position. Further optimization of the combination at *N*-1, C-2, C-5, and C-6 resulted in the identification of compound **56**, which had an excellent potency in both NS5B enzyme (IC_{50} = 0.008 μM) and cell-based replicon (EC_{50} = 0.02 μM) assays and a good oral PK profile with area-under-the curve (AUC) of 14 and 8 μM·h in rats and dogs, respectively. X-ray structure of inhibitor **56** bound to the enzyme was also reported.



HCV NS5B RdRp assay:
IC_{50} = 0.008 μM
HCV replicon cell-based assay:
EC_{50} = 0.02 μM

INTRODUCTION

Hepatitis C virus (HCV) has infected an estimated 3% of the world's population (over 170 million people).[1] The slow progression and mild symptoms of the HCV infection make it a stealth epidemic. Most infections progress to a chronic state that persists for decades and eventually lead to cirrhosis, liver failure, or liver cancer.[2] Currently, there is no anti-HCV vaccine available. The new standard of care (SOC) after recent protease inhibitor approval involves the combination of a protease inhibitor with pegylated α-interferon and the oral nucleoside antiviral agent ribavirin.[3] The outcome of the treatment is defined by the sustained virologic response (SVR) or undetectable HCV RNA in serum at the end of treatment and six months post treatment. The SVR rate for patients with the most difficult to treat genotype-1 HCV is about 70% with the new current therapy.[3] Large pill burden (750–800 mg 3-times a day), relapse (mutations), and discontinuation due to the presence of side effects with these therapies necessitates the need for new and more effective HCV treatments.

Hepatitis C virus was identified more than two decades ago.[4] It belongs to the Flaviviridae family of enveloped viruses. It is a positive-sense single-stranded RNA virus with a single open frame of ~9600 nucleosides. The viral genome encodes a polyprotein of more than 3000 amino acids. The polyprotein is divided into structural and nonstructural precursor regions. The structural protein contains the nucleocapsid core protein (C) and two glycoproteins E1 and E2 (NS1). The nonstructural proteins located downstream are: NS2, NS3, NS4A, NS4B, NS5A, and NS5B. Because of their essential role in the replication of HCV virus, intensive research has been focused on finding drugs directly targeting these nonstructural proteins, and exciting progress has been made in these endeavors.[5] Clinical trials have demonstrated dramatic improvements in SVR rate in patients treated with a direct acting agent in combination with pegylated interferon and ribavirin.[6] Recent approval of the two HCV NS3 protease inhibitors boceprevir[7] (Merck & Co.) and telaprevir[8] (Vertex Pharmaceuticals) by the FDA have generated great excitement for the new treatments of HCV infection. Several other NS3 protease inhibitors and a number of candidates targeting other nonstructural enzymes are also under development.[5]

The HCV NS5B gene encodes an RNA-dependent RNA polymerase (RdRp) whose enzymatic activity is critical to the replication of the viral RNA genome.[9] Replication of the plus-strand RNA viral genome consists of two steps: synthesis of the complementary negative-strand RNA using the positive-strand RNA as a template, and the subsequent synthesis of multiple positive-strand RNA genome using the minus-strand RNA as a template. Besides its direct role in the replication of viral RNA

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genome, the HCV NS5B polymerase is potentially associated with a number of other important functions. It forms a replicase complex (RC) with other HCV nonstructural proteins (NS3, NS4A, NS4B, and NS5A) and a number of cellular cofactors during replication.[10] It was also suggested that NS5B might regulate the function of other HCV enzymes. The crystal structure of the catalytic domain of N5SB has revealed several significant features.[11] The polymerase shares many common features of other polymerases with characteristic thumb, finger, and palm domains analogous with a right hand. In addition to a well-conserved active site (located in the palm domain) for nucleoside binding, mechanistic and structural studies have revealed the existence of multiple allosteric inhibitor binding sites. Binding to these sites interferes with conformational changes required during RNA synthesis. Thumb pockets I and II are two allosteric sites that are located in thumb domain, while palm site I and II are two pockets located adjacent to the active site. These thumb and palm region sites have been popular targets for drug development, which makes the NS5B a particularly druggable target.[12,13]

Extensive efforts directed at inhibiting HCV NS5B polymerase have resulted in numerous drug candidates. They belong to two distinctive classes of inhibitors: nucleoside active site inhibitors (NIs) and non-nucleoside allosteric inhibitors (NNIs).[12,13] They target different stages of RNA synthesis.[14] NNIs were shown to interfere with steps prior to or during the initiation of RNA synthesis. In contrast, the triphosphate form of NIs binds to the active site during elongation. NIs are analogues of natural substrates of the polymerase that are incorporated into the growing RNA chain leading to chain termination. These agents require phosphorylation before being active. Because NS5B is a highly conserved region of the HCV genome, NIs have similar activity against all genotypes and a high genetic barrier to resistance.[15] Several NIs have advanced into clinical trials, including prodrugs valopicitabine (Idenix), R1626 (Roche), RG-7128 (Pharmasset/Roche), PSI-7977 (Pharmasset), PSI-352938 (Pharmasset), IDX-184 (Idenix), and INX-189 (Inhibitex).[16] NNIs, on the other hand, achieve NS5B inhibition by binding to one of at least five allosteric enzyme sites resulting in conformational changes which alter the protein-inhibiting catalytic activity of polymerase. A number of clinical candidates have been identified,[12,13] among them, HCV-796 (Wyeth/Viropharma),[17] filibuvir (Pfizer),[18] VX-222 (Vertex/Virochem),[19] ANA598 (Anadys),[20] BI207127 (Boehringer Ingelheim),[21] and BMS-791325 (Bristol-Myers Squibb)[22] have been progressed to phase II clinical trials. The rapid development of resistant mutants has been observed with NNIs because they bind distantly to the active center of NS5B and mutations at the NNI binding site may not necessary lead to impairment of the enzyme function.

The indole-based lead compound, **1**, was discovered through screening effort at Schering-Plough Research Institute. It had only moderate binding activity (IC_{50} = 0.9 μM) in an RdRp enzyme binding assay.[23] It was inactive in the cell-based replicon assay[24] (EC_{50} > 100 μM). The X-ray structure of **1** in complex with NS5B indicated that it bound to a NS5B apoprotein cavity adjacent to the active site at the "palm" site of the protein.[25] There was an unusual F···H−N hydrogen bonding between the C-3 fluorophenyl and tyrosine-448. Starting from this lead compound, extensive SAR investigations were conducted to improve potency of the indole-based inhibitors. A breakthrough was achieved when C-3 2-fluorophenyl group was replaced with a 3-pyridone moiety, and the benzene ring at *N*-1 substituent was replaced with 4-(2-amino)pyridine, as shown in structure **2** (Figure 1).[25] The

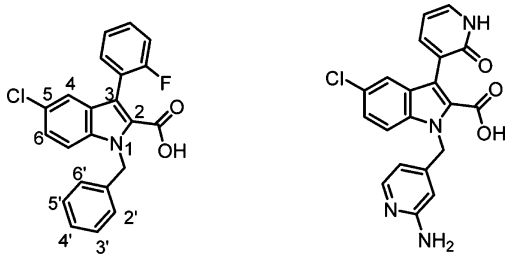
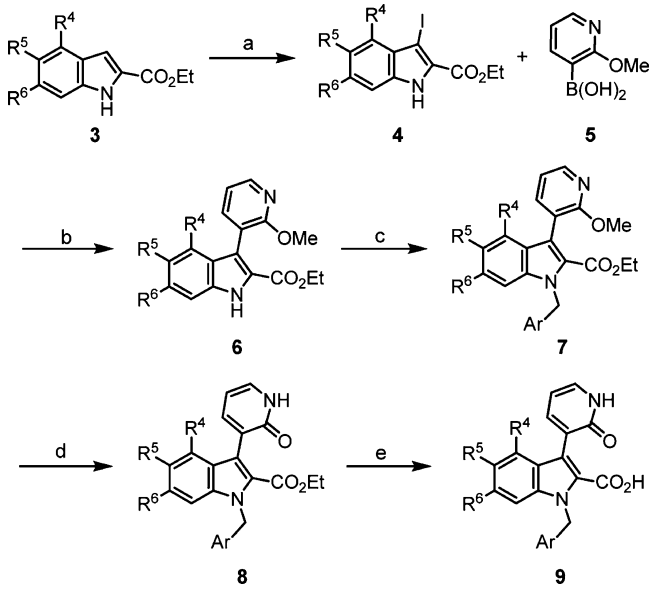


Figure 1. HCV NS5B polymerase inhibitors early lead structures.

pyridone carbonyl and N−H formed strong hydrogen bonds with backbone isoleucine-447 residue, and the amino-pyridine moiety had a hydrogen bonding interaction with serine-367. As a result of the additional interactions, there was a near 20-fold improvement in enzyme potency. More importantly, **2** demonstrated activity in the cellular replicon assay with an EC_{50} of 4.8 μM. Starting from this new lead compound **2**, our task was to develop inhibitors with potency and PK properties desirable in a drug candidate. Herein, we report the SAR development and the discovery of a potent HCV NS5B polymerase inhibitor which exhibited a good PK profile in rats.

Synthesis of HCV NS5B Polymerase Inhibitors. The general synthesis of HCV NS5B polymerase inhibitors is outlined in Schemes 1 and 2. The ethyl indole-2-carboxylate **3**,

Scheme 1[a]



[a]Reagents and conditions: (a) NIS, acetone, quant; (b) $PdCl_2(dppf)_2$, K_2CO_3, 1,2-dimethoxyethane, 50−85%; (c) $ArCH_2Br$ or $ArCH_2Cl$, Cs_2CO_3, DMF, 40−90%; (d) 4 M HCl, *p*-dioxane; (e) LiOH, THF/H_2O 30−60% (2 steps).

with desired substituents at 4,5,6-positions, was either commercially available or could be prepared via known procedures. Intermediate **3** was iodinated at C-3 with *N*-

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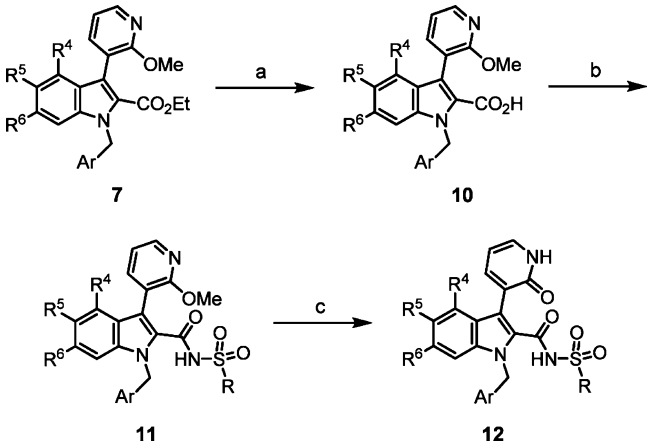
Scheme 2[a]



[a]Reagents and conditions: (a) LiOH, THF/H_2O; (b) CDI, then RSO_2NH_2, DBU, THF, 50−80% (2 steps); (c) 4 M HCl, *p*-dioxane, 30−50%.

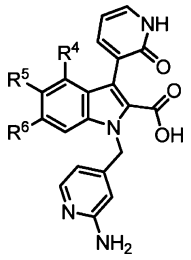
iodosuccinimide (NIS) to give compound **4**. The Suzuki−Miyaura cross-coupling reaction[26] between **4** and 2-methoxy-3-pyridine boronic acid **5** in the presence of a palladium catalyst afforded product **6** in 50−85% yields from **3**. The indole nitrogen was then alkylated to provide compound **7** in 40−90% yields through alkylation with a benzylic halide. The methoxypyridine in **7** was then converted to pyridone product **8** upon treatment with hydrochloric acid. The ethyl ester was finally hydrolyzed to carboxylic acid **9** with yields of 30−60% in two steps, which was tested in RdRp enzyme assay and replicon cell-based assay.

The indole-2-acylsulfonamide series of polymerase inhibitors were prepared according to the procedures shown in Scheme 2. Compound **7** was hydrolyzed to its corresponding carboxylic acid **10**. The acid was activated with carbonyl diimidazole (CDI) and then reacted with a sulfonamide in the presence of diazabicyclicunderdecane (DBU)[27] to give desired acylsulfonamide **11** in 50−80% yields from **7**. Product **11** was then treated with hydrochloric acid to provide pyridone final product **12** in 30−50% purified yields. The inhibitors were evaluated in enzyme and replicon assays to determine their biological activities.

RESULTS AND DISCUSSIONS

Preliminary SAR at Indole C-4, C-5, and C-6 Positions. With the lead compound **2** exhibiting interesting biological activity, an effort was taken to improve the potency via optimization of various substituents at different positions of the indole core. First, the effect of additional substitution at C-4 and C-6 positions was examined and the SAR is summarized in Table 1 (compounds **13**−**15**). Starting from compound **2**, a second chloro substituent was introduced at C-4. Unfortunately, the resulting inhibitor **13** had an IC_{50} of 0.75 μM in an RdRp enzyme binding assay,[23] a 14-fold decrease in activity. The removal of the C-5 chloro group and introduction of a larger bromo atom at C-4 gave **14** (IC_{50} = 4.0 μM) with a 75-fold loss in activity compared to **2**. The detrimental effect of the C-4 substituents was probably caused by its intervention of proper orientation of the C-3 pyridone ring for optimal hydrogen bonding. The investigation was then focused on C-6 modifications. An additional chloro group was introduced to C-6 of compound **2** to give inhibitor **15**. The potency of this compound remained essentially unchanged at approximately 0.05 μM (IC_{50}). It appeared that the C-6 substituent did not adversely affect the potency.

Table 1. Modifications at C-4, C-5, and C-6 of Indole Core



Compound	R^4	R^5	R^6	RdRp IC_{50} (μM)(n = 2)[23]
2	H	Cl	H	0.053
13	Cl	Cl	H	0.75
14	Br	H	H	4.0
15	H	Cl	Cl	0.047
16	H	Br	H	0.044
17	H		H	7.0
18	H		H	0.065
19	H		H	1.4
20	H		H	0.33
21	H		H	0.048
22	H	OH	H	> 20
23	H		H	0.016
24	H	CF_3	H	0.017

The effort was next focused on C-5 optimization (Table 1). Groups were introduced at this position to evaluate the SAR effect of the size, polarity, and H-bonding ability of the substituents. Replacing the chloro atom in **2** with a larger bromo atom gave rise to compound **16**, which had similar activity (IC_{50} = 0.047 μM). A large six-membered pyridone analogue (**17**) was clearly not tolerated with significantly decreased activity (IC_{50} of 7.0 μM). Similar effects were observed for inhibitors (**19**, **20**, and **22**) with other polar substituents, from the larger methyl sulfone in **19**, to medium sized acetyl group in **20**, and to small hydroxyl in **22**. The complete lack of activity of **22** was quite striking. These results

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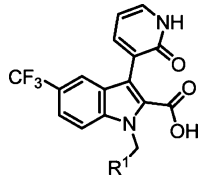
point to the conclusion that the enzyme surface near the C-5 substituent was highly hydrophobic and that polar functionalities were not tolerated. Further evidence of a hydrophobic pocket was provided by the good activity observed with the cyclopropyl analogue **18** and acetylene analogue **21**. Both **18** and **21** had similar potency to that of compound **2**. The breakthrough came from trifluoromethyl and trifluoromethoxy substituted compounds **23** and **24**. These two inhibitors were three times more potent than the lead compound **2** and served as the basis for further optimization at other positions of the indole ring.

SAR Studies of *N*-1 Aromatic Substituents. Although compound **24** had improved binding (IC_{50}), it did not possess a desirable PK profile in rats as exemplified by its negligible oral AUC value (<0.1 μM·h). Most other compounds with the 2-aminopyridinemethyl substituent at indole nitrogen (*N*-1) also displayed poor PK. This structure element was presumably responsible for the lack of exposure of these compounds in vivo. A metabolically more stable moiety was clearly desirable at the *N*-1 position. Thus, a number of *N*-1 benzyl-based substituents were investigated. Because anilines are known to have potential toxicity concerns, we decided to screen benzyl groups with substituents other than a primary amino group. The results of this SAR study are shown in Table 2 with representative compounds.

Table 2. SAR at Indole Core *N*-1 Aromatic Substituent



Compound	R^1	RdRp IC_{50} (μM)(n = 2)[23]
24		0.017
25		0.025
26		0.067
27		0.009
28		0.009
29		0.016
30		0.025
31		0.005
32		0.004
33		0.007

Substitution at different positions of the benzene ring was explored. The *para*-methoxy substituted benzyl analogue **25** was almost equally potent (IC_{50} = 0.025 μM) to compound **24**, while difluoromethoxy analogue **26** was about 3-fold less active. However, simple *ortho*-methyl or fluoro substituted benzyl groups provided more potent compounds **27** and **28**, both with an IC_{50} of 0.009 μM. Additional methyl or fluoro groups at the *para*-position in **29** or **30** caused a small drop in activity (IC_{50} = 0.016 and 0.025 μM, respectively). In contrast, the additional substitution at either *meta*-position enhanced the potency as evidenced in compounds **31** and **32**. Both 2′-fluoro-3′-methyl substituted analogue **31** and the 2′,5′-difluoro structure **32** had excellent activities (IC_{50} = 0.005 and 0.004 μM, respectively). Some polar functionalities such as a primary amide at the 5′-position (**33**) were not only tolerated but improved the potency (IC_{50} = 0.007 μM) compared to **24**. Overall, the *N*-1 substituent SAR exercise improved the potency by 3−4-fold, and more importantly, several aromatic moieties that were more likely to demonstrate good PK properties were discovered. The 2′,5′-difluorobenzyl group in compound **32** was selected for further SAR investigations.

Further Optimization of C-5 Substitution. After the discovery of an *N*-1 benzyl group suitable for continued SAR development, we believed that it was necessary to re-examine C-5 substituents. We learned from earlier C-5 SAR studies (Table 1) that very polar substituents were not suitable for that position. Thus, a select group of nonpolar aliphatic structures were evaluated (Table 3). The compounds bearing trifluoromethoxy (**23**) or trifluoromethyl (**24**) groups were found to be equally potent in Table 1. In contrast, the compound with a C-5 trifluoromethoxy group in the 2′,5′-difluorobenzyl series (**34**, IC_{50} = 0.024 μM) was less active than the corresponding trifluoromethyl analogue (**32**, IC_{50} = 0.004 μM). The methoxy substituted compound **35** fared slightly better (IC_{50} = 0.015 μM), albeit slightly less active. Replacement with the larger ethoxy moiety caused a significant drop in activity in analogue **36** (IC_{50} = 0.079 μM). The four-carbon chain 1-butenyl group, not surprisingly, gave rise to an even greater loss of potency in inhibitor **37** (IC_{50} = 0.15 μM). A few smaller alkyl substituents, however, afforded interesting compounds with excellent IC_{50} values. The 5-methyl analogue **38** had very good activity (IC_{50} = 0.008 μM). The slightly larger ethyl and bromo substituents provided two inhibitors (**39**, **40**) with IC_{50}s of 0.004 and 0.003 μM, respectively. Similar sized trifluoroethyl analogue **41** was also quite active, although with slight loss of potency compared to **32**. The bulkier *tert*-butyl and 1-methylcyclopropyl substitutions were tolerated (**42** and **43**) with IC_{50}s of 0.008 and 0.009 μM, respectively, about 2-fold higher than that of **32**. On the basis of the overall profile of the compounds, the potential liability of the substituents and ease of synthesis, we decided to focus our future SAR studies on three small alkyl C-5 substituents that showed excellent potency: methyl, ethyl, and trifluoromethyl groups.

C-2-Acylsulfonamide SAR Investigation. Although a number of C-2 carboxylic acid inhibitors examined in Tables 1, 2, and 3 demonstrated excellent potency in the enzyme assay, their activities in cellular replicon assay were much less impressive, with an EC_{50} typically in the range of 0.1 μM to greater than 1.0 μM. For example, compound **38** had an EC_{50} of 1.1 μM. The fact that these carboxylic acids were poorly soluble might be a factor in hindering them from cellular uptake because they existed as carboxylate salts under physiological pH. To improve replicon assay potency and solubility of these indole derivatives, the carboxylic acid moiety was replaced by an acylsulfonamide isostere. Thus, a set of simple alkyl acylsulfonamides were prepared (Table 4) and their potency in enzyme and cellular replicon assays was determined.

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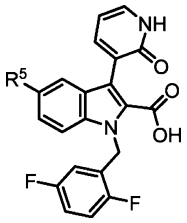
Table 3. Optimization of C-5 Substituent

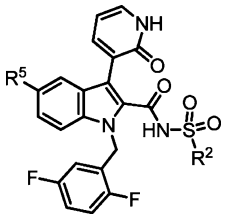


Compound	R^5	RdRp IC_{50} (μM)(n = 2)[23]
32	CF_3	0.004
34		0.024
35		0.015
36		0.079
37		0.15
38	Me	0.008
39		0.004
40	Br	0.003
41		0.008
42		0.008
43		0.009

Compounds **44**–**47** had a C-5 methyl substituent, while compounds **48**–**51** had a C-5 ethyl group. From the C-2 methyl acylsulfonamide (**44**) to ethyl, isopropyl, and cyclopropyl acylsulfonamides (**45**, **46**, and **47**, respectively), the potency against the enzyme remained approximately the same in a narrow range of 0.006–0.010 μM. The potency in cell-based replicon assay, however, varied significantly, but they were all better than the EC_{50} of compound **38** (1.1 μM). The methyl sulfonamide analogue (**44**) was the least potent (EC_{50} = 0.49 μM), but ethyl, isopropyl, and cyclopropyl sulfonamides became progressively more potent (EC_{50} = 0.20, 0.09, 0.09 μM, for compounds **45**, **46**, and **47**, respectively). Similar trends were observed in the C-5-ethyl series. From compounds **48** to **51**, the enzyme assay activity IC_{50} improved 2-fold from 0.006 (for **48**) to 0.003 μM (for **51**). More improvement was observed in replicon activity in this series from methyl acylsulfonamide **48** (EC_{50} = 0.15 μM) to cyclopropyl acylsulfonamide **51** (EC_{50} = 0.06 μM).

Along with the efforts to improve the enzyme and cellular assay potency, the PK profile of the compounds was evaluated and used as an important criterion to select the best compound for further investigation. The AUCs in rats after oral dosing (10 mg/kg) was measured to determine the exposure of the

Table 4. C-2-Acylsulfonamide Optimization



Compound	R^5	R^2	RdRp IC_{50} (μM)(n = 2)[23]	Replicon EC_{50} (μM)	Rat AUC (μM·h)
44	Me	Me	0.007	0.49	22
45			0.010	0.20	11
46			0.007	0.09	6.8
47			0.006	0.09	9.1
48	Et	Me	0.006	0.15	72
49			0.006	0.08	4.6
50			0.005	0.10	0.80
51			0.003	0.06	12

inhibitors in Table 4. All four C-5 methyl acylsulfonamides (**44**–**47**) had good to excellent AUCs ranging from 6.8 to 22 μM·h. In the C-5-ethyl series (**48**–**51**), however, AUCs varied more significantly from extremely high for **48** (72 μM·h) to poor for **50** (0.8 μM·h). Among four more potent compounds with EC_{50} < 0.1 μM in the table, a pair of cyclopropyl acylsulfonamides (**47** and **51**) demonstrated very good PK with AUCs of 9.1 and 12 μM·h, respectively. When both potency and PK factors were considered, the cyclopropyl acylsulfonamide was a better C-2 moiety in a given series with the same C-5 substituent and was used for further SAR studies.

Reoptimization of C-5 and C-6 Substituents. With the *N*-1, C-2, and C-3 moieties optimized, the effects of C-5 and C-6 substituents were then revisited to determine the best combination for compounds with a C-2 acylsulfonamide. Preliminary SAR studies in Table 1 indicated that only compact and nonpolar alkyl groups were tolerated at C-5 position, and C-6 substitution had little impact on potency. Several compact and nonpolar substituents were tested at the C-5 and C-6 (**51**–**60**, Table 5). They all had excellent activity against RdRp enzyme with an IC_{50} between 0.003 and 0.008 μM. The difference in replicon assay potency, however, was significant, with EC_{50} ranging from 0.02 μM (**56**) to 0.6 μM (**54**). Among the six compounds without C-6 substituents (R^6 = H), those with 1-methylcyclopropyl and trifluoroethyl at C-5 were less potent than others in the replicon assay (EC_{50} = 0.6 μM for **54**, and EC_{50} = 0.23 μM for **55**). Both inhibitors with trifluoromethyl and *tert*-butyl C-5 groups were slightly more active in cellular assay, with EC_{50} values around 0.05 μM. Most compounds in this group demonstrated good PK in rats with

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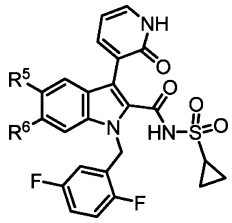
Table 5. Optimization of C-5 and C-6 Substituents



Compound	R^5	R^6	RdRp IC_{50} (μM)(n = 2)[23]	Replicon EC_{50} (μM)	Rat PK AUC (μM·h)
52	CF_3	H	0.003	0.05	4.8
47	Me	H	0.006	0.09	9.1
51		H	0.003	0.04	12
53		H	0.006	0.05	8.0
54		H	0.006	0.6	11
55		H	0.007	0.23	0.67
56	Me	F	0.008	0.02	14
57	Me	Cl	0.005	0.15	NA
58	Me	CF_3	0.007	0.28	0.69
59	CF_3	F	0.007	0.28	NA
60		F	0.005	0.06	6.4

AUCs at or above 5 μM·h, except **55**, which had a poor AUC of 0.67 μM·h.

Five analogues with a C-6 substituent (**56**–**60**) were included in Table 5. Among three compounds with a C-5-methyl group, 6-fluoro analogue **56** was much more potent (EC_{50} = 0.02 μM) than 6-chloro and 6-trifluoromethyl analogues (EC_{50} of 0.15 and 0.28 μM, respectively). If 6-F substitution was combined with 5-CF_3 or 5-ethyl groups (**59** and **60**), the results (EC_{50} = 0.28 and 0.06 μM, respectively) were not as impressive as that of **56**. The rat oral AUC was poor for compound **58** (0.69 μM·h), good for compound **60** (6.4 μM·h), and excellent for compound **56** (14 μM·h). When the overall profiles of these compounds were evaluated, compound **56** had the best combination of potency and PK. It was selected as a potential candidate for further evaluation.

Profile of Compound 56. With a calculated cLogP of 3.8 and polar-surface-area (PSA) of 100, it is not surprising to observe that compound **56** has only moderate permeability of 35 nm/s (apical to basolateral) in a Caco-2 cell permeability assay. When dosed orally in rats at 10 mg/kg, compound **56** had an excellent AUC_{0-24h} of 14 μM·h. It also had a very high concentration of 5850 ng/g in rat livers 6 h after oral dosing. The PK of compound **56** in dogs was also robust, with a good AUC_{0-24h} of 8.0 μM·h in 24 h after 2 mg/kg oral dosing. The clearance of the compound in human hepatocytes was low at <1.0 μL/min/Mcells. The IC_{50}s of compound **56** against the cytochrome P450 (CYP) 2D6, 3A4, 2C9, and 2C19 enzymes (concurrent/preincubation) were all greater than 20 μM. The compound had no P450 3A4 enzyme induction signal at 10 μM in a human PXR assay. There was also no signal at 10 μM in an hERG efflux assay. This compound was selected for more comprehensive studies because of its overall superior profile.

X-ray Structure of Compound 56..[28] The X-ray crystal structure of inhibitor **56** bound to the HCV NS5B polymerase was solved (Figure 2, Protein Data Bank access code 3TYV).[29]

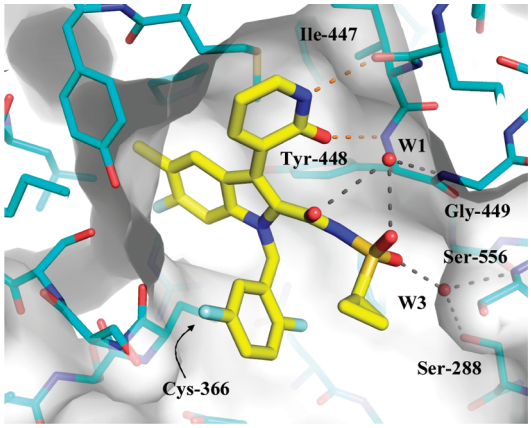


Figure 2. X-ray crystal structure of compound **56** bound to HCV NS5B polymerase.

Similar to the X-ray structure of compound **1** bound to the enzyme, **56** also binds to the "palm" site of NS5B apoprotein within the active site cavity. The indole core makes π-stacking interactions with the benzene ring of protein backbone Tyr-448. The *N*-1 benzyl moiety of the inhibitor stacks atop the thiol side chain of Cys-366. The C-2 acylsulfonamide motif does not have direct binding with the protein, but it interacts indirectly with protein residues Ser-556, Gly-449, and Ser-288 through the formation of a hydrogen bonding network with adjacent water molecules. The indole core and the C-3 pyridone ring have contacts with the side chain of Met-414. There is a pair of hydrogen bonding interaction between the pyridone and the protein backbone, one between the pyridone oxygen and the backbone N–H of Tyr-448, the other between the pyridone N–H and the backbone carbonyl of Ile-447. Because the dihedral angle between the pyridone ring and the indole was not big, any sizable substituent at C-4 position will change the orientation of the pyridone ring and thus disrupt the two hydrogen bonds. The indole C-5 methyl and C-6 fluoro groups reach into a constricted tunnel which extends to protein surface on the other side of the "thumb" subdomain.

CONCLUSION

Starting from compound **2**, an indole-based early lead inhibitor of HCV NS5B polymerase, extensive SAR development on multiple positions of the indole core was performed. While the unique and optimized 3-pyridone moiety at C-3 position was kept constant, substituents at *N*-1, C-2, C-4, C-5, and C-6 positions were varied systemically to optimize potency in both enzyme assays and cell-based replicon assays and to improve PK properties. The C-4 SAR investigation demonstrated that no substitution was tolerated. The best C-5 groups were found to be compact and nonpolar moieties. The C-6 substituents, in

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general, did not provide potency enhancement except in the C-5 methyl and C-6 fluoro combination series. Limited benzyl-type *N*-1 substituent studies indicated that the best substitutions were fluoro or methyl groups at 2′ or 5′ positions. The incorporation of acylsulfonamides as carboxylic acid isosteres at C-2 position improved PK properties. Further optimization of the combination at *N*-1, C-2, C-5, and C-6 positions resulted in the identification of compound **56**, which had excellent enzymatic and cellular assay potencies (IC_{50} = 0.008 μM, EC_{90} = 0.02 μM, respectively) and an excellent PK profile in rats and dogs (AUC = 14 and 8 μM·h, respectively). An X-ray structure of inhibitor **56** bound to the enzyme was also reported. The indole core, C-3 pyridone, C-2 acylsulfonamide, C-5 methyl, and 6-F all contributed to the high potency of compound **56**. This work demonstrated the importance of reoptimization after significant changes were made to other positions of the molecule.

EXPERIMENTAL SECTION

General Methods. Reagents and solvents, including anhydrous solvents, such as THF, dichloromethane, and DMF, were purchased from Aldrich or other commercial sources and were used without further purification. Reactions that were moisture sensitive or using anhydrous solvents were performed under either a nitrogen or an argon atmosphere. Analytical thin layer chromatography (TLC) was performed on precoated silica gel plates obtained from Analtech. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, ethanolic H_2SO_4, or Vaughn's reagent. Some compounds were purified by flash chromatography either on a glass column using Merck silica gel 60 (230−400 mesh) or on an ISCO RediSep disposable silica gel column. More polar compounds were purified by reverse phase HPLC on a Sunfire C18 preparative column (50 mm × 250 mm, 10 μm) running at 30 mL/min using 20−100% acetonitrile/water (both as a 0.1% trifluoroacetic acid solution) as eluent. NMR spectra were recorded at 400 or 500 MHz for ^{1}H and at 100 or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts are given in ppm, referenced to the internal TMS or deuterated solvent signal. HPLC, LC-MS, and/or ^{1}H NMR methods were employed to determine the purity of the synthesized compounds. All new compounds tested had purity at or above 95%.

General procedures for iodination of **3**: The solution of compound **3** (1 mmol) and *N*-iodosuccinimide (NIS) (1.05 mmol) in acetone (15 mL) was stirred at room temperature (rt) for 3 h (h). Saturated aqueous sodium thiosulfate (5 mL) was added. After stirring for 5 min (min), ethyl acetate (30 mL) and water (20 mL) were added. The layers were then separated. The aqueous solution was extracted with ethyl acetate (2 × 25 mL). The combined organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the crude product, which was usually pure enough to be used in the next reaction.

General procedures for Suzuki coupling between **4** and 2-methoxy-3-pyridine boronic acid (**5**): To the suspension of 3-iodoindole (**4**, 10.0 mmol) and [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium ($PdCl_2(dppf)_2$) (1.0 mmol) in dimethoxyethane (DME) (120 mL) at rt was added a solution of boronic acid (**5**) (12.0 mmol) and potassium carbonate (50 mmol) in water (30 mL). The mixture was bubbled with argon gas through a frit glass bubbler for 5 min before it was heated to 90 °C in an oil bath and stirred for 4 h. After cooling to rt, the mixture was diluted with water and ethyl acetate (300 mL each) and the two layers were separated. The aqueous solution was extracted with ethyl acetate (2 × 200 mL). The combined organic solution was washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the crude product, which was purified by silica gel flash chromatography using 0−50% ethyl acetate in hexanes as eluent to give the desired product (**6**) in 50−85% yield.

General procedures for the preparation of **7**: The mixture of **6** (5.0 mmol), arylmethyl bromide (or arylmethyl chloride) (7.5 mmol), and cesium carbonate (7.5 mmol) in anhydrous dimethylformamide (DMF) (80 mL) was vigorously stirred at rt for 16 h. The solution was then diluted with water and ethyl acetate (200 mL each), and the two layers were separated. The aqueous solution was extracted with ethyl acetate (100 mL). The combined organic solution was washed with water (2 × 300 mL), brine, dried over magnesium sulfate, and concentrated in vacuo to give the crude product, which was purified by silica gel flash chromatography using 0−60% ethyl acetate in hexanes as eluent to give the desired product (**7**) in 40−90% yield.

General procedures for the preparation of **8**: Compound **7** (1.0 mmol) was dissolved in a solution of 4 M hydrogen chloride in *p*-dioxane (50 mL) in a sealed tube. The solution was then heated to 90 °C in an oil bath and stirred for 3 h before it was cooled to rt. The solution was then concentrated in vacuo to give the crude product, which was usually pure enough to be used in the next reaction.

General procedures for the preparation of **9**: Compound **8** (1.0 mmol) and lithium hydroxide (4.0 mmol) was dissolved in THF and water (10 mL each). The solution was then heated to 70 °C in an oil bath and stirred for 16 h before it was cooled to rt. The solution was then made acidic (pH ∼ 2) by addition of 1 N hydrochloric acid. Ethyl acetate was added (30 mL), and layers were separated. The aqueous solution was extracted with ethyl acetate (2 × 20 mL). The combined organic solution was washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the crude product, which was purified by reverse phase HPLC to give the desired product (**9**) in 30−60% yield (2 steps).

General procedures for the preparation of **10**: Compound **10** was prepared from compound **7** according to the same procedures described above for the preparation of **9**. The crude product was usually pure enough to be used in the next reaction.

General procedures for the preparation of **11**: The mixture of **10** (0.50 mmol) and carbonyl diimidazole (CDI) (0.60 mmol) in anhydrous THF (5 mL) was heated to 75 °C in an oil bath and stirred 1.5 h. The solution was then cooled to rt, alkyl sulfonamide and DBU (1.5 mmol each) were added, and the resulting mixture was stirred at rt for 18 h. It was then diluted with water and ethyl acetate (30 mL each), and the two layers were separated. The aqueous solution was extracted with ethyl acetate (2 × 15 mL). The combined organic solutions were washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the crude product, which was purified by silica gel flash chromatography using 0−80% acetone in dichloromethane as eluent to give the desired product (**11**) in 50−80% yield.

General procedures for the preparation of **12**: Compound **12** was prepared from compound **11** according to the same procedures described above for the preparation of **8**. The crude product was purified by reverse phase HPLC to give the desired product (**12**) in 30−50% yield.

1-[(2-Amino-4-pyridinyl)methyl]-4,5-dichloro-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1*H*-indole-2-carboxylic Acid (13). Compound **13** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 11.36 (s, 2 H), 7.76 (d, J = 5.2 Hz, 1 H), 7.36 (q, J = 1.9 Hz, 1 H), 7.27 (s, 2 H), 7.18 (q, 8.9 Hz, 2 H), 6.28 (d, J = 5.4 Hz, 1 H), 6.14 (s, 2 H), 5.79 (s, 2 H), 5.60 (d, J = 16.8 Hz, 1 H). ^{13}C NMR (125 MHz, DMSO-d_6), 168.9, 163.8, 160.7, 148.5, 147.9, 145.0, 143.3, 135.6, 134.3, 127.5, 111.4, 111.1, 110.9, 110.0, 106.2, 98.7, 67.9, 47.4, 27.8, 19.2. HRMS calcd for $C_{20}H_{15}Cl_2N_4O_3$ $(M + H)^+$, 429.0521; found, 429.0517.

1-[(2-Amino-4-pyridinyl)methyl]-4-bromo-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1*H*-indole-2-carboxylic Acid (14). Compound **14** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.44 (s, 1 H), 11.58 (s, 1 H), 7.90 (d, J = 6.0 Hz, 1 H), 7.76 (s, 2 H), 7.59 (d, J = 8.1 Hz, 1 H), 7.40−7.38 (m, 3 H), 7.24 (t, J = 7.9 Hz, 1 H), 6.58 (d, J = 5.8 Hz, 1 H), 6.30 (s, 1 H), 6.24 (t, J = 6.4 Hz, 1 H), 4.38 (q, J = 18.0 Hz, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.3, 163.0, 156.1, 155.7, 142.1, 139.7, 138.2, 135.3, 128.5, 127.1, 126.9,

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126.3, 125.0, 120.8, 116.1, 111.5, 111.2, 109.0, 105.4, 48.2. HRMS calcd for $C_{20}H_{16}N_4O_3Br$ $(M + H)^+$, 439.0406; found LRMS, 439.0.

1-[(2-Amino-4-pyridinyl)methyl]-5-bromo-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1*H*-indole-2-carboxylic Acid (16). Compound **16** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 11.97 (s, 1 H), 8.44 (s, 1 H), 7.74 (s, 1 H), 7.58 (s, 1 H), 7.42 (s, 2 H), 7.18 (s, 2 H), 6.27 (s, 2 H), 6.20 (s, 1 H) 5.80 (s, 2 H), 5.59 (s, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 165.8, 162.9, 160.7, 149.3, 148.5, 141.6, 135.0, 129.7, 124.4, 123.8, 113.0, 112.4, 111.4, 109.7, 109.5, 106.4, 100.4, 97.0, 96.4, 93.5, 81.5, 74.3, 62.6, 52.1, 48.3, 47.4, 44.1. HRMS calcd for $C_{20}H_{16}N_4O_3Br$ $(M + H)^+$, 439.0406; found, 439.0398.

5-Acetyl-1-[(2-amino-4-pyridinyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1*H*-indole-2-carboxylic Acid (20). Compound **20** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 11.96 (s, 2 H), 8.12 (s, 1 H), 8.00 (s, 2 H), 7.95−7.91 (m, 3 H), 7.66 (d, J = 8.9 Hz, 1 H), 7.57 (d, J = 7.8 Hz, 1 H), 6.56 (d, J = 6.0 Hz, 1 H), 6.40 (d, J = 6.6 Hz, 1 H), 6.36 (s, 1 H), 5.87 (s, 2 H), 2.59 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 198.1, 163.5, 162.1, 156.4, 155.4, 141.5, 140.6, 137.4, 135.6, 131.5, 129.4, 126.5, 125.7, 125.5, 123.5, 120.3, 111.8, 111.2, 109.4, 106.1, 48.2, 27.6. LRMS calcd for $C_{22}H_{19}N_4O_4$ $(M + H)^+$, 403.1; found: 403.2.

1-[(2-Amino-4-pyridinyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(trifluoromethyl)-1*H*-indole-2-carboxylic Acid (24). Compound **24** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.78 (s, 1 H), 11.90 (s, 1 H), 8.03 (s, 2 H), 7.92 (d, J = 6.7 Hz, 1 H), 7.82 (s, 1 H), 7.80 (s, 1 H), 7.66−7.61 (m, 2 H), 7.47 (q, J = 1.9 Hz,1 H), 6.63 (q, J = 1.3 Hz, 1 H), 6.38−6.35 (m, 2 H), 5.90 (s, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.4, 162.0, 156.8, 155.0, 141.7, 139.7, 136.9, 135.8, 129.7, 125.1, 124.8, 122.9, 122.6, 122.1, 119.7, 119.5, 119.4, 113.0, 111.2, 109.5, 106.0, 48.2. LRMS calcd for $C_{21}H_{16}F_3N_4O_3$ $(M + H)^+$, 429.1; found, 429.0.

1-[(2,4-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(trifluoromethyl)-1*H*-indole-2-carboxylic Acid (30). Compound **30** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.25 (s, 1 H), 11.83 (s, 1 H), 7.84 (d, J = 8.8 Hz, 1 H), 7.75 (s, 1 H), 7.62−7.60 (m, 2 H), 7.44 (dd, J = 1.9, 1.5 Hz,1 H), 7.30−7.25 (m, 1 H), 7.00−6.96 (m, 1 H), 6.81−6.77 (m, 1 H), 6.34 (t, J = 6.6 Hz, 1 H), 5.91 (s, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 162.1, 161.5, 161.4, 159.6, 159.5, 141.4, 139.6, 135.6, 130.3, 130.2, 130.1, 127.0, 126.1, 125.2, 124.8, 122.6, 122.4, 122.3, 122.2, 122.1, 121.7, 119.2, 119.1, 119.0, 113.0, 112.6, 112.5, 112.4, 106.0, 105.1, 104.9, 104.7, 42.4. LRMS calcd for $C_{22}H_{14}F_5N_2O_3$ $(M + H)^+$, 449.1; found, 449.0.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(trifluoromethyl)-1*H*-indole-2-carboxylic Acid (32). Compound **32** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.28 (s, 1 H), 11.83 (s, 1 H), 7.85 (d, J = 7.5 Hz, 1 H), 7.76 (s, 1 H), 7.64 (s, 2 H), 7.45 (s, 1 H), 7.32−7.38 (m, 1 H), 7.16 (d, J = 2.7 Hz, 1 H), 6.50 (s, 1 H), 6.35 (s, 1 H), 5.93 (s, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 162.1, 159.9, 157.6, 141.4, 139.5, 135.6, 129.8, 128.0, 127.0, 126.1, 121.8, 119.3, 119.2, 118.0, 117.9, 116.6, 116.5, 115.4, 115.3, 115.2, 113.0, 106.0, 42.9. LRMS calcd for $C_{22}H_{14}F_5N_2O_3$ $(M + H)^+$, 449.1; found, 449.1.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(trifluoromethoxy)-1*H*-indole-2-carboxylic Acid (34). Compound **34** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 11.82 (s, 1 H), 7.72 (d, J = 9.2 Hz, 1 H), 7.59 (dd, J = 1.94, 1.77 Hz, 1 H), 7.43 (dd, J = 1.90, 1.54 Hz, 1 H), 7.36 (s, 1 H), 7.32−7.28 (m, 1 H), 7.18−7.14 (m, 1 H), 6.54−6.50 (m, 1 H), 6.34 (t, J = 6.6 Hz, 1 H), 5.88 (s, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.7, 162.1, 161.1, 159.1, 158.0, 150.7, 143.8, 141.2, 138.2, 137.2, 128.1, 127.0, 118.1, 118.0, 117.9, 117.8, 116.4, 115.3, 113.6, 113.3, 103.0, 94.5, 67.2, 42.7, 30.0, 24.2. HRMS calcd for $C_{22}H_{14}F_5N_2O_4$ $(M + H)^+$, 465.0874; found, 465.0868.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(methoxy)-1*H*-indole-2-carboxylic Acid (35). Compound **35** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.98 (s, 1 H), 11.79 (s, 1 H), 7.59 (q, J = 2.4 Hz, 1 H), 7.50 (d, J = 9.2 Hz, 1 H), 7.41 (q, J = 3.6 Hz, 1 H), 7.31−7.27 (m, 1 H), 7.16−7.11 (m, 1 H), 6.98 (q, J = 2.6 Hz, 1 H), 6.86 (d, J = 2.5 Hz, 1 H), 6.37−6.33 (m, 2 H), 5.83 (s, 2 H), 3.73 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.4, 172.8, 166.0, 151.3, 145.4, 144.1, 138.8, 137.6, 137.0, 128.4, 128.2, 127.3, 126.7, 125.6, 125.4, 123.2, 120.8, 116.6, 112.4, 66.7, 52.9. LRMS calcd for $C_{22}H_{17}F_2N_2O_4$ $(M + H)^+$, 411.1; found, 411.2.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethoxy)-1*H*-indole-2-carboxylic Acid (36). Compound **36** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**.^{1}H NMR (500 MHz, DMSO-d_6), 11.76 (s, 1 H), 7.58 (q, J = 2.1 Hz, 1 H), 7.48 (d, J = 9.1 Hz, 1 H), 7.40 (q, J = 2.0 Hz, 1 H), 7.30−7.27 (m, 1 H), 7.15−7.11 (m, 1 H), 6.97 (q, J = 2.6 Hz, 1 H), 6.84 (d, J = 2.1 Hz, 1 H), 6.38−6.33 (m, 2 H), 5.82 (s, 2 H), 3.99−3.95 (m, 2 H), 1.30 (t, J = 6.9 Hz, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.4, 172.8, 170.4, 168.5, 167.9, 166.0, 165.2, 151.4, 145.4, 144.0, 139.2, 138.8, 137.7, 136.9, 128.4, 128.2, 127.6, 126.8, 126.7, 125.6, 125.4, 123.3, 123.1, 121.0, 120.8, 116.7, 113.4, 74.8, 52.9, 26.1. LRMS calcd for $C_{23}H_{19}F_2N_2O_4$ $(M + H)^+$, 425.1; found, 425.1.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxylic Acid (38). Compound **38** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.0 (s, 1 H), 11.8 (s, 1 H), 7.56 (dd, J = 2.2, 1.9 Hz, 1 H), 7.5 (d, J = 8.6 Hz, 1 H), 7.41 (d, J = 6.3 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.24 (s, 1 H), 7.17−7.08 (m, 2 H), 6.37−6.33 (m, 2 H), 5.83 (s, 2 H), 2.36 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 181.8, 181.4, 177.0, 174.5, 172.8, 152.8, 151.5, 147.3, 145.6, 141.2, 138.2, 137.5, 136.3, 131.2, 128.4, 121.9, 113.7, 113.1, 112.4, 78.4, 66.3, 59.9, 41.8, 36.6, 36.5, 32.4. LRMS calcd for $C_{22}H_{17}F_2N_2O_3$ $(M + H)^+$, 395.1; found, 395.0.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-1*H*-indole-2-carboxylic Acid (39). Compound **39** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.97 (s, 1 H), 11.78 (s, 1 H), 7.58 (dd, J = 1.8, 2.2 Hz, 1 H), 7.49 (d, J = 8.5 Hz, 1 H), 7.42 (d, J = 4.8 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.24 (s, 1 H), 7.20 (d, J =8.4 Hz, 1 H), 7.17−7.11 (m, 1 H), 1.18 (t, J =7.6 Hz, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 164.0, 162.3, 159.4, 158.0, 157.5, 155.6, 141.0, 137.4, 137.0, 135.0, 128.0, 127.0, 126.6, 119.5, 118.1, 118.0, 117.9, 117.8, 117.7, 116.4, 116.3, 116.2, 116.1, 115.2, 115.0, 106.1, 29.1, 17.1. HRMS calcd for $C_{23}H_{19}F_2N_2O_4$ $(M + H)^+$, 409.1364; found, 409.1358.

5-Bromo-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1*H*-indole-2-carboxylic Acid (40). Compound **40** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.14 (s, 1 H), 11.80 (s, 1 H), 7.62−7.58 (m, 3 H), 7.45 (dd, J = 1.9, 1.9 Hz, 1 H), 7.42 (dd, J = 1.7, 1.9 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.18−7.14 (m, 1 H), 6.45−6.41 (m, 1 H), 6.33 (t, J = 6.6 Hz, 1 H), 5.86 (s, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 162.1, 160.5, 147.2, 141.4, 136.9, 129.2, 128.5, 128.3, 123.6, 119.3, 119.1, 117.9,

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117.7, 115.3, 114.3, 114.0, 109.1, 106.2, 55.9, 48.8, 42.6. LRMS calcd for $C_{21}H_{14}F_2N_2O_3Br$ $(M + H)^+$, 459.0, 461.0; found, 461.3.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(1,1-dimethylethyl)-1*H*-indole-2-carboxylic Acid (42). Compound **42** was prepared according to the reaction sequence outlined in Scheme 1 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.99 (s, 1 H), 11.83 (s, 1 H), 7.58 (q, J = 1.9 Hz, 1 H), 7.50 (d, J = 8.8 Hz, 1 H), 7.45−7.41 (m, 2 H), 7.36 (s, 1 H), 7.32−7.29 (m, 1 H), 7.17−7.12 (m, 1 H), 6.44−6.40 (m, 1 H), 6.36 (t, J = 6.7 Hz, 1 H), 5.83 (s, 2 H), 1.29 (s, 9 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.5, 172.8, 170.4, 168.5, 168.0. 166.1, 154.7, 151.5, 147.1, 145.5, 139.1, 138.4, 136.9, 135.0, 129.0, 128.5, 128.4, 128.3, 128.2, 126.9, 126.8, 126.7, 125.8, 125.7, 125.6, 125.5, 121.8, 116.6, 52.8, 45.7, 42.8. LRMS calcd for $C_{25}H_{23}F_2N_2O_3$ $(M + H)^+$, 437.2; found, 437.3.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-*N*-methylsulfonyl)-1*H*-indole-2-carboxamide (44). Compound **44** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.77 (s, 1 H), 12.64 (s, 1 H), 7.82 (d, J = 9.7 Hz, 1 H), 7.66 (s, 1 H), 7.50 (d, J = 8.5 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.23 (s, 1 H), 7.19 (d, J = 8.6 Hz, 1 H), 7.18−7.14 (m, 1 H), 6.60 (s, 2 H), 5.71 (s, 2 H), 3.25 (s, 3 H), 2.36 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 159.9, 158.0, 157.6, 155.7, 136.9, 136.4, 131.2, 128.1, 127.2, 125.8, 120.7, 118.0, 117.9, 117.8, 117.7, 116.7, 116.6, 116.5, 116.4, 115.9, 115.7, 111.6, 108.2, 44.0, 42.5, 42.1, 41.0, 31.3, 21.9. HRMS calcd for $C_{23}H_{20}F_2N_3O_4S$ $(M + H)^+$, 472.1143; found, 472.1137.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-*N*-(ethylsulfonyl)-5-methyl-1*H*-indole-2-carboxamide (45). Compound **45** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.73 (s, 1 H), 7.82 (d, J = 6.0 Hz, 1 H), 7.68 (s, 1 H), 7.54 (d, J = 9.5 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.21 (s, 2 H), 7.18−7.13 (m, 1 H), 6.63 (t, J = 6.3 Hz, 1 H), 6.56- 6.52 (m, 1 H), 5.72 (s, 2 H), 3.35 (q, J = 7.3 Hz, 2 H), 2.36 (s, 3 H), 1.05 (t, J = 7.3 Hz, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.5, 162.0, 159.0, 158.0, 157.6, 155.7, 145.1, 140.1, 136.6, 131.3, 128.2, 127.9, 127.2, 120.7, 118.0, 117.9, 117.8, 117.7, 115.8, 115.5, 111.6, 108.3, 52.4, 47.7, 42.4, 31.3, 30.1, 21.9, 8.3. HRMS calcd for $C_{24}H_{22}F_2N_3O_4S$ $(M + H)^+$, 486.1299; found, 486.1295.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-*N*-[(1-methylethyl)sulfonyl)-1*H*-indole-2-carboxamide (46). Compound **46** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.78 (s, 1 H), 12.70 (s, 1 H), 7.83 (d, J = 7.0 Hz, 1 H), 7.70 (s, 1 H), 7.55 (d, J = 8.6 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.22−7.12 (m, 3 H), 6.64 (t, J = 6.60 Hz, 1 H), 6.53−6.49 (m, 1 H), 5.73 (s, 2 H), 3.64−3.56 (m 1 H), 2.36 (s, 3 H), 1.14 (d, J = 6.9 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 162.0, 160.0, 157.6, 155.7, 145.2, 137.1, 136.6, 131.3, 129.4, 128.3, 127.2, 123.8, 120.7, 118.0, 117.9, 117.8, 117.7, 116.6, 116.5, 116.4, 115.9, 115.7, 115.5, 111.6, 108.4, 53.6, 42.4, 21.9, 16.1. LRMS calcd for $C_{25}H_{24}F_2N_3O_4S$ $(M + H)^+$, 500.1; found, 500.3

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (47).** Compound **47** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.79 (s, 1 H), 12.71 (s, 1 H), 7.81 (q, J = 1.9 Hz, 1 H), 7.69 (s, 1 H), 7.54−7.52 (m, 1 H), 7.32−7.27 (m, 1 H), 7.21−7.20 (m, 2 H), 7.18−7.13 (m, 1 H), 6.64−6.16 (m, 1 H), 6.60−6.56 (m, 1 H), 5.73 (s, 2 H), 2.97−2.91 (m, 1 H), 2.36 (s, 3 H), 0.97 (d, J = 6.3 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.5, 161.9, 160.0, 158.0, 157.6, 155.7, 145.0, 136.9, 136.5, 131.2, 128.1, 127.9, 127.2, 123.9, 120.7, 118.0, 117.9, 117.8, 117.7, 116.6, 115.9, 115.8, 111.6, 108.3, 42.4, 31.5, 21.9, 6.3. LRMS calcd for $C_{25}H_{22}F_2N_3O_4S$ $(M + H)^+$, 498.1; found, 498.3.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-*N*-(methylsulfonyl)-1*H*-indole-2-carboxamide (48). Compound **48** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.77 (s, 1 H), 12.64 (s, 1 H), 7.82 (d, J = 6.4 Hz, 1 H), 7.66 (s, 1 H), 7.53 (d, 8.9 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.25 (s, 1 H), 7.24 (s, 1 H), 7.18−7.13 (m, 1 H), 6.63−6.6 (m, 1 H), 5.7 (s, 2 H), 3.25 (s, 3 H), 2.66 (q, J = 7.5 Hz, 2 H), 1.18 (t, J = 7.4 Hz, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.4, 159.9, 158.0, 157.6, 155.7, 155.6, 144.7, 137.9, 137.0, 136.4, 127.1, 119.5, 118.0, 117.8, 117.7, 116.7, 116.6, 116.0, 116.4, 115.9, 115.8, 115.7, 111.7, 108.2, 42.5, 42.0, 29.1, 17.0. LRMS calcd for $C_{24}H_{22}F_2N_3O_4S$ $(M + H)^+$, 486.1; found, 486.0.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-*N*-(ethylsulfonyl)-1*H*-indole-2-carboxamide (49). Compound **49** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.72 (s, 2 H), 7.83 (dd, J = 1.7, 1.8 Hz, 1 H), 7.69 (s, 1 H), 7.56 (d, J = 8.5 Hz, 1 H), 7.32−7.27 (m, 1 H), 7.25 (d, J = 8.9 Hz, 1 H), 7.21 (s, 1 H), 7.19−7.14 (m, 1 H), 6.65−6.57 (m, 2 H), 5.72 (s, 1 H), 3.35 (q, J = 7.3 Hz, 2 H), 2.66 (q, J = 7.5 Hz, 2 H), 1.18 (t, J = 7.6 Hz, 3 H), 1.05 (t, J = 7.4 Hz, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.5, 162.1, 159.9, 158.0, 157.6, 155.7, 145.1, 140.0, 137.9, 137.1, 136.6, 127.2, 119.5, 118.0, 117.9, 117.8, 117.7, 116.7, 116.6, 116.5, 115.8, 115.6, 111.7, 108.4, 47.7, 42.5, 31.3, 29.1, 17.0, 8.3. LRMS calcd for $C_{25}H_{24}F_2N_3O_4S$ $(M + H)^+$, 500.1; found, 500.0.

1-[(2,5-Difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-*N*-[(1-methylethyl)sulfonyl)-1*H*-indole-2-carboxamide (50). Compound **50** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.79 (s, 1 H), 12.70 (s, 1 H), 7.83 (d, J = 5.4 Hz, 1 H), 7.70 (s, 1 H), 7.57 (d, J = 8.4 Hz, 1 H), 7.32−7.25 (m, 2 H), 7.20 (s, 1 H), 7.18−7.14 (m, 1 H), 6.64 (t, J = 6.6 Hz, 1 H), 6.56−6.53 (m, 1 H), 5.73 (s, 2 H), 3.62−3.57 (m, 1 H), 2.66 (q, J = 7.5 Hz, 2 H), 1.18 (d, J = 7.5 Hz, 3 H), 1.15 (d, J = 6.6 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 162.0, 160.0, 158.0, 157.6, 155.7, 145.3, 137.9, 137.2, 136.7, 128.0, 127.2, 123.8, 119.5, 118.0, 117.9, 117.8, 117.7, 116.7, 116.5, 116.4, 116.0, 115.8, 111.7, 108.5, 53.6, 42.5, 29.1, 17.0, 16.1. LRMS calcd for $C_{26}H_{26}F_2N_3O_4S$ $(M + H)^+$, 514.2; found, 514.1.

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)-methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-1*H*-indole-2-carboxamide (51).** Compound **51** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.79 (s, 1 H), 12.71 (s, 1 H), 7.82 (q, J = 1.8 Hz, 1 H), 7.69 (s, 1 H), 7.56 (d, J = 8.6 Hz, 1 H), 7.32−7.15 (m, 4 H), 6.65−6.60 (m, 2 H), 5.73 (s, 2 H), 2.96−2.91 (m, 1 H), 2.68−2.64 (m, 2 H), 1.17 (t, J = 7.5 Hz, 3 H), 0.98 (d, J = 6.3 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.5, 161.9, 159.9, 158.0, 157.7, 155.7, 145.0, 137.9, 137.1, 136.6, 129.5, 127.2, 127.1, 123.9, 119.5, 118.0, 117.8, 116.7, 116.5, 115.9, 115.8, 115.7, 111.7, 108.3, 42.3, 31.6, 29.1, 17.0, 6.3. LRMS calcd for $C_{26}H_{24}F_2N_3O_4S$ $(M + H)^+$, 512.1; found, 512.1.

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(trifluoromethyl)-1*H*-indole-2-carboxamide (52).** Compound **52** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.78 (s, 2 H), 7.90 (d, J = 8.8 Hz, 1 H), 7.81 (q, J = 2.2 Hz, 1 H), 7.70 (s, 1 H), 7.66 (q, J = 1.6 Hz, 2 H), 7.32−7.27 (m, 1 H), 7.20−7.16 (m, 1 H), 6.71−6.68 (m, 1 H), 6.58 (t, J = 6.7 Hz, 1 H), 5.80 (s, 2 H), 2.95−2.90 (m, 1 H), 0.99 (s, 2 H), 0.90−0.85 (m, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 163.0, 161.6, 160.0, 155.8, 144.6, 139.5, 136.8, 132.4, 129.5, 126.2, 123.0, 122.1, 119.6, 118.0, 117.9, 116.9, 116.2, 116.0, 113.1, 107.9, 68.3, 42.7, 32.9, 31.5, 30.7, 24.1, 23.2, 11.7, 6.3, 5.8. LRMS calcd for $C_{25}H_{19}F_5N_3O_4S$ $(M + H)^+$, 552.1; found, 552.0.

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***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(1,1-dimethylethyl)-1*H*-indole-2-carboxamide (53).** Compound **53** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 7.83 (q, J = 2.5 Hz, 1 H), 7.70 (d, J = 5.9 Hz, 1 H), 7.57 (d, J = 8.8 Hz, 1 H), 7.49 (q, J = 2.3 Hz, 1 H), 7.32 (d, J = 1.4 Hz, 1 H), 7.29 (q, J = 4.3 Hz, 1 H), 7.20−7.14 (m, 1 H), 6.65 (t, J = 7.4 Hz, 2 H), 5.73 (s, 2 H), 2.98−2.91 (m, 1 H), 1.28 (s, 9H), 0.99 (d, J = 5.7 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.0, 172.4, 170.4, 168.5, 168.1, 166.2, 155.6, 155.2, 147.2, 139.9, 138.5, 138.3, 137.1, 135.5, 134.3, 128.5, 128.3, 126.9, 126.8, 126.5, 126.3, 122.0, 118.8, 52.8, 45.7, 42.7, 42.0, 16.8. LRMS calcd for $C_{28}H_{28}F_2N_3O_4S$ $(M + H)^+$, 540.2; found, 540.1.

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-(1-methylcyclopropyl)-1*H*-indole-2-carboxamide (54).** Compound **54** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.81 (s, 1 H), 12.72 (s, 1 H), 7.84 (q, J = 1.6 Hz, 1 H), 7.69 (d, J = 4.7 Hz, 1 H), 7.58 (d, J = 8.9 Hz, 1 H), 7.47−7.45 (m, 1 H), 7.33 (s, 1 H), 7.32−7.29 (m, 1 H), 7.19−7.15 (m, 1 H), 6.64−6.62 (m, 2 H), 5.74 (s, 2 H), 3.86 (s, 3 H), 2.97−2.92 (m, 1 H), 1.98 (s, 2 H), 1.74 (d, J = 6.5 Hz, 2 H), 0.98 (d, J = 6.3 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 173.9, 172.3, 168.5, 168.1, 166.2, 155.5, 151.1, 148.4, 148.0, 147.1, 146.6, 140.2, 138.3, 137.4, 136.6, 135.1, 134.2, 132.6, 129.3, 128.5, 128.3, 128.0, 126.9, 126.4, 126.2, 122.0, 118.8, 52.9, 42.0, 37.5, 31.3, 27.0, 26.3, 25.6, 16.8. LRMS calcd for $C_{28}H_{26}F_2N_3O_4S$ $(M + H)^+$, 538.2; found, 538.1.

(*N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-6-fluoro-5-(methyl)-1*H*-indole-2-carboxamide (56). Compound **56** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 7.78 (dd, J = 7.2, 2.0 Hz, 1 H), 7.67−7.65 (m, 1 H), 7.53 (d, J = 10.8 Hz, 1 H), 7.29−7.24 (m, 2 H), 7.18−7.12 (m, 1 H), 6.61−6.57 (m, 2 H), 5.69 (s, 2 H), 2.93−2.85 (s, 1 H), 2.26 (s, 3 H), 0.95 (d, J = 6.4 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 162.3, 160.7, 158.9, 157.0, 154.8, 144.0, 135.7, 122.5, 122.3, 119.2, 117.0, 116.8, 115.8, 115.6, 115.1, 114.9, 114.7, 107.3, 96.9, 96.7, 42.7, 31.5, 15.6, 15.5. HRMS calcd for $C_{25}H_{21}F_3N_3O_4S$ $(M + H)^+$, 516.1205; found, 516.1200.

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-6-fluoro-5-(trifluoromethyl)-1*H*-indole-2-carboxamide (59).** Compound **59** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.70 (s, 1 H), 12.56 (s, 1 H), 7.99 (d, J = 12.2 Hz, 1 H), 7.81−7.79 (m, 2 H), 7.66 (d, J = 5.7 Hz, 1 H), 7.33−7.28 (s, 1 H), 7.23−7.17 (m, 1 H), 6.74−6.69 (m, 1 H), 6.56 (t, J = 6.8 Hz, 1 H), 5.77 (s, 2 H), 2.93−2.87 (m, 1 H), 0.98 (d, J = 6.1 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 173.4, 171.7, 170.6, 170.4, 169.3, 168.5, 168.3, 167.4, 166.4, 155.1, 150.5, 147.5, 142.0, 137.4, 133.1, 133.0, 132.1, 128.6, 128.5, 128.4, 128.0, 127.3, 126.8, 126.6, 118.3, 110.8, 110.6, 53.6, 42.0, 16.8. LRMS calcd for $C_{25}H_{18}F_6N_3O_4S$ $(M + H)^+$, 570.1; found, 570.1.

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-6-fluoro-1*H*-indole-2-carboxamide (60).** Compound **60** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **12**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.71 (s, 2 H), 7.80 (q, J = 1.9 Hz, 1 H), 7.69 (s, 1 H), 7.55 (d, J = 11.7 Hz, 1 H), 7.32−7.27 (m, 2 H), 7.21−7.14 (m, 1 H), 6.66 (s, 2 H), 5.71 (s, 2 H), 2.95−2.88 (m, 1 H), 2.67 (q, J = 7.4 Hz, 2 H), 1.16 (t, J = 7.2 Hz, 3 H), 0.97 (d, J = 6.9 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 173.8, 172.1, 170.4, 170.2, 168.5, 168.2, 166.3, 155.5, 147.8, 147.2, 140.1, 138.2, 137.0, 134.0, 132.3, 128.5, 128.3, 127.1, 126.8, 126.5, 126.3, 118.8, 108.6, 108.4, 53.2, 42.0, 33.6, 26.1, 16.8. LRMS calcd for $C_{26}H_{23}F_3N_3O_4S$ $(M + H)^+$, 530.1; found, 530.0.

Abbreviations Used

HCV, hepatitis C virus; PK, pharmacokinetics; SAR, structure−activity relationship; AUC, area-under-the-curve; SOC, standard of care; SVR, sustained virologic response; RdRp, RNA-dependent RNA polymerase; RC, replicase complex; NIs, nucleoside inhibitors; NNIs, non-nucleoside inhibitors; NIS, *N*-iodosuccinimide; CDI, carbonyl diimidazole; DBU, diazabicyclicunderdecane; CYP, cytochrome P450; hERG, the human ether-à-go-go-related gene; PXR, pregnane X receptor; TLC, thin layer chromatography; rt or RT, room temperature; min, minutes; DME, dimethoxyethane; DMF, dimethylformamide; DMSO, methyl sulfoxide; THF, tetrahydrofuran

■ ASSOCIATED CONTENT

Accession Codes

Protein Data Bank ID 3TYV.

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(28) Figure 2 was generated using the program PyMOL (The PyMOL Molecular Graphics System, version 1.2r1, Schrödinger, LLC.).
(29) X-Ray crystal structure data of HCV NS5B in complex with **56** have been deposited in the Protein Data Bank with access code 3TYV.