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Pyridofuran substituted pyrimidine derivatives as HCV replication (replicase) inhibitors

Frank Bennett [a,*], Hollis S. Kezar III [b,*], Vinay Girijavallabhan [a], Yuhua Huang [a], Regina Huelgas [a], Randall Rossman [a], Neng-Yang Shih [a], John J. Piwinski [a], Malcolm MacCoss [a], Cecil D. Kwong [b], Jeremy L. Clark [b], Anita T. Fowler [b], Feng Geng [b], Abhijit Roychowdhury [b], Robert C. Reynolds [b], Joseph A. Maddry [b], Subramaniam Ananthan [b], John A. Secrist III [b], Cheng Li [a], Robert Chase [a], Stephanie Curry [a], Hsueh-Cheng Huang [a], Xiao Tong [a], F. George Njoroge [a], Ashok Arasappan [a]

[a] Merck Research Laboratories, 2015 Galloping Hill Rd, Kenilworth, NJ 07033, USA
[b] Southern Research Institute, Birmingham, AL 35205, USA

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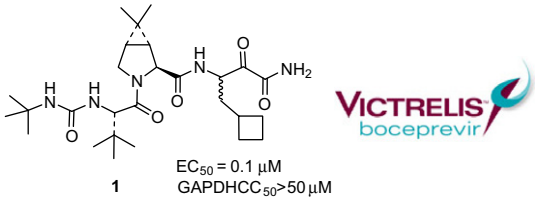
ABSTRACT

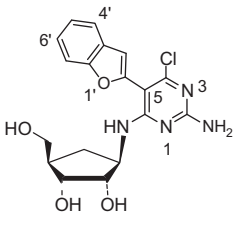
Introduction of nitrogen atom into the benzene ring of a previously identified HCV replication (replicase) benzofuran inhibitor **2**, resulted in the discovery of the more potent pyridofuran analogue **5**. Subsequent introduction of small alkyl and alkoxy ligands into the pyridine ring resulted in further improvements in replicon potency. Replacement of the 4-chloro moiety on the pyrimidine core with a methyl group, and concomitant monoalkylation of the C-2 amino moiety resulted in the identification of several inhibitors with desirable characteristics. Inhibitor **41**, from the monosubstituted pyridofuran and inhibitor **50** from the disubstituted series displayed excellent potency, selectivity (GAPDH/MTS CC_{50}) and PK parameters in all species studied, while the selectivity in the thymidine incorporation assay (DNA·CC_{50}) was low.

An estimated 180 million people worldwide are infected with hepatitis C virus (HCV), a positive stranded RNA virus belonging to the *Flaviviridae* family.[1] HCV is the leading cause of liver fibrosis, cirrhosis and hepatocellular carcinoma leading to liver failure in humans. Until recently, the standard-of-care (SOC), pegylated α-interferon and ribavirin combination therapy, provided less than 50% response rate among patients infected with the most prevalent strain in North America, Europe and Japan.[2] However, response rates of approximately 70% are achieved in patients infected with genotypes 2 and 3.[3] In addition to the relative ineffectiveness against genotype 1, this SOC is associated with serious side effects[4] underscoring the need for improved therapies, stimulating intensive research worldwide for orally available small molecule drug candidates that directly target various viral proteins.[5] Recently, the introduction of the NS3 protease inhibitor Victrelis®,[6] (**1**, Fig. 1) from our own laboratories and Incivek®,[7] from Vertex has provided renewed hope to HCV infected patients. The search for even more effective treatment continues. In addition to the NS3 protease inhibitors, NS5B nucleoside and non-nucleoside



1 EC_{50} = 0.1 μM, GAPDH CC_{50} >50 μM

Figure 1. Victrelis (boceprevir) **1**.



2 EC_{50} = 0.3 uM

Figure 2. Benzofuran inhibitor **2**.

* Corresponding authors. Tel.: +1 732 594 2602; fax: +1 732 594 2210.
E-mail address: frank.bennett@merck.com (F. Bennett).

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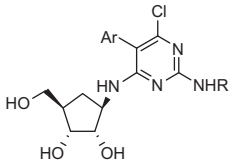
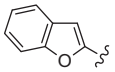
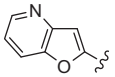
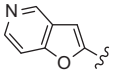
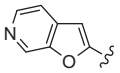
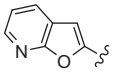
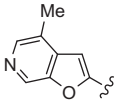
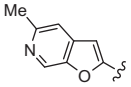
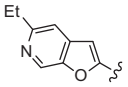
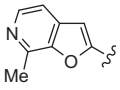
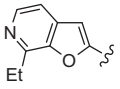
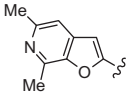
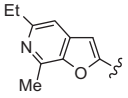
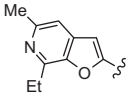
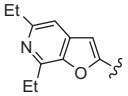
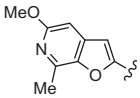
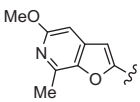
Table 1
Evaluation of 5-pyridofuran substitution



Compound #	Ar	R	EC_{50} (μM)	MTS/GAPDH CC_{50} (μM)	AUC[a] (μM.h)
2		H	0.3	>25/—	—
3		H	>25	21/—	—
4		H	1.7	25/—	—
5		H	0.07	25/25	0
6		H	0.4	19/—	—
7		H	1.4	>25/25	—
8		H	0.03	25/9	—
9		H	0.01	22/12	0.07
10		H	0.12	>25/>25	—
11		H	0.02	24/19	0.05
12		H	0.01	22/15	0.01
13		H	0.01	16/13	0.01
14		H	0.03	20/9	0
15		H	0.02	16/25	0.17
16		H	0.01	25/—	0.1
17		Me	0.02	25/>25	6.5

[a] $AUC_{0-6\ h}$ or $AUC_{0-24\ h}$, po (10 mpk), vehicle −0.4% mc.

inhibitors, and NS5A inhibitors are in various stages of clinical studies.[5]

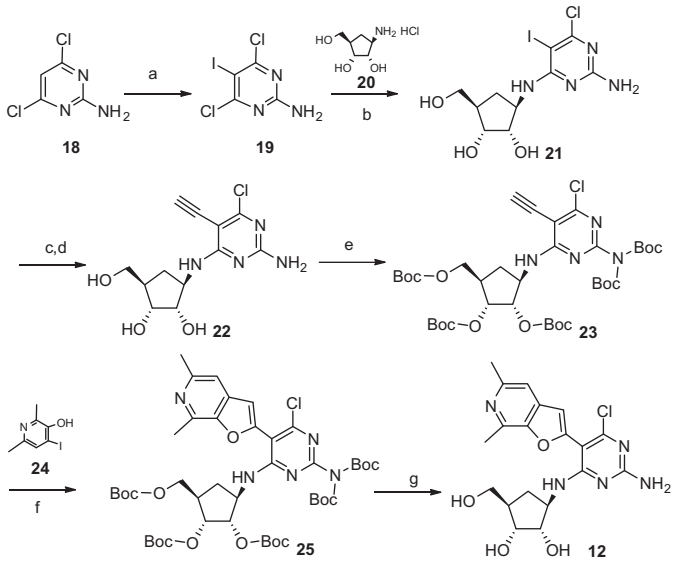
Our previous efforts[8] towards identification of novel small molecule inhibitors of hepatitis C virus replication resulted in carbanucleoside-like compounds (e.g., **2**; Fig. 2) with modest potency in the HCV genotype 1b subgenomic replicon assay.[9]

In this manuscript we will present the syntheses and activities of a series of analogues derived from logical modifications on the benzofuran **2**, directed towards obtaining more potent anti-HCV agents with good selectivity relative to cell toxicity, demonstrated by the MTS and GAPDH assay, and acceptable PK parameters.

Early in this study we decided to introduce nitrogen into the benzene ring of the benzofuran **2** and synthesized the pyridofuran analogues (**3–6**; Table 1). As can be seen from Table 1, while substitution at the 4′-position **3** resulted in complete loss of activity, the 5′-position isomer **4** resulted in a 5- to 6-fold decrease in potency relative to **2**. Potency was retained with the 7′-aza derivative **6**. We were encouraged to find significant improvement in the replicon activity after introduction of nitrogen at the 6′-position **5**, and as a result the majority of subsequent modifications were conducted with this bicyclic ring system.

We next introduced small alkyl groups into the pyridine ring (**7–15**). Methylation at the 4′-position **7** resulted in loss of potency. We were pleased to find that we could increase the potency with substitution at the 5′-position **8**, although this compound did exhibit slightly lower GAPDH CC_{50}. The ethyl analogue **9** was more potent but displayed similar CC_{50} profile. Retention of potency was observed through methylation at the 7′-position **10**. Interestingly, simply substituting the 7′-methyl group in **10** with an ethyl substituent **11**, a 5-fold increase in potency was realized. Extending to 5′,7′-disubstituted analogues (**12–17**) further improvements in potency could be achieved. As can be seen in Table 1, the majority of our most potent inhibitors were accompanied with differing levels of cell toxicity demonstrated in the MTS and GAPDH assays; a notable exception being the 7-methyl analogue **10**. This undesired effect could be somewhat alleviated with the incorporation of 5′-alkoxy ligand (**16**–**17**) rather than alkyl groups. Initial rat pharmacokinetic studies[10] proved to be disappointing, particularly for inhibitors **11**, **13** and **14**. Measurable, albeit low exposure, was observed in the case of 5′-ethyl, 5′,7′-diethyl and 5′-methoxy-7′-methyl derivatives (**9**, **15** and **16**, respectively). Tremendous improvements in rat PK parameters were observed with the introduction of a methyl group on the 2-amino functionality on the pyrimidine ring (**16** vs **17**). This modification resulted in a potent inhibitor, **17** with good selectivity profiles (MTS EC_{50}/CC_{50} >1000) and excellent plasma exposure in rats. Unfortunately, the liver concentration[11] of **17**, another important parameter for HCV inhibitors, was determined to be low, only 274 ng/g at 6 h post-dose. The low liver concentration clearly demonstrated that further derivatization was necessary to realize a molecule with an overall acceptable profile.

The syntheses of the vast majority of the compounds (**3–15**), exemplified by inhibitor **12**, is shown in Scheme 1. Iodination of commercially available dichloropyrimidine **18** with iodine monochloride gave the 5-iodo product **19**. Displacement of one of the identical chloro-substituents in **19** with commercially available carbasugar **20** was achieved in refluxing ethanol in the presence of triethylamine, producing adduct **21**. Subsequent Sonogashira type coupling with trimethylsilylacetylene and treatment of the resulting intermediate with fluoride provided the terminal acetylene **22**. We found it advantageous to ease the handling of these polar intermediates by protecting the triol **22** with excess di-tert-butylpyrocarbonate to give the workable fully protected **23**. A second Sonogashira reaction, this time in the presence of an appropriately substituted 3-hydroxy-4-iodopyridine **24** installed the required pyridofuran bicyclic moiety on the 5-position of the

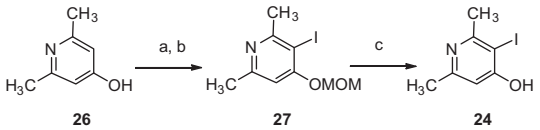


Scheme 1. Reagents and conditions: (a) ICl, AcOH (79%); (b) Et_3N, EtOH, reflux (80%); (c) Trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, dioxane, 50 °C (56%); (d) $Et_4NF{\cdot}H_2O$ (94%); (e) Boc_2O, DMAP; (f) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, dioxane, 50 °C; g) 4 N·HCl in dioxane.

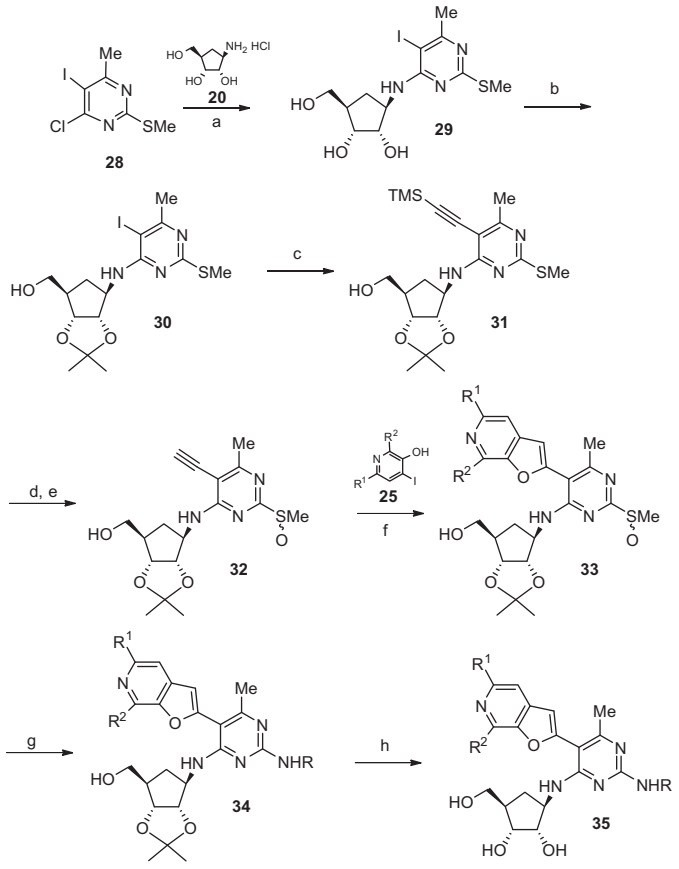
pyrimidine ring **25**. Finally, exposure to hydrogen chloride in dioxane gave the target compound **12**, as the hydrochloride salt.

The hydroxypyridine **24** was prepared in 3 steps, as shown in Scheme 2. Commercially available 2,6-dimethyl-4-hydroxypyridine **26** was converted to the intermediate methoxymethyl ether. *Ortho*-lithiation and treatment of the resulting anion with molecular iodine gave the desired iodide **27**. The MOM protecting group was removed with trifluoroacetic acid followed by liberation of the resulting salt with triethylamine to give the desired 3-iodo-4-hydroxypyridine **24**.

In line with our previous studies,[8] we replaced the 4-chloro moiety on the pyrimidine ring with a methyl group. Furthermore, as shown in Table 1, to realize acceptable exposure in rats, it was necessary to incorporate at least a small alkyl group on the amino functionality at the 2-position on the pyrimidine ring. To accommodate these requirements a new synthetic route had to be devised. To this end we envisioned that the methyl sulfide functionality **28** (Scheme 3) once coupled to **20**, (as previously shown for the preparation of **21** in Scheme 1) would provide **29**, and the sulfide could be oxidized to the corresponding sulfone or sulfoxide at a late stage to be conveniently displaced by a variety of amines. Indeed, when **28** was exposed to **20** in refluxing ethanol in the presence of excess triethylamine the desired adduct **29** was obtained. Protection of the 1,2-diol was achieved via the isopropylidene group to provide **30**. The next step in the sequence involved conversion of the iodide to an acetylene under Sonogashira type conditions with trimethylsilylacetylene to give **31**. The methylsulfide was oxidized to the intermediate sulfoxide prior to deprotection of the alkyne with fluoride, providing **32**. As previously shown in Scheme 1, the 5-pyridofuran moiety was constructed



Scheme 2. Reagents and conditions: (a) MOMCl, Et_3N, CH_2Cl_2; (b) *n*BuLi, followed by I_2, THF; (c) CF_3CO_2H, CH_2Cl_2 followed by Et_3N.



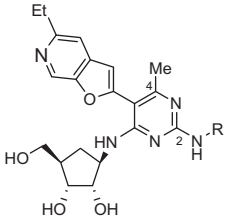
Scheme 3. Reagents and conditions: (a) Et_3N, EtOH, reflux; (b) 2,2-dimethoxypropane, MsOH (79% for 2 steps); (c) trimethylsilylacetylene, $Pd(PPh_3)_3Cl_2$, CuI, dioxane, 50–100 °C; (d) MCPBA (1 equiv), CH_2Cl_2; (e) $Et_4NF{\cdot}H_2O$, MeCN; (f) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, dioxane, 50–100 °C; (g) RNH_2, heat; (h) HCl, dioxane-H_2O.

using the appropriately substituted 3-hydroxy-4-iodopyridine, again under Sonogashira type conditions. The amino functionality was conveniently installed by treating the sulfoxide **33** with a variety of amines in refluxing acetonitrile. For less nucleophilic substrates (e.g., 1,1,1-trifluoroethylamine) it was found necessary to heat the sulfoxide with neat amine in a sealed tube. Regardless of the conditions used, the desired products were usually obtained in good yield. Deprotection of the isopropylidene group to the final products **35** was conducted with HCl in aqueous dioxane.

Using compounds **9**, from mono-substituted pyridofuran series, and **16**, from di-substituted pyridofuran series, as the basis (potency, selectivity window and PK parameters) for further studies, a selection of the inhibitors prepared and evaluated are shown in Tables 2 and 3 respectively.

Compounds **36–43** (Table 2) are 4-methyl analogues of **9**, differentiated by the substituent on the amino functionality at the pyrimidine 2-position. Immediately, we saw that the methyl group was an effective replacement for the 4-chloro moiety. The N-methyl analogue **36** retained most of the potency and showed an excellent profile in the cytotoxicity assays. There was a significant improvement in the rat plasma exposure when compared with **9**. The n-pentyl derivative **37** had similar potency but less desirable effects in the toxicity assays, and less exposure in rats. The iso-butyl derivative **38**, while displaying good PK properties (AUC), had a lower potency and selectivity window. Introduction of the neopentyl moiety **39** restored the potency; however the rest of the profile was similar to that of **38**. The N-ethoxyethyl derivative **40** was highly potent with moderate PK parameters, and displayed lower GAPDH CC_{50} levels. The 1,1,1-trifluoroethyl inhibitor **41** retained good potency, exhibited excellent rat PK properties (both, plasma exposure and liver concentration) and an acceptable selectivity window. A similar profile, albeit with a lower AUC, was seen for the 1,1,1-trifluorobutyl inhibitor, **42**. The most potent inhibitor in this series was the 3,5-dimethoxy analogue **43**, with an excellent selectivity window. Unfortunately, this promising derivative displayed extremely poor rat in vivo parameters.

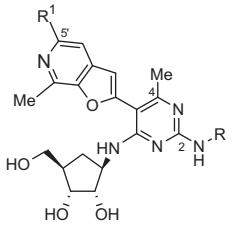
Table 2
Evaluation of C-2 amino substituent on pyrimidine C-4 Me derivatives of inhibitor **9**

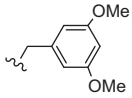
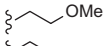
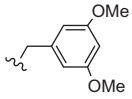


Compound	R	EC_{50} (μM)	MTS/GAPDH CC_{50} (μM)	AUC[a] (μM.h)	Liver[b] $C_{6\ h}$ (ng.g)
36	—Me	0.03	>25/>25	0.9	936
37	Me	0.03	3/3	0.48	31
38		0.08	12/7	4.5	251
39	$C(CH_3)_3$	0.03	11/10	4.0	315
40	OEt	0.004	16/4	1.2	492
41	CF_3	0.02	15/13	5.3	1732
42	CF_3	0.02	>25/17	0.9	1000
43	OMe, OMe	0.003	20/>25	0.1	20

[a] $AUC_{0-6\ h}$, po (10 mpk), vehicle −0.4% mc.
[b] Liver conc at 6 h.

Table 3
Evaluation of C-2 amino substituent on inhibitor-type **16**



Compound	R	R^1	EC_{50} (μM)	MTS/GAPDH CC_{50} (μM)	AUC[a] (μM.h)	Liver[b] $C_{6\ h}$ (ng.g)
44		OMe	0.06	25/>25	2.7	310
45		OMe	0.04	21/17	4.4	1185
46		OMe	0.01	25/>25	0.5	178
47		OEt	0.05	>25	1.4	238
48		OEt	0.05	23/12	6.8	2334
49		OEt	0.15	18/24	18.5	8981
50		OEt	0.02	25/25	45.5	10226
51		OEt	0.009	>25/>25	1.1	285
52		OEt	0.02	>25/>25	8.9	3015
53		OEt	0.002	>25/>25	0.35	238

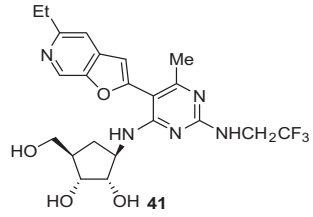
[a] $AUC_{0-6\ h}$, po (10 mpk), vehicle −0.4% mc.
[b] Liver conc at 6 h.

Table 3 shows the C-2 SAR studies carried out on the 4-methyl pyrimidine derivatives of type **16**. As seen before in Table 2, the 4-methyl group was well tolerated on 5′-alkoxy pyridofuran targets of type **16**. The C-2 N-methyl analogues **44** and **47** exhibited good replicon potency, selectivity indices and moderate plasma exposure (AUC). However, the liver concentrations at the 6 h time point were poor for inhibitors **44** and **47**. The neopentyl inhibitors **45** and **48** showed similar potency, improved plasma exposure and liver concentrations, and moderate selectivity windows. Again the most potent derivatives proved to be the 3,5-dimethoxybenzyl analogues **46** and particularly **53**, in the 5′-methoxy and 5′-ethoxy series respectively. Similar to prior results with the 5′-ethyl benzofuran **43**, the PK parameters were poor for both 5′-alkoxy derivatives, **46** and **53**.

The N-phenyl analogue **49** proved to be comparatively less active. The cyclopropylmethyl derivative **50**, methoxyethyl target **51** and 1,1,1-trifluoroethyl compound **52** all displayed excellent potencies and selectivity windows. Of particular interest was the cyclopropylmethyl derivative **50**, distinguished by its exceptional rat plasma exposure and liver concentration.

Based on the potency, selectivity, and rat exposure, both plasma and liver concentration, inhibitors **41** and **50** from the respective 5′-pyridofuran series were selected for further studies in higher species. The full profile of the two inhibitors, **41** and **50** are shown in Figure 3. Both molecules exhibited excellent plasma concentration and bioavailability in monkeys. While **41** displayed high exposure in dogs, the corresponding data for inhibitor **50** proved to be slightly lower. It should be noted that both compounds showed a similar, albeit lower, selectivity profile in the more stringent thymidine incorporation assay (DNA·CC_{50}).

In summary, introduction of a nitrogen atom at the 6′-position of the 5-benzofuran moiety of a carbanucleoside-like HCV replicase inhibitor **2** resulted in measurable improvements in replicon potency. Modifications were carried out at the 5-pyridofuran moiety and the pyrimidine core. These studies identified inhibitors **41** and **50** with excellent potency, selectivity with respect to the GAPDH/MTS CC_{50} and desirable PK parameters in all species studied. However, inhibitors **41** and **50** displayed lower selectivity in the thymidine incorporation assay. Studies directed towards overcom-



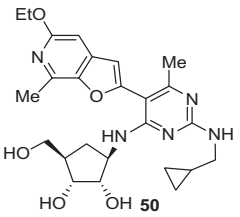
41

Replicon (1b) EC50 = 0.02 μM
MTS/GAPDH CC50 =15/13 μM
DNA CC50 = 4 μM

PK Data: (HCl salt)
Rat (po,10 mpk):
AUC = 5.3μM.h; F = 39%
t1/2 = 4.2 hr; Cl = 27 ml/min/kg
Liver C(6h) = 1732 ng/g

Monkey (po, 3 mpk):
AUC = 5.2 μM.h; F = 69%
t1/2 = 7 hr; Cl = 15 ml/min/kg

Dog (po, 3 mpk):
AUC = 6.4 μM.h; F = 83%
t1/2 = 2.1 hr; Cl = 13 ml/min/kg



50

Replicon (1b) EC50 = 0.02 μM
MTS/GAPDH CC50 =25/25 μM
DNA CC50 = 5 μM

PK Data: (HCl salt)
Rat (po, 10 mpk):
AUC = 45.5μM.h; F = 97%
t1/2 = 3.3 hr; Cl = 8 ml/min/kg
Liver C(6h) = 10226 ng/g

Monkey (po, 3 mpk):
AUC =10.2 μM.h; F =100 %
t1/2 = 2.8 hr; Cl = 16 ml/min/kg

Dog (po, 3 mpk):
AUC = 1.6 μM.h; F =15 %
t1/2 = 2.1 hr; Cl = 9.5 ml/min/kg

Figure 3. Profile of inhibitors **41** and **50**.

ing this obstacle while retaining the desirable characteristics of the aforementioned inhibitors will be the subject of future communications.

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(b) To measure cell-based anti-HCV activity, replicon cells (1b-Con1) are seeded at 5000 cells/well in 96-well plates one day prior to inhibitor treatment. Various concentrations of an inhibitor in DMSO are added to the replicon cells, with the final concentration of DMSO at 0.5% and fetal bovine serum at 10% in the assay media. Cells are harvested three days post dosing. The replicon RNA level is determined using real-time RT-PCR (Taqman assay) with GAPDH RNA as endogenous control. EC_{50} values are calculated from experiments with 10 serial twofold dilutions of the inhibitor in triplicate. To measure cytotoxicity in replicon cells of an inhibitor, an MTS assay is performed according to the manufacturer's protocol for CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Cat # G3580) three days post dosing on cells treated identically as in replicon activity assays. CC_{50} is the concentration of inhibitor that yields 50% inhibition compared to vehicle-treated cells. Effect of an inhibitor on cellular DNA synthesis is determined by a scintillation proximity assay. Replicon cells are seeded in 96-well Cytostar-T Scintillating Microplates (PerkinElmer, Cat # RPNQ0163) one day prior to inhibitor treatment. Various concentrations of an inhibitor in triplicate are added with [methyl-^{14}C]-thymidine (PerkinElmer, Cat # NEC568050UC, final concentration 0.5 μCi/mL media) and incubated for three days. DNA CC_{50} is the inhibitor concentration that yields 50% inhibition of labeled thymidine incorporation as measured by Packard TopCount compared to vehicle-treated cells.
10. Inhibitors were dosed orally at 10 mpk ($n = 2$ for each compound). The formulation vehicle was 0.4% MC and plasma samples were taken at determined time points up to 6 h. Rat plasma exposure is reported as $AUC_{0-6\ h}$.
11. The animals were sacrificed at 6 h and the liver was harvested and processed to measure the compound concentration (liver C_{6h}).