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A Novel Class of Highly Potent Irreversible Hepatitis C Virus NS5B Polymerase Inhibitors

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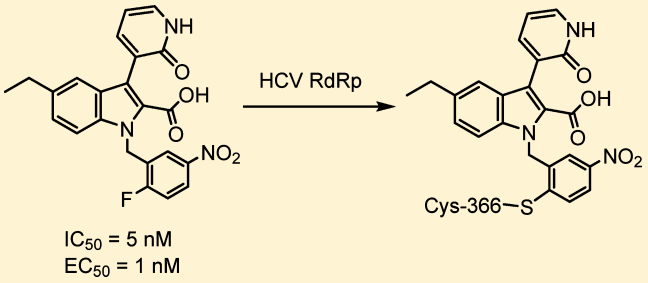
ABSTRACT: Starting from indole-based C-3 pyridone HCV NS5B polymerase inhibitor **2**, structure−activity relationship (SAR) investigations of the indole N-1 benzyl moiety were performed. This study led to the discovery of irreversible inhibitors with *p*-fluoro-sulfone- or *p*-fluoro-nitro-substituted N-1 benzyl groups which achieved breakthrough replicon assay potency (EC_{50} = 1 nM). The formation of a covalent bond with adjacent cysteine-366 thiol was was proved by mass spectroscopy and X-ray crystal structure studies. The C-5 ethyl C-2 carboxylic acid derivative **47** had an excellent oral area-under-the-curve (AUC) of 18 μM·h (10 mg/kg). Its oral exposure in monkeys and dogs was also very good. The NMR ALARM assay, mass spectroscopy experiments, in vitro counter screening, and toxicology assays demonstrated that the covalent bond formation between compound **47** and the protein was highly selective and specific. The overall excellent profile of **47** made it an interesting candidate for further investigation.



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] The new standard of care (SOC) after recent protease inhibitor approval involves the combination of a protease inhibitor with pegylated-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, three times a day), relapse (mutations), and discontinuation due to the presence of side effects with these therapies necessitates the need for safe 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 (NS) 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 which directly target these nonstructural proteins.[5] Exciting progress has been made in these endeavors.[5] Recent clinical trials have demonstrated dramatic improvements in SVR rate in patients treated with a direct-acting agent in combination with standard of care.[6] Recent approval of the two HCV NS3 protease inhibitors boceprevir[7] (Victrelis, Merck & Co.) and telaprevir[8] (Incivek, 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 genome, the HCV NS5B polymerase is potentially associated

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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.[12,13] Binding to these sites interferes with conformational changes required during RNA synthesis. Thumb pockets I and II are two allosteric sites thatz 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 the targets for drug development, which makes the NS5B a particularly drugable 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 four 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 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.

Our early research effort on the optimization of an indole-based screening hit led to compound **1**.[23] It had good potency (IC_{50} = 53 nM) in an NS5B RdRp enzyme assay.[24] It was active in a cell-based replicon assay[25] with an EC_{50} of 4800 nM). 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.[23] Further SAR studies were carried out to improve potency, especially replicon assay potency. Since compound **1** had poor pharmacokinetic (PK) properties with very low AUC value when dosed orally in rats, it was also desirable to improve PK properties through structural modifications. Because the X-ray structure of **1** also revealed that the C-3 pyridone carbonyl and N-H formed hydrogen bonds with the backbone isoleucine-447 residue, it was decided to leave this moiety intact while changes were made at other substitutions at the indole core. After considerable effort, new structures such as compound **2** with much improved potency and PK profiles emerged as the new lead series.[26] The indole core of inhibitor **2** was substituted by a methyl at C-5 and a cyclopropyl acylsulfonamide at C-2. The indole nitrogen was substituted by a 2′,5′-difluorobenzyl group. A 7-fold improvement in enzyme potency to an IC_{50} of 6 nM was observed, probably as a result of increased hydrophobic interactions with the enzyme surface from the C-5 and N-1 substituents. More importantly, this compound demonstrated greatly improved (50-fold) activity in replicon assay with an EC_{50} of 90 nM. Compound **2** also had an improved AUC of 9.1 μM·h in rats following 10 mg/kg oral dosing. Despite its good overall profile, further improvement in compound **2** is required to have a drug candidate, particularly in the potency in the cellular assay. Thus, the optimization of the indole series of inhibitors continued. Herein, we report our SAR development and the discovery of a novel class of irreversible highly potent HCV NS5B polymerase inhibitors. (see Figure 1.)

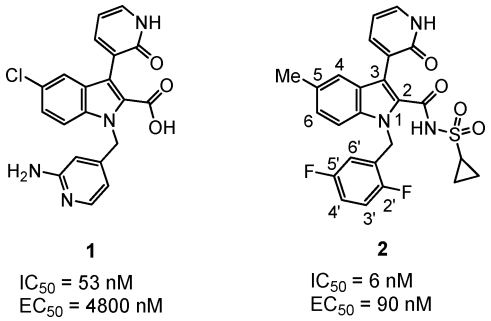


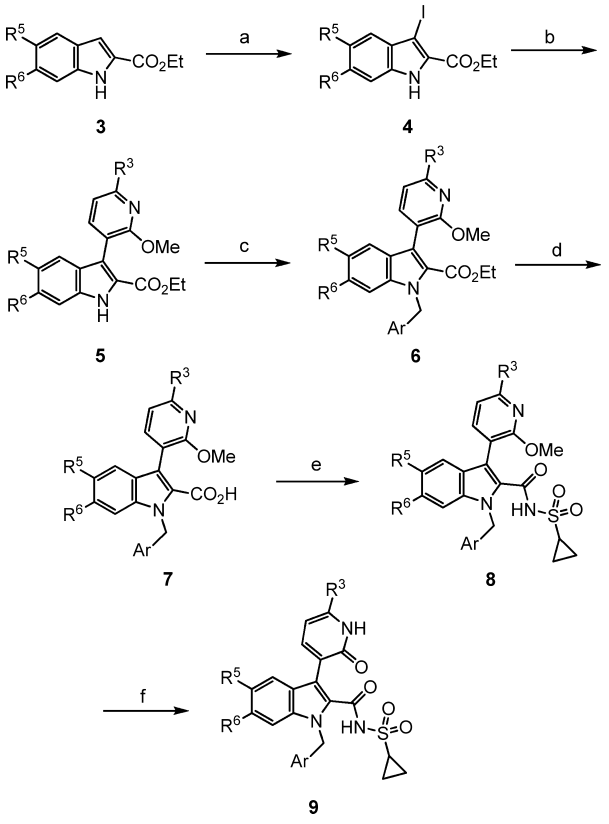
Figure 1. Lead compounds from early SAR studies.

SYNTHESIS OF HCV NS5B POLYMERASE INHIBITORS

The general synthesis of inhibitors is outlined in Schemes 1 and 2. The ethyl indole-2-carboxylate starting material **3**, with desired substituents at the 4-, 5-, and 6-position, was either commercially available or could be prepared via known procedures. The intermediate **3** was iodinated at C-3 with *N*-iodosuccinimide (NIS) to give compound **4**. The Suzuki–Miyaura cross-coupling reaction[27] between **4** and 2-methoxy-3-pyridineboronic acid in the presence of a palladium catalyst afforded product **5**. The indole nitrogen was then alkylated to provide compound **6** through alkylation with a benzylic halide. The ethyl ester was hydrolyzed to its corresponding carboxylic acid **7**, which, after activation by carbonyl diimidazole (CDI), was converted to an acylsulfonamide **8** through reaction with a primary alkylsulfonamide.[28] The methoxypyridine **8** was then demethylated to afford pyridone **9** upon treatment with hydrochloric acid. The final compounds were tested in RdRp enzyme assay and replicon cell-based assay.

Alternatively, the indole-2-acylsulfonamide series of polymerase inhibitors were prepared according to the procedures shown in Scheme 2. Compound **5** was treated with hydrochloric acid to provide C-3 pyridone product **10**, which was hydrolyzed to its corresponding C-2 carboxylic acid **11**. The acid was activated with EDC and cyclized intramolecularly to the pyridone to form the tetracyclic lactone **12**. The indole nitrogen was then alkylated with either a benzylic halide similar to the conversion of **5** to **6**,

Scheme 1. [a]



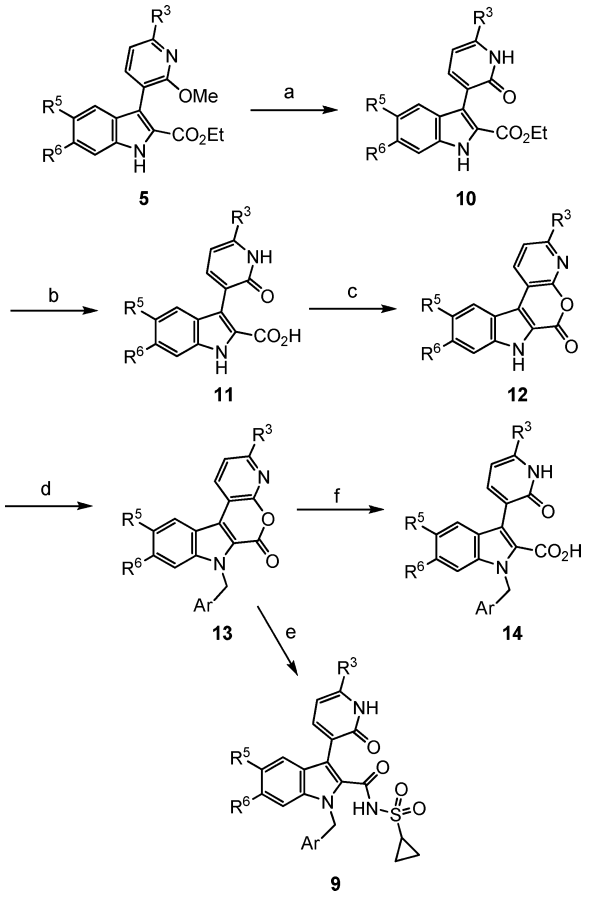
[a]Reaction conditions: (a) NIS, $CHCl_3$; (b) 2-methoxy-3-pyridineboronic acid, $PdCl_2(dppf)_2$, K_2CO_3, 1,2-dimethoxyethane, 50−85% (two steps); (c) $ArCH_2Br$ (or $ArCH_2Cl$), Cs_2CO_3, DMF, 40−90%; (d) LiOH, THF/H_2O, 60−90%; (e) CDI, then RSO_2NH_2, DBU, THF, 50−80%; (f) 4 M HCl, dioxane, 30−60%.

or via a Mitsunobu reaction[29] with a benzylic alcohol. The resulting lactone product **13** was either reacted with a sulfonamide in the presence of 1,8-diazabicyclocundec-7-ene (DBU) to give the desired acylsulfonamide analogue **9**, or hydrolyzed with lithium hydroxide to carboxylic acid inhibitor **14**. The products **9** and **14** were then evaluated in enzyme and replicon assays to determine their biological activities.

RESULTS AND DISCUSSION

Although lead compound **2** had an overall good profile, better potency was desired for a potential drug development candidate. Several modifications attempted at the indole core failed to improve the potency of the resulting inhibitors. We decided to keep the indole core intact but to make changes at substituents that attached to various positions of the indole ring system. Thus, to optimize the indole N-1 substituent, a number of benzyl type structures with various substitution patterns were examined. Results from selected examples are shown in Table 1. Compound **15** had only one fluoro group at the 2′-position and had slightly better potency in both RdRp enzyme (EC_{50} = 50 nM) and replicon cell-based assays (EC_{50} = 50 nM) than that of the benchmark compound **2** which had two fluoro substitutions at the 2′,5′-positions. When an additional methyl was introduced to C-3′ of **15**, the activity of compound **16** against enzyme improved even further to an IC_{50} of 4 nM. However, its replicon assay potency decreased to an EC_{50} of 380 nM probably due to some unfavorable interactions from

Scheme 2. [a]



[a]Reaction conditions: (a) 4 M HCl, dioxane; (b) LiOH, THF/H_2O; (c) EDC, NEt_3, DMF, 60−90%; (d) $ArCH_2Br$ (or $ArCH_2Cl$), Cs_2CO_3, DMF; or $ArCH_2OH$, DIAD, PPh_3, THF, 60−90%; (e) RSO_2NH_2, DBU, DMF, 30−60%; (f) LiOH, THF/H_2O, 20−60%.

3′-methyl group. Analogue **17** had the same N-1 4-(2′-aminopyridine)methyl moiety as that of compound **1**. It maintained enzyme activity (IC_{50} = 6 nM) but lost even more cellular potency (EC_{50} = 500 nM). Further exploration at the 5′-substitution was also conducted to search for a group that was capable of establishing additional interactions with nearby protein backbone residues. Toward that end, moieties with different functionalities were examined. A 5′-nitrile group in compound **18** caused a drop in both enzyme and replicon potency (IC_{50} = 9 nM, EC_{50} = 220 nM) when compared to 5′-nonsubstituted **15**. The 5′-primary amide analogue **19** faired better in the enzyme assay (IC_{50} = 4 nM) but lost activity in the replicon assay by 3-fold.

Several analogues with a sulfonyl moiety para to the fluoro group were also prepared (**20** to **22**). The compound bearing a C-5′-methyl sulfone (**20**) maintained a good IC_{50} of 6 nM while losing significant cellular assay activity (EC_{50} = 580 nM). The primary sulfonamide derivative **21** did not provide any improvement, having almost the same IC_{50} and EC_{50}. When an additional nitrile group was introduced at C-3′ however, dramatic changes in potency were observed. The 3′-nitrile-5′-methyl sulfone analogue **22** not only gave a better IC_{50} of 3 nM but also provided a much better EC_{50} (5 nM) in the replicon assay. The 100-fold improvement in cellular potency compared to **20** cannot be easily explained by any extra simple contact(s) established by the nitrile group, and there was a possibility that

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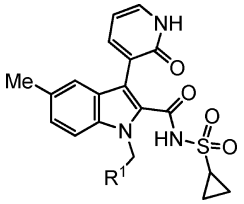
Table 1. SAR of Substitution at the Benzyl Group at the Indole Core N-1 Position

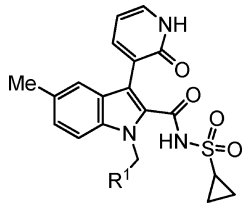


Compound	R^1	IC_{50} (nM) (n≥2)	EC_{50} (nM) (n=2)
2		6	90
15		5	50
16		4	380
17		6	500
18		9	220
19		4	140
20		5	580
21		5	550
22		3	5

some unusual interaction(s) had occurred when this additional nitrile group was present. Looking at the structure, one could make an educated guess from chemistry knowledge and literature that two strong electron-withdrawing-groups at para-and ortho-positions could have made the fluoro substituent very susceptible to attack by an adjacent nucleophile. By checking out the amino acid residues of the enzyme backbone in the region to where the benzyl group was bound, cysteine-366 appeared to be within attacking distance. The thiol of cysteine-366 could replace the fluoro atom to form a covalent irreversible sulfide bond with the benzene ring. Although reversible covalent bond formation with cysteine-366 has been reported previously,[30] no irreversible inhibitors were ever identified. If the irreversible sulfide bond formation could be confirmed, a new class of covalently bonded HCV NS5B polymerase inhibitors was then discovered. We decided to conduct further investigation into this class of compounds.

Exploration of Potential Irreversible Inhibitors. To explore other phenyl rings that had the potential to form a covalent bond with cysteine-366, a number of N-1 benzyl groups with multiple electron-withdrawing groups were studied (Table 2). Leaving groups other than fluoride, such as chloride, were also examined. In compound **23**, the benzene ring was substituted by 2′-chloro and strongly electron-withdrawing 5′-trifluoromethyl sulfone. However, with an IC_{50} of 46 nM and an EC_{50} of 900 nM, **23** was even less potent than that for most

Table 2. NS5B Polymerase Inhibitors[a]



Compound	R^1	IC_{50} (nM) (n≥2)	EC_{50} (nM) (n=2)
23		46	900
24		14	3400
25		14	270
26*		2	60
27*		3	3
28*		6	1400
29		14	3300
30		7	16
31		6	1

[a]An asterisk denotes that the C5 substituent was an ethyl group for these inhibitors.

noncovalent inhibitors in Table 1 and definitely much less potent than that for potential covalent inhibitor **22**. The less electron-withdrawing trifluoromethyl sulfoxide **24** was, not surprisingly, even less potent in the replicon assay (EC_{50} = 3400 nM) than that of **23**. The 2′,3′,6′-trifluoro-5′-methylsulfonylbenzene analogue **25** was also not very potent, although it had a better EC_{50} (270 nM) than those of **23** and **24**. Apparently, the two extra fluorines were not strong enough electron-withdrawing groups. Similarly, an additional 4′-chloro substituent in **26** was not sufficient to make the compound susceptible to covalent bonding. But both its enzymatic and cellular potency (IC_{50} = 2 nM, EC_{50} = 60 nM) improved significantly over other sulfones **23**–**25**, probably because of favorable interaction(s) with the 4′-substituent. When the 4′-position was substituted with a cyano group (**27**), however, the potency was dramatically improved to an EC_{50} of 3 nM, even better than that of compound **22**. Again, it was speculated that the strong electron-withdrawing sulfone and cyano groups made the nucleophilic replacement of 2′-F much easier. The two multisubstituted 5′-sulfonamide or primary amide analogues **28** and **29** were, as expected, much less potent inhibitors (EC_{50} = 1400 and 3300 nM, respectively) due to the weaker electron-withdrawing ability of sulfonamides, amides and halogens. The fact that C-5 was substituted by an ethyl group in compounds **26**, **27**, and **28** instead of a methyl group in other compounds might have small impact on the IC_{50}s and EC_{50}s of these inhibitors, but the difference in potency should be quite small based on SAR from other series.[26]

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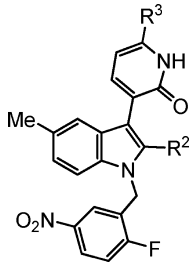
Because the nitro group is known to be one of the strongest electron-withdrawing groups, we hoped that it would provide facile covalent bond formation. Indeed, in the presence of the C-5′ nitro group, significant improvement in potency for the 2′-chloro analogue **30** (EC_{50} = 16 nM) was observed compared to **23** and **24**. The 5′-nitro-2′-fluoro analogue **31** had an EC_{50} of 1 nM. Simple hydrophobic interactions are unlikely to explain the significant difference in potency among the inhibitors in Tables 1 and 2. Evidence for covalent bond formation from mass spectroscopy and X-ray crystal structure studies will be discussed in the later section.

Optimization of Substitutions at C-2 and the Pyridone Ring. Because of the increased potency of *p*-nitro-fluoro-benzyl-substituted compound **31**, further investigation of more compounds with this N-1 substituent was conducted. Thus, analogues with a series of C-2 moieties were prepared and tested (Table 3). The C-2 carboxylic acid derivative **32** although it was found to be unstable in PK studies and possessed no appreciable AUC in rats.

Our early SAR studies indicated that introduction of a methyl substitution at the indole C-3 pyridone ring could improve rodent PK, probably due to less metabolism at the pyridone. A methyl group was introduced to pyridone C-6 of compounds **31**–**33** to give compounds **37**, **38**, and **39**, respectively. Unfortunately, acid analogue **37** lost substantial activity in both enzyme and cellular assays. Although methyl acylsulfonamide **38** was as active as **33** in the enzyme assay, it was 8-fold less potent in replicon assay. The cyclopropyl acylsulfonamide analogue **39** also lost potency in both the enzyme assay (by 2-fold) and replicon assay (by 9-fold). The decrease in potency of in these methyl-substituted pyridone analogues could be attributed to the unfavorable interactions between methyl and the protein backbone (see X-ray structure in Figure 2 for protein surface). On the basis of these results, we decided to continue to use the C-2 cyclopropyl acylsulfonamide moiety in further SAR development.

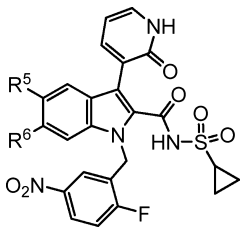
SAR Development of C-5 and C-6 Substituents. Keeping the N-1, C-2, and C-3 moieties constant, the effects of varying C-5 and C-6 substituents were studied and the results are shown in Table 4. Because our previous SAR indicated that the C-5-position cannot tolerate large groups, a number of small and compact substituents were tested in compounds **40**–**45**. Compared to benchmark methyl-substituted analogue **31**, the smaller C-5 substituent such as fluoro decreased activity in the replicon assay (**40**, EC_{50} = 6 nM). A similar loss of potency was observed in nonsubstituted compound **41**. Although the size of a chloro atom is close to that of a methyl group, C-5 chloro derivative **42** was still five times less active than that of **31**. The slightly larger trifluoromethyl group in compound **43** faired better (EC_{50} = 2 nM) with only a 2-fold loss of potency compared to **31**. The large *tert*-butyl group in inhibitor **44** was tolerated as evidenced by the same IC_{50} of 6 nM as that of compound **31**. However, the fact that a 10-fold loss in cellular assay activity (EC_{50} of 10 vs 1 nM) was observed could not be easily explained. The slightly larger ethyl group gave excellent potency in both the enzyme and replicon assays (IC_{50} = 4 nM and EC_{50} = 1 nM, respectively). In summary, the C-5-position preferred small

Table 3. C-2 and Pyridone Ring Substituent Modifications



Compound	R^2	R^3	IC_{50} (nM) (n≥2)	EC_{50} (nM) (n=2)
32		H	3	1
33		H	6	1
31		H	6	1
34		H	4	3
35		H	7	2
36		H	5	0.5
37		Me	13	30
38		Me	6	8
39		Me	12	9

Table 4. Optimization of C-5 Substitution and the Effect of the C6 Substituent



compound	R^5	R^6	IC_{50} (nM) ($n \geq 2$)	EC_{50} (nM) ($n = 2$)
31	Me	H	6	1
40	F	H	5	6
41	H	H	4	6
42	Cl	H	6	5
43	CF_3	H	4	2
44	*t*-Bu	H	6	10
45	Et	H	4	1
46	Me	F	7	4

maintained the excellent replicon potency (EC_{50} = 1 nM) of compound **31**. The methyl acylsulfonamide **33** also proved to be an equally potent inhibitor. Isopropyl acylsulfonamide **35** and acylsulfinamide **36**, however, were slightly less potent than that of **31**–**33**. Dimethyl acylsulfonylurea inhibitor **36**, surprisingly, had subnanomolar replicon potency (EC_{50} = 0.5 nM),

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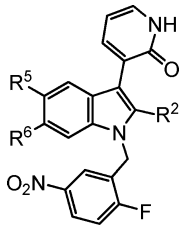
alkyl substituents such as methyl, ethyl, and trifluoromethyl groups. To explore the effect of C-6 substitution, a small fluoro atom was introduced to **31**, which resulted in compound **46**. Unfortunately, this substitution caused a 4-fold drop in replicon potency to an EC_{50} of 4 nM.

Investigation of PK Properties of Best Compounds. With the discovery of a number of potent inhibitors with superb potency, the next step was to evaluate their pharmacokinetic properties. Thus, a series of potent and representative compounds were selected for rat PK studies, and some results are shown in Table 5. It was very disappointing to find out that all four C-5 methyl derivatives (**31**, **33**, **36**, and **32**) with various C-2 acylsulfonamide or carboxylic acid moieties had very low or no AUC when dosed orally in rats at 10 mg/kg. The C-6 fluoro-substituted analogue **46** was slightly better with an AUC of 0.35 μM·h. The C-5 chloro and ethyl compounds (**42** and **45**, respectively) did not provide good exposure with AUCs of 0.30 and 0.17 μM·h, respectively, although they were somewhat better than that of the C-5 methyl compound **31**. However, it was very gratifying to see good oral exposure (18 μM·h) with compound **47**, a C-2 carboxylic acid inhibitor. The combination of both potency and good oral exposure in rats led us to further evaluate this compound.

Table 5. Evaluation of Pharmacokinetic Property of Selective Compounds



Compound	R^2	R^5	R^6	EC_{50} (nM) (n=2)	Rat oral AUC (10 mg/kg) (μM·h)
31		Me	H	1	0.01
33		Me	H	1	0.19
36		Me	H	0.5	0
32		Me	H	1	0.14
46		Me	F	4	0.35
42		Cl	H	5	0.30
45		Et	H	1	0.17
47		**Et**	**H**	**1**	**18**

Profile of Lead Compound 47. In addition to good oral exposure in rats at 10 mg/kg, the C-5 ethyl C-2 carboxylic acid derivative **47** demonstrated a bioavailability of 37% with an IV half-life of 8.2 h in rats (Table 6). In monkey PK studies with a 3 mg/kg oral dosing, it also exhibited a good AUC of 3.4 μM·h along with 30% bioavailability. The PK profile was even more impressive in dogs: an oral AUC of 11 μM·h coupled with 95% bioavailability when dosed at 3 mg/kg. The half-lives of compound **47** in monkeys and dogs are on the short side (1.3 and 1.2 h, respectively). The clearance rates (CL) were moderate for **47** in all three species. It had a good liver concentration of 200 ng/g (0.46 μM) in rats 6 h after dosing. It was stable for up to 3 h in rat, monkey, dog, and human plasma. No inhibition of CYP P450 enzymes 2D6, 3A4, and 2C9 was observed when tested up to 30 μM. It was also clean in a P450 enzyme induction assay at 10 μM. Overall, compound **47** exhibited very favorable potency, PK, and selectivity. It was considered as a potential candidate for further evaluation.

Table 6. PK Parameters of Compound 47

compound **47**	rat	monkey	dog
AUC(PO) (μM·h)	18[a]	3.4[b]	11[b]
bioavailability (F)	37%	30%	95%
$t_{1/2}$ (IV) (h)	8.2	1.3	1.2
CL (mL/min/kg)	8.0	10	10
rat liver concn (μM @ 6 h)	0.46	–	–

[a]10 mg/kg. [b]3 mg/kg. All administered with 0.4% MC.

X-ray Structure of Compound 47.[31] The X-ray crystal structure of compound **47** bound to HCV NS5B polymerase was solved (Figure 2[32]). Similar to the X-ray structure of compound **1** bound to the protein, **47** is bound to the 'palm' site of NS5B apoprotein within the active site cavity. The refined structure showed a bond between the side chain of Cys-366 and the phenyl ring of the compound with a 1.8 to 1.9 Å S−C distance. This covalent bond is a result of the side chain thiol attacking the electron-deficient benzene ring at the position para to the nitro group with departure of the fluoro group via a presumed SNAr reaction. The indole core and the C3 pyridone ring contact the side chain of Met-414 while the C3 pyridone forms two hydrogen bond interactions with the backbone of Ile-447 and Tyr-448. The C-2 carboxylic acid does not make direct interactions with the protein, but it rather interacts indirectly with amino acid residues Gly-449 and Ser-556 via hydrogen bonds with bridging water molecules. The indole C5 ethyl group projects into a constricted tunnel which extends to the protein surface on the other side of the 'thumb' subdomain.

Selectivity and Toxicity Concerns about Irreversible Inhibitors. Although we were very excited by the potency brought by the covalent bonding with the *p*-fluoro-nitro-benzyl moiety, and covalently bonded irreversible inhibitors have been seen in numerous drugs and drug candidates,[33] the reactive nature of these compounds is always raising an alarm of potentially nonselective bond formations. The potential side reactions from this nitro-fluoro series of irreversible inhibitors was also a concern we felt we needed to address. A NMR spectroscopy-based assay (ALARM NMR)[34] which was designed to identify thiol reactive compounds was used to evaluate these inhibitors. In this assay, the highly reactive thiols in the cysteines of the human La antigen were exploited to detect nonspecific covalent bonding activity. When compounds **45** and **47** were tested in the ALARM assay, no reactions between these two compounds and the reactive thiols in the human La antigen were detected by NMR studies. This indicated that the reactivity of the *p*-fluoro-nitro-substituted benzene moiety was not indiscriminately reactive toward any

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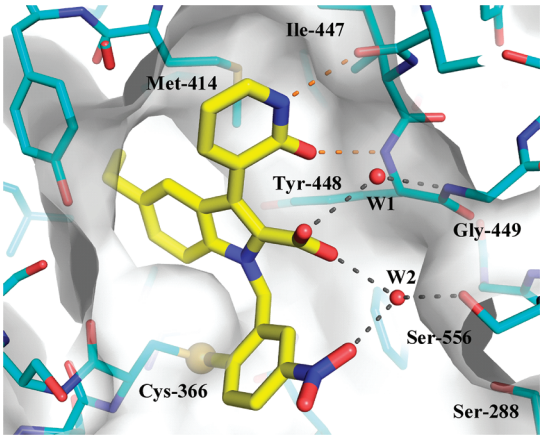

Figure 2. X-ray structure of compound **47** bound to the palm site of HCV NS5B polymerase. The compound (yellow) is covalently attached to the protein (cyan) via a bond between the side chain sulfur of Cys-366 (depicted as a small sphere) and the 5′-position of the compound. The electron density for the compound and residue Cys-366 is best modeled as two alternate conformations and are included as such in the deposited PDB entry 3TYQ. A single conformation is shown here for clarity. The compound makes identical interactions as observed in the complex between inhibitor **1** and NS5B,[23] with a pair of hydrogen bonds from the C3-pyridone group to the backbone of residues Ile-447 and Tyr-448. Furthermore, the interaction of the C2-carboxylate group with the protein is mediated by a pair of water molecules, shown as red spheres. Besides the backbone atoms of Ile-447 and Tyr-448, all side-chain atoms within 5 Å of the ligand are depicted as sticks.

cysteines present in the protein. Rather, the reaction was selective and specific toward cysteine-366 which existed in close proximity. The specificity was also confirmed by mass spectroscopy studies. When compound **33** was incubated with NS5B enzyme, only tryptic peptide sequence 346−379 was modified by changing from 3533 Da to 4012 Da, which was consistent with covalent bonding with cysteine-366. No other tryptic peptide was found to be modified by 480.0 Da. All these studies suggested that this class of irreversible inhibitors was highly selective and specific in the formation of a covalent adduct, reacting only with cysteine-366. The SAR trends of these irreversible inhibitors also indicated that the covalent bonding was induced by proximity after the inhibitor bound to the enzyme surface through traditional binding interactions.

Even though there are several marketed drugs that contain nitro-aromatic moieties, some concerns still exist about the potential safety issues arising from the metabolites of the nitrobenzene. The partial reduction of the nitro group could lead to the formation of nitro-anion radical or a nitroso group, which could cause cytotoxic effects.[35] To address this concern, compound **45** and **47** were tested in the *Salmonella*/mammalian microsome Mini-AMES reverse mutation assay. No bacterial mutagenicity was observed with or without metabolic activation. Both compounds were also evaluated in the in vitro micronucleus induction assay. No chromosome damage was found with either **45** or **47**. Inhibitor **47** was also found to be clean in counterscreens of kinases and protease panels. In summary, the fact that compound **47** was clean in all in vitro assays discussed above and no toxicity was observed in single dose animal PK studies demonstrated that no evidence for potential safety issues related to the aromatic nitro moiety has been found.

CONCLUSION

Starting from lead compound **2**, extensive SAR development on the indole N-1 benzyl moiety was performed. The effort led to the discovery of irreversible inhibitors of NS5B polymerase with significant improvements in cellular replicon assay potency. Some indole-based inhibitors with *p*-fluoro-sulfone-substituted N-1 benzyl groups were speculated to be capable of forming a covalent bond with the cysteine-366 thiol of the NS5B polymerase. The compounds with a *p*-nitro-fluoro-benzyl substituent at the indole nitrogen were the most potent inhibitors found which achieved impressive replicon potencies with EC_{50}s down to 1 nM. The effect of C-3 pyridone substitution and variation of C-2 moieties were also investigated. While all C-2 carboxylic acid, acylsulfonamide, and acylsulfonylurea analogues had superior potency, the methyl substitution at the C-3 pyridone decreased activity of the corresponding inhibitors. The C-5 SAR investigation demonstrated that small alkyl groups such as methyl and ethyl groups were preferred. The addition of a C-6 fluoro substituent did not improve either potency or PK profile. A selected group of the most promising compounds were evaluated in rat PK studies. Most of them exhibited disappointing oral exposure (AUC < 0.4 μM·h when dosed at 10 mg/kg). However, the C-5 ethyl C-2 carboxylic acid analogue **47** stood out, having an excellent oral AUC of 18 μM·h at 10 mg/kg. Further studies proved that **47** also had good oral exposure and bioavailability in monkeys and dogs. The NMR ALARM assay and mass spectroscopy experiments demonstrated that compound **47** was highly selective and specific in the formation of a covalent bond with cystaine-366 thiol. The concerns about the potential nonselective reactivity and reactive intermediates from the partially reduced nitro group were largely alleviated by the negative results in several in vitro counter screens and toxicology assays. The overall profile of **47** in terms of potency, PK, and safety made it a good candidate for further evaluation.

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 × 250 mm, 10 μm) running at 30 mL/minute 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 all 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 the mixture was stirred for 5 min (min), ethyl acetate (30 mL) and water (20 mL) were added. Layers were 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 directly.

*General Procedures for Suzuki Coupling between **4** and 2-Methoxy-3-pyridineboronic Acids.* 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 (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 being cooled 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 **5** in 50−85% yield (two steps).

*General Procedures for the Preparation of **6**.* The mixture of **5** (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. It 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) and 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 **6** in 40−90% yield.

*General Procedures for the Preparation of **7**.* Compound **6** (1.0 mmol) and lithium hydroxide (4.0 mmol) were 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 the 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 **7** in 60−90% yield, which was used without further purification.

*General Procedures for the Preparation of **8**.* The mixture of **7** (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. It was then cooled to rt, alkyl sulfonamide and 1,8-diazabicyclocundec-7-ene (DBU) (1.5 mmol each) were added, and the resulting mixture was stirred at rt for 18 h. The mixture 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 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 0−80% acetone in dichloromethane as eluent to give the desired product **8** in 50−80% yield.

*General Procedures for the Preparation of **9**.* Compound **8** (1.0 mmol) was dissolved in a solution of 4 M hydrogen chloride in *p*-dioxane (50 mL) in a pressure glass tube. The solution was then sealed and 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 purified by reverse phase HPLC to give the desired product **9** in 30−60% yield.

Compound **10** was prepared from compound **5** according to the same procedures described above for the preparation of **9** from compound **8**.

Compound **11** was prepared from compound **10** according to the same procedures described above for the preparation of **7**. The crude product was used in the next reaction without further purification.

*General Procedures for the Preparation of **12**.* To the solution of compound **11** (1.0 mmol) in anhydrous DMF (5 mL) at rt was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (442 mg, 2.3 mmol) and triethylamine (0.69 mL, 5.0 mmol). The mixture was stirred overnight (17 h). EtOAc (15 mL), 5% aqueous phosphoric acid solution (7 mL), and water (7 mL) were added. After the layers were separated, the aqueous solution was extracted with EtOAc (3 × 22 mL). The combined organic solution was dried over magnesium sulfate and concentrated in vacuo to give the crude product in 60−90% yield, which was used without further purification.

Compound **13** was prepared from compound **12** according to the same general procedures described above for the preparation of **6** from **5**. The crude product was used in the next reaction without further purification.

*Alternative General Method for Preparation of **13** from **12** through a Mitsunobu Reaction.* To the mixture of **12** (2.0 mmol), arylmethyl alcohol (2.8 mmol), and triphenylphosphine (3.8 mmol) in anhydrous THF (50 mL) was added diisopropyl azodicarboxylate (DIAD) (3.8 mmol). The resulting mixture was stirred at rt for 16 h. It was 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 **13** in 60−90% yield.

*General Procedures for the Preparation of **9** from **13**.* To the solution of compound **13** (0.2 mmol) and appropriate alkyl primary sulfonamide (0.4 mmol) in anhydrous THF (5 mL) at rt was added sodium hydride (30 mg, 1.5 mmol, 60% in mineral oil). The mixture was heated to 70 °C in an oil bath for 1.5 h. After the mixture was cooled to rt, EtOAc (30 mL) and water (40 mL) were added. After the layers were separated, the aqueous solution was extracted with EtOAc (2 × 40 mL). The combined organic solution was dried over magnesium sulfate and concentrated in vacuo to give the crude product in 30−60% yield.

*General Procedures for the Preparation of **14**.* Compound **14** (0.3 mmol) and lithium hydroxide (2.0 mmol) were dissolved in THF and water (10 mL each). The solution was stirred at rt for 4 h. EtOAc (40 mL) and 5% aqueous phosphoric acid solution (20 mL) were added. After the layers were separated, the aqueous solution was extracted with ethyl acetate (2 × 30 mL). The combined organic solution was 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 (**14**) in 20−60% yield.

***N*-(Cyclopropylsulfonyl)-1-[(2,5-difluorophenyl)methyl]-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (2).** Compound **2** 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), 12.79 (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 $C_{25}H_{22}N_3O_4F_2S$ $(M + H)^+$, 498.1; found, 498.3.

***N*-(Cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1-[(2-fluorophenyl)methyl]-5-methyl-1*H*-indole-2-carboxamide (15).** Compound **15** 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), 12.80 (s, 1 H), 12.74 (s, 1 H), 7.7 9(d, *J* = 2.2 Hz, 1 H), 7.69 (t, *J* = 4.4 Hz, 1 H), 7.52 (d, *J* = 9.0 Hz, 1 H), 7.33−7.28 (m, 1 H), 7.23−7.18 (m, 3 H), 7.10−7.06 (m, 1 H), 6.87−6.82 (m, 1 H), 6.30 (t, *J* = 6.6 Hz, 1 H), 5.75 (s, 2 H), 2.93−2.89 (m, 1 H), 2.35 (s, 3 H), 0.96 (d, *J* = 6.3 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.0, 172.4, 172.0, 170.1, 166.0, 162.8, 157.4, 155.6, 150.5, 147.5, 141.6, 140.8, 140.0, 138.5, 137.7, 135.9, 131.1, 126.8, 126.6, 122.2, 118.9, 116.5, 78.4, 52.8, 45.7, 42.0, 41.8, 32.4, 16.8. LRMS calcd for $C_{25}H_{23}N_3O_4FS$ $(M + H)^+$, 480.1; found, 480.3.

***N*-(Cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1-[(2-fluoro-3-methylphenyl)methyl]-5-methyl-1*H*-indole-2-carboxamide (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), 12.79 (s, 1 H), 12.73 (s, 1 H), 7.78 (q, *J* = 1.8 Hz, 1 H), 7.69 (t, *J* = 5.8 Hz, 1 H), 7.51 (d, *J* = 9.0 Hz, 1 H), 7.19−7.15 (m, 3 H), 6.95 (t, *J* = 7.6 Hz, 1 H), 6.64 (q, *J* = 5.0 Hz, 2 H), 5.73

(s, 2 H), 2.95−2.90 (m, 1 H), 2.35 (s, 3 H), 2.22 (s, 3 H), 0.97 (d, J = 6.6 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.0, 172.4, 155.6, 147.5, 147.0, 145.4, 142.0, 141.5, 140.0, 138.4, 137.6, 137.4, 136.2, 135.3, 134.4, 131.0, 125.9, 122.2, 118.8, 52.9, 45.9, 42.0, 41.8, 32.4, 25.4, 16.8. LRMS calcd for $C_{26}H_{25}N_3O_4FS$ (M + H)$^+$, 494.2; found, 494.3.

1-[(2-Amino-4-pyridinyl)methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (17). Compound **17** 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), 13.45 (s, 1 H), 12.84 (s, 2 H), 7.94 (s, 1 H), 7.90 (d, J = 6.6 Hz, 1 H), 7.81 (q, J = 1.9 Hz, 1 H), 7.74 (s, 1 H), 7.45 (d, J = 8.2 Hz, 1 H), 7.24 (s, 2 H), 6.68 (t, J = 6.9 Hz, 1 H), 6.60 (d, J = 7.0 Hz, 1 H), 6.41 (s, 1 H), 5.67 (s, 2 H), 2.98−2.93 (m, 1 H), 2.37 (s, 3 H), 1.01 (d, J = 7.9 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.0, 172.1, 167.3, 165.4, 155.8, 147.5, 142.1, 139.9, 139.0, 137.8, 134.1, 131.3, 130.0, 126.2, 122.0, 121.9, 120.4, 119.1, 109.3, 58.6, 42.6, 42.1, 41.8, 33.4, 32.4, 25.3, 16.8. LRMS calcd for $C_{24}H_{24}N_5O_4S$ (M + H)$^+$, 478.2; found, 478.3.

1-[(5-Cyano-2-fluorophenyl)methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (8). Compound **18** 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), 12.73 (s, 1 H), 12.65 (s, 1 H), 7.90−7.86 (m, 1 H), 7.82 (d, J = 6.4 Hz, 1 H), 7.67 (s, 1 H), 7.56−7.49 (s, 2 H), 7.23−7.20 (m, 3 H), 6.61 (t, J = 6.2 Hz, 1 H), 5.76 (s, 2 H), 2.93−2.87 (m, 1 H), 2.37 (s, 3 H), 0.95 (d, J = 7.6 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 164.1, 163.9, 163.4, 162.1, 161.9, 161.5, 146.4, 144.9, 143.8, 140.1, 139.7, 139.4, 136.9, 136.4, 135.3, 134.8, 133.5, 133.2, 131.4, 129.6, 129.4, 128.2, 127.2, 125.8, 123.8, 123.0, 120.8, 118.8, 118.2, 118.1, 115.9, 111.5, 108.7, 108.3, 107.2, 98.3, 42.2, 35.2, 34.8, 32.9, 31.5, 31.3, 30.1, 29.9, 21.9, 6.3, 5.9, 5.1. LRMS calcd for $C_{26}H_{22}N_4O_4FS$ (M + H)$^+$, 505.1; found, 505.3.

1-[[5-(Aminocarbonyl)-2-fluorophenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (19). Compound **19** 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), 12.82 (s, 1 H), 12.78 (s, 1 H), 7.89 (s, 1 H), 7.84−7.80 (m, 2 H), 7.70 (s, 1 H), 7.55 (d, J = 7.8 Hz, 2 H), 7.38 (s, 1 H), 7.30 (t, J = 9.3 Hz, 1 H), 7.21−7.18 (m, 2 H), 6.65−6.62 (m, 1 H), 5.76 (s, 2 H), 2.96−2.89 (m, 1 H), 2.36 (s, 3 H), 0.96 (d, J = 6.0 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 167.6, 163.5, 161.9, 161.5, 152.5, 145.3, 137.2, 136.6, 131.6, 131.1, 130.0, 129.7, 128.1, 127.2, 125.9, 125.8, 125.2, 123.9, 120.7, 116.2, 116.0, 115.6, 111.7, 108.4, 67.9, 42.7, 35.3, 31.6, 31.3, 30.1, 28.9, 16.0, 24.9, 21.9, 6.3. LRMS calcd for $C_{26}H_{24}N_4O_5FS$ (M + H)$^+$, 523.1; found, 523.3.

1-[[5-(Aminosulfonyl)-2-fluorophenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (21). Compound **21** 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), 12.84 (s, 2 H), 7.82−7.78 (m, 2 H), 7.73−7.71 (m, 1 H), 7.54 (d, J = 8.1 Hz, 1 H), 7.49−7.43 (m, 2 H), 7.38 (s, 2 H), 7.21 (d, J = 8.8 Hz, 1 H), 7.19 (s, 1 H), 6.66 (t, J = 8.0 Hz, 1 H), 5.78 (s, 2 H), 2.96−2.91 (m, 1 H), 2.36 (s, 3 H), 0.97 (d, J = 6.1 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 163.2, 161.8, 161.2, 152.3, 145.4, 141.4, 137.3, 136.7, 131.3, 128.5, 127.5, 127.3, 126.9, 125.8, 120.8, 117.3, 115.7, 111.6, 108.6, 67.9, 42.8, 31.6, 31.3, 30.1, 26.0, 21.9. LRMS calcd for $C_{25}H_{24}N_4O_6FS_2$ (M + H)$^+$, 559.1; found, 559.3.

1-[[3-Cyano-2-fluoro-5-(methylsulfonyl)phenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (22). Compound **22** 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), 12.89 (s, 1 H), 12.83 (s, 1 H), 8.32 (d, J = 9.5 HZ, 1 H), 7.82 (q, J = 1.6 Hz, 1 H), 7.72 (s, 1 H), 7.62 (q, J = 6.8 Hz, 2 H), 7.24 (d, J = 8.9 Hz, 1 H), 7.22 (s, 1 H), 6.67−6.62 (m, 1 H), 5.87 (s, 2 H), 3.31 (s, 3 H), 2.93−2.89 (m, 1 H), 2.37 (s, 3 H), 0.97 (s, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 161.8, 161.4, 158.8, 157.0, 152.8, 145.4, 139.5, 137.3, 136.8, 133.1, 131.6, 131.2, 131.1, 128.5, 127.3, 120.8, 116.1, 115.3, 111.9, 111.5, 108.6, 44.0, 43.1, 31.6, 31.3, 21.9, 6.3. LRMS calcd for $C_{27}H_{24}N_4O_6FS_2$ (M + H)$^+$, 583.1; found, 583.0.

1-[[2-Chloro-5-[(trifluoromethyl)sulfonyl]phenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (23). Compound **23** 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), 13.03 (s, 1 H), 12.88 (s, 1 H), 8.08−8.02 (m, 2 H), 7.84 (dd, J = 1.6, 1.5 Hz, 1 H), 7.76−7.74 (m, 1 H), 7.50 (d, J = 8.8 Hz, 1 H), 7.24 (s, 1 H), 7.21 (d, J = 8.8 Hz,1 H), 7.06 (d, J = 1.8 Hz, 1 H), 6.68 (t, J = 6.6 Hz, 1 H), 5.87 (s, 2 H), 2.86−2.81 (m, 1 H), 2.37 (s, 3 H), 0.92 (d, J = 6.0 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 161.7, 156.9, 145.6, 142.4, 140.1, 137.1, 136.8, 133.0, 131.8, 131.6, 131.1, 129.6, 129.4, 128.6, 127.3, 123.5, 120.9, 116.0, 111.4, 109.8, 108.8, 16.6, 31.5, 21.9, 6.2, 5.1. LRMS calcd for $C_{26}H_{22}N_3O_6ClF_3S_2$ (M + H)$^+$, 628.0; found, 628.3.

1-[[2-Chloro-5-[(trifluoromethyl)sulfinyl]phenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (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), 12.93 (s, 1 H), 12.83 (s,1 H), 7.89 (d, J = 1.6 Hz, 1 H), 7.84−7.81 (m, 1 H), 7.73 (s,1 H), 7.43 (d, J = 8.8 Hz, 1 H), 7.23 (s,1 H), 7.18 (d, J = 8.4 Hz, 1 H), 6.91 (s, 1 H), 6.87 (s, 1 H), 6.71−6.63 (m, 1 H), 5.83 (s, 2 H), 2.88−2.80 (m, 1 H), 2.36 (s, 3 H), 1.35 (d, J = 1.6 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 152.3, 145.4, 140.0, 138.8, 137.8, 136.7, 135.5, 131.8, 131.5, 129.6, 128.9, 128.3, 127.3, 127.2, 126.7, 125.8, 125.2, 120.9, 111.5, 108.6, 108.5, 46.6, 35.2, 31.5, 31.3, 30.1, 21.9, 61.2. LRMS calcd for $C_{26}H_{22}N_3O_5ClF_3S_2$ (M + H)$^+$, 612.1; found, 612.3.

1-[[4-Chloro-2-fluoro-5-(methylsulfonyl)phenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (26). Compound **26** 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), 12.89 (s, 1 H), 12.83 (s, 1 H), 7.87 (d, J = 9.8 Hz, 1 H), 7.81 (d, J = 6.4 Hz, 1 H), 7.76 (d, J = 7.6 Hz, 1 H), 7.72 (s, 1 H), 7.61 (d, J = 8.4 Hz, 1 H), 7.28 (d, J = 8.5 Hz, 1 H), 7.20 (s, 1 H), 6.66 (t, J = 6.7 Hz, 1 H), 5.79 (s, 2 H), 3.31 (s, 3 H), 2.95−2.90 (m, 1 H), 2.67 (q, J = 7.5 Hz, 2 H), 1.18 (t, J = 7.6 Hz, 3 H), 0.99 (d, J = 8.5 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 164.2, 163.5, 162.1, 161.8, 161.4, 157.0, 145.5, 138.1, 137.4, 136.7, 136.3, 135.2, 133.1, 132.1, 129.2, 127.4, 127.1, 126.4, 126.3, 123.6, 120.7, 120.4, 119.5, 116.1, 111.6, 108.7, 43.3, 42.4, 31.6, 29.9, 29.4, 29.0, 17.0, 6.3, 5.9, 5.1. LRMS calcd for $C_{27}H_{26}N_3O_6ClFS_2$ (M + H)$^+$, 606.1; found, 606.3.

1-[[4-Cyano-2-fluoro-5-(methylsulfonyl)phenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (27). Compound **27** 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), 12.90 (s, 1 H), 12.85 (s, 1 H), 8.33 (d, J = 9.4 Hz, 1 H), 7.83 (d, J = 5.7 Hz, 1 H), 7.72 (s, 1 H), 7.67 (d, J = 6.7 Hz, 1 H), 7.62 (d, J = 8.8 Hz, 1 H), 7.29 (d, J = 9.0 Hz, 1 H), 7.22 (s, 1 H), 6.66 (s, 1 H), 5.87 (s, 2 H), 3.32 (s, 3 H), 2.90 (s, 1 H), 2.67 (q, J = 7.5 Hz, 2 H), 1.18 (t, J = 7.3 Hz, 3 H), 0.97 (s, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.4, 163.0, 162.4, 161.4, 161.0, 156.4, 155.6, 147.0, 141.3, 140.4, 139.7, 134.4, 132.5, 132.4, 130.9, 130.0, 128.2, 124.5, 124.3, 122.6, 122.0, 121.0, 114.4, 112.6, 85.4, 69.1, 67.0, 63.0, 44.0, 32.9, 29.8, 29.2, 28.3, 24.7, 22.6, 17.2, 6.1, 5.8, 4.7. LRMS calcd for $C_{28}H_{26}N_4O_6FS_2$ (M + H)$^+$, 597.1; found, 597.3

1-[[5-(Aminosulfonyl)-2,4-dichlorophenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-ethyl-1*H*-indole-2-carboxamide (28). Compound **28** 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), 12.87 (s, 2 H), 7.94 (s, 1 H), 7.83

(q, J = 1.8 Hz, 1 H), 7.75−7.73 (m, 1 H), 7.62 (s, 2 H), 7.44 (d, J = 8.6 Hz, 1 H), 7.29 (s, 1 H), 7.25 (q, J = 1.7 Hz, 1 H), 7.23 (s, 1 H), 6.69 (t, J = 6.6 Hz, 1 H), 5.72 (s, 2 H), 2.93−2.88 (m, 1 H), 2.67 (q, J = 7.5 Hz, 2 H), 1.18 (t, J = 7.6 Hz, 3 H), 0.98−0.96 (m, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 163.6, 161.6, 147.0, 145.5, 141.0, 139.6, 138.1, 137.5, 136.8, 136.4, 136.1, 132.5, 130.7, 128.6, 127.5, 127.3, 123.7, 119.7, 115.9, 111.6, 108.7, 46.6, 31.6, 30.2, 29.1, 16.9, 6.3. LRMS calcd for $C_{26}H_{25}N_4O_6Cl_2S_2$ $(M + H)^+$, 623.1; found, 624.8.

1-[[3-(Aminocarbonyl)-2,5,6-trifluorophenyl]methyl]-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (29). Compound **29** 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), 12.67 (s, 1 H), 12.64 (d, J = 5.0 Hz, 1 H), 7.81 (s, 1 H), 7.75 (s, 1 H), 7.73 (q, J = 1.8 Hz, 1 H), 7.70−7.64 (m, 2 H), 7.54 (d, J = 8.5 Hz, 1 H), 7.23 (d, J = 8.6 Hz, 1 H), 7.17 (s, 1 H), 6.59 (t, J = 6.6 Hz, 1 H), 5.86 (s, 2 H), 2.98−2.93 (m, 1 H), 2.35 (s, 3 H), 1.06−0.99 (m, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 164.1, 163.3, 162.0, 152.3, 144.8, 137.0, 136.5, 131.1, 129.8, 128.0, 127.2, 125.8, 123.8, 120.8, 118.0, 117.9, 115.9, 111.2, 108.1, 67.9, 37.5, 31.3, 26.0, 21.8, 6.3. LRMS calcd for $C_{26}H_{22}N_4O_5F_3S$ $(M + H)^+$, 559.1; found, 559.3.

1-[(2-Chloro-5-nitrophenyl)methyl]-*N*-(-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxamide (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), 12.88 (s, 1 H), 12.77 (s, 1 H), 8.15 (q, J = 2.8 Hz, 1 H), 7.86−7.84 (m, 2 H), 7.72 (s, 1 H), 7.48 (d, J = 8.5 Hz, 1 H), 7.27 (s, 2 H), 7.21 (d, J = 8.6 Hz, 1 H), 6.65 (t, J = 6.6 Hz, 1 H), 5.80 (s, 2 H), 2.88 − 2.82 (m, 1 H), 2.38 (s, 3 H), 0.92 (d, J = 6.2 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 164.4, 163.8, 163.5, 161.8, 148.7, 147.5, 145.2, 139.1, 138.8, 137.1, 136.6, 131.8, 131.5, 130.7, 128.5, 127.3, 124.6, 122.8, 120.9, 111.5, 108.4, 46.6, 31.5, 21.9, 6.2. LRMS calcd for $C_{25}H_{22}N_4O_6ClS$ $(M + H)^+$, 541.1; found, 541.3.

***N*-(Cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-5-methyl-1*H*-indole-2-carboxamide (31).** Compound **31** 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), 12.86 (s, 1 H), 12.76 (s, 1 H), 8.25 −8.22 (m, 1 H), 7.82 (d, J = 7.2 Hz, 1 H), 7.75−7.73 (m, 1 H), 7.70 (s, 1 H), 7.58 (q, J = 7.8 Hz, 2 H), 7.23 (d, J = 6.5 Hz, 2 H), 6.66−6.61 (m, 1 H), 5.82 (s, 2 H), 2.93−2.87 (s, 1 H), 2.37 (s, 3 H), 0.96 (d, J = 5.7 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 165.1, 163.5, 163.1, 161.9, 145.2, 144.9, 137.1, 136.6, 131.4, 129.5, 128.3, 128.1, 128.0, 127.3, 126.3, 125.2, 123.8, 120.8, 118.1, 117.9, 115.9, 108.4, 42.6, 31.6, 21.9, 6.3. LRMS calcd for $C_{25}H_{22}N_4O_6FS$ $(M + H)^+$, 525.1; found, 525.3.

1-[(2-Fluoro-5-nitrophenyl)methyl]-3-(2-hydroxy-3-pyridinyl)-5-methyl-1*H*-indole-2-carboxylic acid (32). Compound **32** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **14**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.04 (s, 1 H), 11.81 (s, 1 H), 8.23−8.20 (m, 1 H), 7.61 (q, J = 2.85 Hz, 1 H), 7.58−7.53 (m, 3 H), 7.42 (q, J = 1.9 Hz, 1 H), 7.26 (s, 1 H), 7.19−7.17 (m, 1 H), 6.35 (t, J = 6.6 Hz, 1 H), 5.91 (s, 2 H), 2.37 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 174.5, 173.5, 172.7, 164.6, 155.4, 151.6, 149.1, 147.4, 145.6, 141.5, 141.3, 139.3, 138.3, 137.6, 136.6, 135.0, 131.3, 128.7, 128.5, 128.3, 121.9, 116.6, 108.9, 87.0, 52.9, 41.8, 36.8, 32.4. LRMS calcd for $C_{22}H_{17}N_3O_5F$ $(M + H)^+$, 422.1; found, 422.2.

3-(1,2-Dihydro-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-5-methyl-*N*-(methylsulfonyl)-1*H*-indole-2-carboxamide (33). Compound **33** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.83 (s, 1 H), 12.71 (s, 1 H), 8.25−8.22 (m, 1 H), 7.83 (d, J = 6.9 Hz, 1 H), 7.76−7.74 (m, 1 H), 7.68 (s, 1 H), 7.59−7.53 (m, 2 H), 7.24 (s, 2 H), 6.65−6.62 (m, 1 H), 5.80 (s, 2 H), 3.25 (s, 3 H), 2.37 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 65.1, 163.3, 163.1, 162.2, 155.6, 145.0, 140.1, 137.0, 136.4, 134.2, 131.5, 128.4, 128.0, 127.9, 115.8, 111.5, 108.4, 42.6, 42.0, 35.2, 31.3, 30.1, 22.5, 21.8. LRMS calcd for $C_{23}H_{20}N_4O_6FS$ $(M + H)^+$, 499.1; found, 499.3.

3-(1,2-Dihydro-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-5-methyl-*N*-[(1-methylethyl)sulfonyl]-1*H*-indole-2-carboxamide (34). Compound **34** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.81 (s, 1 H), 12.74 (s, 1 H), 8.25−8.22 (m, 1 H), 7.84−7.83 (m, 1 H), 7.71 (q, J = 8.8 Hz, 2 H), 7.61−7.54 (m, 2 H), 7.24 (s, 1 H), 7.22 (s, 1 H), 6.65 (t, J = 6.6 Hz, 1 H), 5.81 (s, 2 H), 3.61−3.55 (m, 1 H), 2.37 (s, 3 H), 1.13 (d, J = 6.9 Hz, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6), 165.2, 163.6, 163.1, 162.0, 145.4, 144.9, 137.2, 136.7, 131.5, 129.3, 128.5, 128.2, 128.1, 127.2, 126.4, 126.3, 125.1, 123.7, 120.8, 118.1, 117.9, 116.1, 111.5, 108.5, 53.6, 42.7, 31.3, 21.9, 16.1. LRMS calcd for $C_{25}H_{24}N_4O_6FS$ $(M + H)^+$, 527.1; found, 527.3.

3-(1,2-Dihydro-6-methyl-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-5-methyl-1*H*-indole-2-carboxylic acid (37). Compound **37** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **14**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.20 (s, 1 H), 11.91 (s, 1 H), 8.24−8.20 (m, 1 H), 7.62−7.61 (m, 1 H), 7.58−7.47 (m, 3 H), 7.24 (s, 1 H), 7.18 (d, J = 8.5 Hz, 2 H), 6.19 (d, J = 6.9 Hz, 1 H), 5.90 (s, 2 H), 2.36 (s, 3 H), 2.26 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 175.2, 174.6, 173.4, 155.4, 152.2, 150.9, 147.5, 141.2, 139.2, 138.3, 137.7, 135.1, 133.0, 131.4, 128.7, 128.5, 128.3, 121.8, 116.1, 108.5, 78.4, 32.4, 29.8. LRMS calcd for $C_{23}H_{19}N_3O_5F$ $(M + H)^+$, $C_{23}H_{18}N_3O_5F$: 436.1; found, 436.2.

3-(1,2-Dihydro-6-methyl-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl)methyl]-5-methyl-*N*-(methylsulfonyl)-1*H*-indole-2-carboxamide (38). Compound **38** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.12(s, 1 H), 12.83 (s, 1 H), 8.25−8.22 (m, 1 H), 7.77−7.75 (m, 2 H), 7.58−7.54 (m, 2 H), 7.23−7.21 (m, 2 H), 6.50 (d, J = 7.2 Hz, 1 H), 5.80 (s, 2 H), 3.27 (s, 3 H), 2.37 (d, J = 1.8 Hz, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6), 172.8, 163.6, 162.9, 158.2, 156.1, 155.2, 147.7, 141.8, 139.7, 138.9, 136.9, 135.7, 133.5, 133.4, 133.2, 131.5, 131.3, 130.3, 129.9, 129.8, 129.6, 128.4, 126.4, 123.4, 122.0, 118.7, 66.3, 52.6, 45.8, 32.4, 29.8, 27.2. LRMS calcd for $C_{24}H_{22}N_4O_6FS$ $(M + H)^+$, 513.1; found, 513.3.

***N*-(Cyclopropylsulfonyl)-3-(1,2-dihydro-6-methyl-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-5-methyl-1*H*-indole-2-carboxamide (39).** Compound **39** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 13.12 (s, 1 H), 12.87 (s, 1 H), 8.26−8.22 (m, 1 H), 7.76 (s, 2 H), 7.59−7.55 (m, 2 H), 7.23−7.20 (m, 2 H), 6.50 (s, 1 H), 5.82 (s, 2 H), 2.92 (s, 1 H), 2.36 (s, 6 H), 0.97 (s, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 164.0, 163.1, 162.0, 147.8, 145.7, 144.9, 137.2, 134.4, 131.4, 129.3, 128.4, 128.1, 128.0, 127.4, 126.4, 125.1, 123.0, 120.9, 119.8, 118.1, 117.9, 116.0, 111.5, 108.3, 45.6, 31.6, 26.1, 25.2, 24.6, 21.8, 19.3, 6.2, 5.8. LRMS calcd for $C_{26}H_{24}N_4O_6FS$ $(M + H)^+$, 539.1; found, 539.3.

5-Chloro-*N*-(cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-1*H*-indole-2-carboxamide (42). Compound **42** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.81 (s, 1 H), 12.64 (s, 1 H), 8.26−8.23 (m, 1 H), 7.81 (q, J = 1.8 Hz, 1 H), 7.79−7.77 (m, 2 H), 7.67 (s, 1 H), 7.56 (t, J = 9.5 Hz, 1 H), 7.47 (d, J = 1.9 Hz, 1 H), 7.41 (q, J=2.2 Hz, 1 H), 6.59 (t, J = 6.8 Hz, 1 H), 5.85 (s, 2 H), 2.92−2.87 (m, 1 H), 0.97 (d, J = 5.9 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 165.2, 163.1, 161.6, 152.3, 145.0, 144.8, 136.9, 128.0, 127.6, 127.5, 127.0, 126.6, 126.5, 126.4, 125.8, 125.4, 125.3, 120.9, 118.1, 117.9, 115.7, 113.7, 108.2, 42.8, 35.2, 31.5, 31.3, 22.6, 6.3. LRMS calcd for $C_{24}H_{18}N_4O_6ClFS$, 544.1; found, 544.8.

***N*-(Cyclopropylsulfonyl)-3-(1,2-dihydro-2-oxo-3-pyridinyl)-1-[(2-fluoro-5-nitrophenyl)methyl]-5-(trifluoromethyl-1*H*-indole-2-carboxamide (43).** Compound **43** was prepared according to the reaction sequence outlined in Scheme 2 and the general procedures

described above for the preparation of generic compound **9**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.80 (s, 1 H), 12.60 (s, 1 H), 8.27−8.23 (m, 1 H), 7.97 (d, J = 8.8 Hz, 1 H), 7.84−7.81 (m, 2 H), 7.80 (s, 1 H), 7.69 (q, J = 1.4 Hz, 2 H), 7.56 (t, J = 9.2 Hz, 1 H), 6.59 (t, J = 6.6 Hz, 1 H), 5.9 (s, 2 H), 2.92−2.87 (m, 1 H), 0.97 (d, J = 5.2 Hz, 4 H). ^{13}C NMR (125 MHz, DMSO-d_6), 165.3, 163.2, 163.0, 161.6, 152.3, 145.0, 144.7, 139.7, 136.9, 128.9, 127.4, 127.2, 126.9, 126.6, 126.3, 125.8, 125.5, 124.7, 122.9, 122.3, 119.7, 118.2, 118.0, 117.0, 113.1, 108.0, 42.9, 31.5, 31.3, 30.1, 6.3. LRMS calcd for $C_{25}H_{18}N_4O_6F_4S$, 578.1; found, 578.8.

3-(1,2-Dihydro-2-oxo-3-pyridinyl)-5-ethyl-1-[(2-fluoro-5-nitrophenyl)methyl]-1*H*-indole-2-carboxylic Acid (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 **14**. ^{1}H NMR (500 MHz, DMSO-d_6), 12.99 (s, 1 H), 11.80 (s, 1 H), 8.23−8.20 (m, 1 H), 7.65 (q, J = 2.9 Hz, 1 H), 7.58−7.54 (m, 3 H), 7.42 (q, J = 1.9 Hz, 1 H), 7.26 (s, 1 H), 7.23 (d, J = 8.4 Hz, 1 H), 6.36 (t, J = 6.9 Hz, 1 H), 5.91 (s, 2 H), 2.67 (q, J = 7.6 Hz, 2 H), 1.18 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), 165.0, 164.0, 163.0, 162.3, 144.9, 141.1, 137.5, 137.1, 135.1, 128.8, 128.6, 127.9, 127.1, 126.8, 126.2, 126.1, 124.7, 124.6, 119.6, 118.4, 118.0, 117.8, 111.5, 106.2, 42.4, 29.1, 17.1. LRMS calcd for $C_{23}H_{19}N_3O_5F$ $(M + H)^+$, 436.1; found, 436.0.

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ABBREVIATIONS USED

HCV, hepatitis C virus; PK, pharmacokinetics; SAR, structure−activity relationship; NS, nonstructural; 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, 1,8-diazabicyclocundec-7-ene; DIAD, diisopropyl azodicarboxylate; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; CL, clearance rate; CYP, cytochrome P450; hERG, the human ether-a- 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

REFERENCES

(1) (a) Cohen, J. The scientific challenge of hepatitis C. *Science* **1999**, *285*, 26−30. (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. (c) Cuthbert, J. A. Hepatitis C: Progress and problems. *Clin. Microbiol. Rev.* **1994**, *7*, 505−532.

(2) (a) Shepard, C. W.; Finelli, L.; Alter, M. J. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* **2005**, *5*, 558−567. (b) Monto, A.; Wright, T. L. The epidemiology and prevention of hepatocellular carcinoma. *Semin. Oncol.* **2001**, *28*, 441−449.

(3) (a) Sheridan, C. New Merck and Vertex drugs raise standard of care in hepatitis C. *Nat. Biotechnol.* **2011**, *29*, 553−554. (b) Fried, M. W.; Shiffman, M. L.; Reddy, D. K. R.; Smith, C.; Marinos, G.; Gonçales, F. L.; Häussinger, D.; Diago, M.; Carosi, G.; Dhumeaux, D.; Craxi, A.; Lin, A.; Hoffman, J.; Yu, J. PEGinterferon-α-2a plus ribavirin for chronic hepatitis C virus infection. *New Engl. J. Med.* **2002**, *347*, 975−982. (c) Di Bisceglie, A. M.; McHutchison, J.; Rice, C. M. New therapeutic strategies for hepatitis C. *Hepatology* **2002**, *35*, 224−231.

(4) Choo, Q. L.; Kuo, G.; Weiner, A. J.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **1989**, *244*, 359−362.

(5) (a) Chen, K. X.; Njoroge, F. G. A review of HCV protease inhibitors. *Curr. Opin. Invest. Drugs* **2009**, *10*, 821−837. (b) Watkins, W. J.; Ray, A. S.; Chong, L. S. HCV NS5B polymerase inhibitors. *Curr. Opin. Drug Discovery Dev.* **2010**, *13*, 441−465. (c) Legrand-Abravanel, F.; Nicot, F.; Izopet, J. New NS5B polymerase inhibitors for hepatitis C. *Expert Opin. Investig. Drugs* **2010**, *19*, 963−975. (d) 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.

(6) (a) McHutchison, J. G.; Everson, G. T.; Gordon, S. C.; Jacobson, I. M.; Sulkowski, M.; Kauffman, R.; McNair, L.; Alam, J.; Muir, A. J.; Afdhal, N.; Arora, S.; Balan, V.; Vargas, H.; Bernstein, D.; Black, M.; Brown, R.; Bzowej, N.; Davis, G.; Di Bisceglie, A.; Dienstag, J.; Everson, G.; Faruqui, S.; Franco, J.; Fried, M.; Ghalib, R.; Gordon, S. C.; Gross, J.; Jacobson, I. M.; Jensen, D.; Kugelmas, M.; Kwo, P.; Lawitz, E.; Lee, W.; Martin, P.; Nelson, D.; Northup, P.; Patel, K.; Poordad, F.; Reddy, R. K.; Rodriguez-Torres, M.; Rustgi, V.; Schiff, E.; Sherman, K.; Shiffman, M.; Sulkowski, M.; Szabol, G.; Younossi, Z. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N. Eng. J. Med.* **2009**, *360*, 1827−1838. (b) Kwo, P. Y.; Lawitz, E. J.; McCone, J.; Schiff, E. R.; Vierling, J. M.; Pound, D.; Davis, M. N.; Galati, J. S.; Gordon, S. C.; Ravendhran, N.; Rossaro, L.; Anderson, F. H.; Jacobson, I. M.; Rubin, R.; Koury, K.; Pedicone, L. D.; Brass, C. A.; Chaudhri, E.; Albrecht, J. K. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* **2010**, *376*, 705−716.

(7) Perni, R. B.; Almquist, S. J.; Byrn, R. A.; Chandorkar, G.; Chaturvedi, P. R.; Courtney, L. F.; Decker, C. J.; Dinehart, K.; Gates, C. A.; Harbeson, S. L.; Heiser, A.; Kalkeri, G.; Kolaczkowski, E.; Lin, K.; Luong, Y.-P.; Rao, B. G.; Taylor, W. P.; Thomson, J. A.; Tung, R. D.; Wei, Y.; Kwong, A. D.; Lin, C. Preclinical profile of VX-950, a potent, selective, and orally, bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob. Agents Chemother.* **2006**, *50*, 899−909.

(8) (a) Njoroge, F. G.; Chen, K. X.; Shih, N.-Y.; Piwinski, J. J. Challenges in modern drug discovery: A case study of boceprevir, an HCV protease inhibitor for the treatment of hepatitis C virus infection. *Acc. Chem. Res.* **2008**, *41*, 50−59. (b) Venkatraman, S.; Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y.-T.; Lovey, R.; Hendrata, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, A.; Agarwal, S.; Ingravallo, P.; Pichardo, J.; Kong, R.; Baroudy, B.; Malcolm, B.; Guo, Z.; Prongay, A.; Madison, B. L.; Cui, X.; Cheng, K.-C.; Hsieh, T. Y.; Brisson, J.-M.; Prelusky, D.; Kormacher, W.; White, R.; Bogonowich-Knipp, S.; Pavlovsky, A.; Prudence, B.; Saksena, A. K.; Ganguly, A.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. Discovery of (1*R*,5*S*)-*N*-[3-amino-1-(cyclobutylmethyl)-2−3-dioxopropyl]-3-[2(*S*)-[[[(1,1-dimethylethyl)-amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (SCH 503034), a selective, potent, orally bioavailable, hepatitis C virus NS3 protease inhibitor: A potential therapeutic agent for the treatment of hepatitis C infection. *J. Med. Chem.* **2006**, *49*, 6074−6086.

(9) (a) Behrens, S. E.; Tomei, L.; De Francesco, R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J.* **1996**, *15*, 12−22. (b) Lohmann, V.; Korner, F.; Herian, U.; Bartenschlager, R. Biochemical properties of hepatitis c virus ns5b RNAdependent RNA polymerase and identification of amino acid sequence motifs essential for enzymatic activity. *J. Virol.* **1997**, *71*, 8416−8428.

(10) Quinkert, D.; Bartenschlager, R.; Lohmann, V. Quantitative analysis of the hepatitis C virus replication complex. *J. Virol.* **2005**, *79*, 13594−13605.

(11) (a) Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. C. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. *Nat. Struct. Biol.* **1999**, *6*, 937−943. (b) Ago, H.; Adachi, T.; Yoshida, A.; Yamamoto, M.; Habuka, N.; Yatsunami, K.; Miyano, M. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. *Structure* **1999**, *7*, 1417−1426.

(12) Reviews of non-nucleoside HCV NS5B polymerase inhibitors: (a) Liu, Y.; He, Y.; Molla, A. Hepatitis C virus polymerase as a target for antiviral drug intervention: Non-nucleoside inhibitors. In *Antiviral Research: Strategies in Antiviral Drug Discovery*; LaFemina, R. L., Ed.; ASM Press: Washington, DC, 2009; pp 137−151. (b) Beaulieu, P. L. Non-nucleoside inhibitors of the hcv ns5b polymerase: Progress in the discovery and development of novel agents for the treatment of hcv infections. *Curr. Opin. Investig. Drugs* **2007**, *8*, 614−634.

(13) Reviews of HCV NS5B polymerase inhibitors: (a) Watkins, W. J.; Ray, A. S.; Chong, L. S. HCV NS5B polymerase inhibitors. *Curr. Opin. Drug Discovery Dev.* **2010**, *13*, 441−465. (b) Legrand-Abravanel, F.; Nicot, F.; Izopet, J. New NS5B polymerase inhibitors for hepatitis C. *Expert Opin. Investig. Drugs* **2010**, *19*, 963−975. (c) Beaulieu, P. L. Recent advances in the development of NS5B polymerase inhibitors for the treatment of hepatitis C virus infection. *Expert Opin. Ther. Pat.* **2009**, *19*, 145−164.

(14) Ma, H.; Leveque, V.; De Witte, A.; Li, W.; Hendricks, T.; Clausen, S. M.; Cammack, N.; Klumpp, K. Inhibition of native hepatitis C virus replicase by nucleotide and non-nucleoside inhibitors. *Virology* **2005**, *332*, 8−15.

(15) Manns, M. P.; Foster, G. R.; Rockstroh, J. K.; Zeuzem, S.; Zoulim, F.; Houghton, M. The way forward in hcv treatment--finding the right path. *Nat. Rev. Drug Discovery* **2007**, *6*, 991−1000.

(16) (a) Pierra, C.; Amador, A.; Benzaria, S.; Cretton-Scott, E.; D'Amours, M.; Mao, J.; Mathieu, S.; Moussa, A.; Bridges, E. G.; Standring, D. M.; Sammadossi, J.-P.; Storer, R.; Gosselin, G. Synthesis and pharmacokinetics of valopicitabine (NM283), an efficient prodrug of the potent anti-HCV agent 20-C-methylcytidine. *J. Med. Chem.* **2006**, *49*, 6614−6620. R1626: (b) Smith, D. B.; Kalayanov, G.; Sund, G.; Winqvist, A.; Maltseva, T.; Leveque, V. J.-P.; Rajyagurr, S.; Le Pogam, S.; Najer, I.; Benkestock, K.; Zhou, X.-X.; Kaiser, A. C.; Maag, H.; Cammack, N.; Martin, J. A.; Swallow, S.; Johannsson, N. G.; Klumpp, K.; Smith, M. The design, synthesis, and antiviral activity of monofluoro and difluoro analogues of 40-azidocytidine against hepatitis C virus replication: The discovery of 40-azido-20-deoxy-20-fluorocytidine and 40-azido-20-dideoxy-20,20-difluorocytidine. *J. Med. Chem.* **2009**, *52*, 2971−2978. PSI-6130: (c) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. Design, synthesis, and antiviral activity of 20-deoxy-20-fluoro-20-c-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005**, *48*, 5504−5508. (d) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H. R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. Discovery of a β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* **2010**, *53*, 7202−18. (e) Reddy, P. G.; Bao, D.; Chang, W.; Chun, B.-K.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Ross, B. S.; Zhang, H.-R.; Bansal, S.; Espiritu, C. L.; Keilman, M.; Lam, A. M.; Niu, C.; Steuer, H. M.; Furman, P. A.; Otto, M. J.; Sofia, M. J. 20-Deoxy-20-a-fluoro-20-b-C-methyl 30,50-cyclic phosphate nucleotide prodrug analogues as inhibitors of HCV NS5B polymerase: Discovery of PSI-352938. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7376−7380. (f) Cretton-Scott, E.; Perigaud, C.; Peyrottes, S.; Licklider, L.; Camire, M.; Larsson, M.; La Colla, M.; Hildebrand, E.; Lallos, L.; Bilello, J.; McCarville, J.; Seifer, M.; Liuzzi, M.; Pierra, C.; Badaroux, E.; Gosselin, G.; Surleraux, D.; Standring, D. N. In vitro antiviral activity and pharmacology of IDX184, a novel and potent inhibitor of HCV replication. *J. Hepatol.* **2008**, *48*, S220. (g) McGuigan, C.; Madela, K.; Aljarah, M.; Gilles, A.; Brancale, A.; Zonta, N.; Chamberlain, S.; Vernachio, J.; Hutchins, J.; Hall, A.; Ames, B.; Gorovits, E.; Ganguly, B.; Kolykhalov, A.; Wang, J.; Muhammadb, J.; Patti, J. M.; Henson, G. Design, synthesis and evaluation of a novel double pro-drug: INX-08189. A new clinical candidate for hepatitis C virus. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4850−4854.

(17) Kneteman, N. M.; Howe, A. Y.; Gao, T.; Lewis, J.; Pevear, D.; Lund, G.; Douglas, D.; Mercer, D. F.; Tyrrell, D. L.; Immermann, F.; Chaudhary, I.; Speth, J.; Villano, S. A.; O'Connell, J.; Collett, M. HCV796: A selective nonstructural protein 5B polymerase inhibitor with potent anti-hepatitis C virus activity in vitro, in mice with chimeric human livers, and in humans infected with hepatitis C virus. *Hepatology* **2009**, *49*, 745−52.

(18) Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Jewell, T.; Patel, L.; Ludlum, S.; Drowns, M.; Rahavendran, S. V.; Skor, H.; Hunter, R.; Shi, S. T.; Herlihy, K. J.; Parge, H.; Hickey, M.; Yu, X.; Chau, F.; Nonomiya, J.; Lewis, C. Discovery of (*R*)-6-cyclopentyl-6-(2-(2,6-diethylpyridin-4-yl)ethyl)-3-((5,7-dimethyl-[1,2,4]triazolo[1,5-*a*]-pyrimidin-2-yl)methyl)-4-hydroxy-5,6-dihydropyran-2-one (PF-00868554) as a potent and orally available hepatitis C virus polymerase inhibitor. *J. Med. Chem.* **2009**, *52*, 1255−1258.

(19) Rodriguez-Torres, M.; Lawitz, E.; Conway, B.; Kaita, K.; Sheikh, A. M.; Ghalib, R.; Adrover, R.; Cooper, C.; Silva, M.; Rosario, M.; Bourgault, B.; Proulx, L.; McHutchison, J. G. Safety and antiviral activity of the non-nucleoside polymerase inhibitor VX-222 in treatment naïve genotype 1 HCV infected patients. *J. Hepatol.* **2010**, *52*, S14.

(20) Lawitz, E.; Rodrigues-Torres, M.; DeMicco, T.; Nguyen, T.; Godofsky, E.; Appleman, J.; Rahimy, M.; Crowley, C.; Freddo, J. Antiviral activity of ANA598, a potent non-nucleoside polymerase inhibitor, in chronic hepatitis C patients. *J. Hepatol.* **2009**, *50*, S384.

(21) Larrey, D.; Benhamou, Y.; Lohse, A. W.; Trepo, C.; Moelleken, C.; Bronowicki, J.-P.; Heim, M. H.; Arasteh, K.; Zarski, J.-P.; Bourlière, M.; Wiest, R.; Calleja, J. L.; Enríquez, J.; Erhardt, A.; Wedemeyer, H.; Gerlach, T.; Berg, T.; Stern, J. O.; Wu, K.; Abdallah, N.; Nehmiz, G.; Boecher, W. O.; Berger, F. M.; Steffgen, J. BI 207127 is a potent HCV RNA polymerase inhibitor during 5 days monotherapy in patients with chronic hepatitis C. *Hepatology* **2009**, *50*, 1044A.

(22) http://clinicaltrials.gov/ct2/show/NCT01193361?term=bms-791325&rank=4.

(23) Anilkumar, G. N.; Lesburg, C. A.; Selyutin, O.; Rosenblum, S. B.; Zeng, Q.; Jiang, Y.; Chan, T.-Y.; Pu, H.; Vaccaro, H.; Wang, L.; Bennett, F.; Chen, K. X.; Duca, J.; Gavalas, S.; Huang, Y.; Pinto, P.; Sannigrahi, M.; Velazquez, F.; Venkatraman, S.; Vibulbhan, B.; Agrawal, S.; Butkiewicz, N.; Feld, B.; Ferrari, E.; He, Z.; Jiang, C.-K.; Palermo, R. E.; Mcmonagle, P.; Huang, H.-C.; Shih, N.-Y.; Njoroge, G.; Kozlowski, J. A. Novel HCV NS5B polymerase inhibitors: Discovery of indole 2-carboxylic acids with C3-heterocycles. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5336−5341.

(24) Ferrari, E.; He, Z.; Palermo, R. E.; Huang, H.-C. Hepatitis C virus NS5B polymerase exhibits distinct nucleotide requirements for initiation and elongation. *J. Biol. Chem.* **2008**, *283*, 33893−33901.

(25) 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.

(26) Chen, K. X.; Vibulbhan, B.; Yang, W.; Sannigrahi, M.; Velazquez, F.; Chan, T. Y.; Venkatraman, S.; Anilkumar, G. N.; Zeng, Q.; Bennet, F.; Jiang, Y.; Lesburg, C. A.; Duca, J.; Pinto, P.; Gavalas, S.; Huang, Y.; Wu, W.; Selyutin, O.; Agrawal, S.; Feld, B.; Huang, H. C.; Li, C.; Cheng, K. C.; Shih, N. Y.; Kozlowski, J. A.; Rosenblum, S. B.; Njoroge, F. G. Structure−activity relationship development and discovery of potent indole-based inhibitors of the hepatitis C virus NS5B polymerase. *J. Med. Chem.* **2012**, *55*, 754−765.

(27) Ishiyama, T.; Murata, M.; Miyaura, N. Palladium(0)-catalyzed cross-coupling reaction of alkoxydiboron with haloarenes: A direct procedure for arylboronic esters. *J. Org. Chem.* **1995**, *60*, 7508−7510.

(28) Jansen, M.; Dannhardt, G. Variations of acidic functions at position 2 and substituents at positions 4, 5 and 6 of the indole moiety and their effect on NMDA-glycine site affinity. *Eur. J. Med. Chem.* **2003**, *38*, 855−865.

(29) (a) Hughes, D. L. The Mitsunobu Reaction. *Org. Reactions* **1992**, *42*, 335. (b) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1−28.
(30) Powers, J. P.; Piper, D. E.; Li, Y.; Mayorga, V.; Anzola, J.; Chen, J. M.; Jaen, J. C.; Lee, G.; Liu, J.; Peterson, M. G.; Tonn, G. R.; Ye, Q.; Walker, M. P. C; Wang, Z. SAR and mode of action of novel non-nucleoside inhibitors of hepatitis C NS5b RNA polymerase. *J. Med. Chem.* **2006**, *49*, 1034−1046.
(31) Figure 2 was generated using the program PyMOL (The PyMOL Molecular Graphics System, Version 1.2r1, Schrödinger, LLC.).
(32) X-Ray crystal structure data of HCV NS5B in complex with **47** have been deposited in the Protein Data Bank with access code 3TYQ.
(33) (a) Potashman, M. H.; Duggan, M. E. Covalent Modifiers: An Orthogonal Approach to Drug Design. *J. Med. Chem.* **2009**, *52*, 1231−1246. (b) Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. Irreversible inhibitors of serine, cysteine, and threonine proteases. *Chem. Rev.* **2002**, *102*, 4639−4750.
(34) Huth, J. R.; Song, D.; Mendoza, R. R.; Black-Schaefer, C. L.; Mack, J. C.; Dorwin, S. A.; Ladror, U. S.; Severin, J. M.; Walter, K. A.; Bartley, D. M.; Hajduk, P. J. Toxicological evaluation of thiol-reactive compounds identified using a La assay to detect reactive molecules by nuclear magnetic resonance. *Chem. Res. Toxicol.* **2007**, *20*, 1752−1759.
(35) Boelsterli, U. A.; Ho, H. K.; Zhou, S.; Leow, K. Y. Bioactivation and hepatotoxicity of nitroaromatic drugs. *Curr. Drug Metab.* **2006**, *7*, 715−727.