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5,5′- and 6,6′-Dialkyl-5,6-dihydro-1*H*-pyridin-2-ones as potent inhibitors of HCV NS5B polymerase

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ABSTRACT

The discovery of 5,5′- and 6,6′-dialkyl-5,6-dihydro-1*H*-pyridin-2-ones as potent inhibitors of the HCV RNA-dependent RNA polymerase (NS5B) is described. Several of these agents also display potent antiviral activity in cell culture experiments (EC_{50} <0.10 μM). In vitro DMPK data for selected compounds as well as crystal structures of representative inhibitors complexed with the NS5B protein are also disclosed.

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 180 million individuals, 3% of the world's population, are chronically infected with HCV and 3–4 million people are newly infected each year.[1] Currently, there is no vaccine available to prevent hepatitis C, nor an HCV-specific antiviral agent approved for treatment of chronic hepatitis C. The current standard of care is comprised of a combination of pegylated interferon (peg-IFN) with the nucleoside analog ribavirin (RBV).[2] Low response rates, in particular for patients infected with genotype 1 HCV, along with significant side-effects of peg-IFN/RBV therapies necessitate the identification of more effective anti-HCV agents.[3]

The activity of the virally encoded HCV NS5B polymerase is essential for HCV replication making it an attractive target for the development of novel HCV treatments.[4] A number of nucleoside and non-nucleoside NS5B inhibitors have been described in the literature. Most small molecule, non-nucleoside inhibitors of NS5B bind to a number of pockets that are distinct from the active site.[5,6] Among these, the NS5B inhibitor development efforts at Anadys have focused on compounds which bind to the 'palm' site of the NS5B protein.

We recently reported that molecules containing a 1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazine moiety linked to a bicyclic 5,6-dihydro-1*H*-pyridin-2-one (e.g., structure **1**, Fig. 1), are potent inhibitors of the NS5B polymerase with significantly improved oral bioavailability relative to our previously reported NS5B inhibitors.[7] Additionally, monocyclic pyridinones (e.g., structure **2**) have been reported as potent NS5B polymerase inhibitors.[8] Encouraged by the favorable biological profile of **1**, we wished to explore related monocyclic 5,6-dihydro-1*H*-pyridin-2-ones (e.g., structure **3**) in the hope of identifying additional potent antiviral agents with favorable pharmacokinetic (PK) properties. The results of these efforts are described below.

Synthetic routes to produce 5-mono-alkylated and 5,5′-di-alkylated pyridinones are shown in Scheme 1. In cases where disubstituted dialkyl malonates **4** were not commercially available, we began our synthesis from a variety of mono substituted malonates. Anion formation of mono substituted malonates using sodium hydride followed by treatment with alkyl halides led to the di-substituted intermediates **4**. De-symmetrization of the di-substituted malonates was achieved by treatment with diisobutylaluminium

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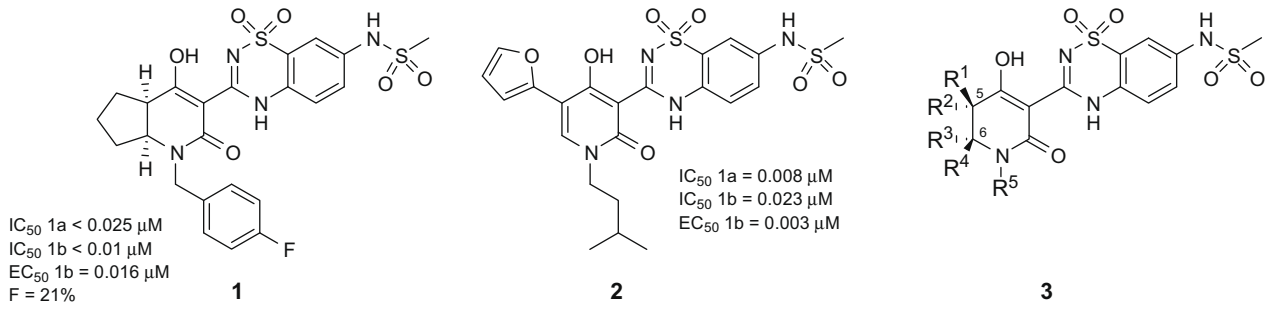
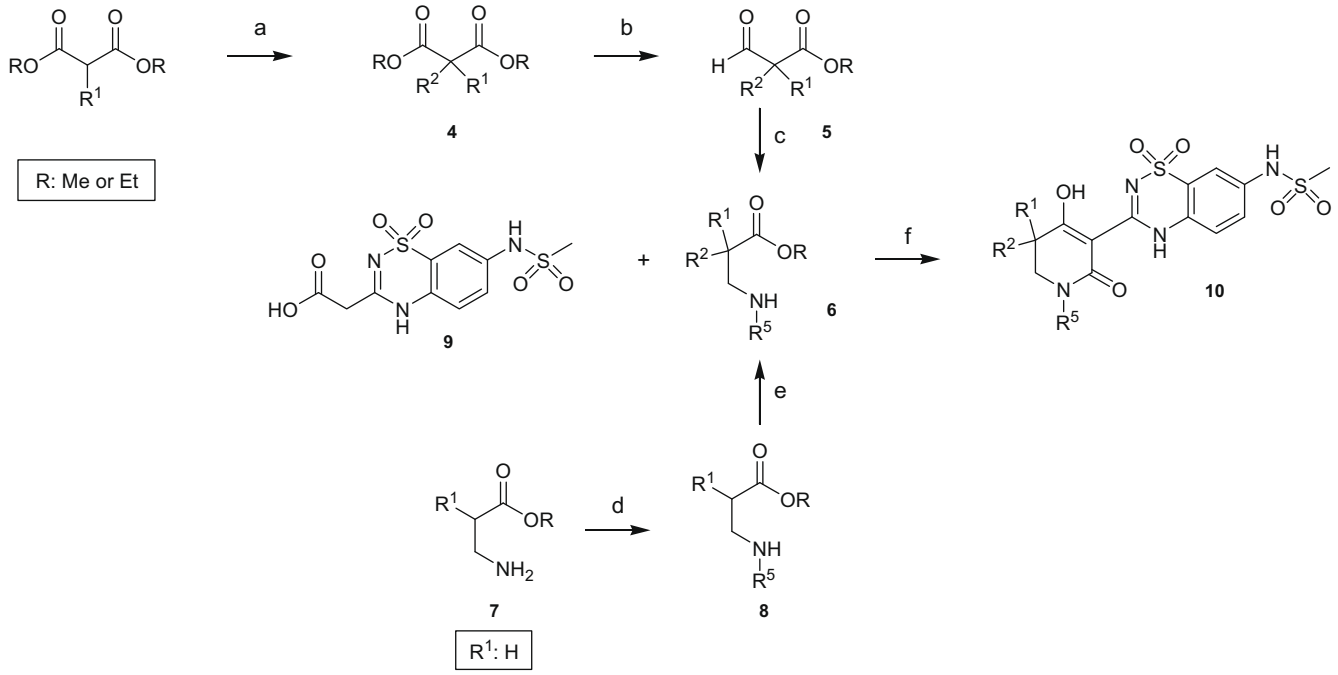


Figure 1. HCV polymerase inhibitors.



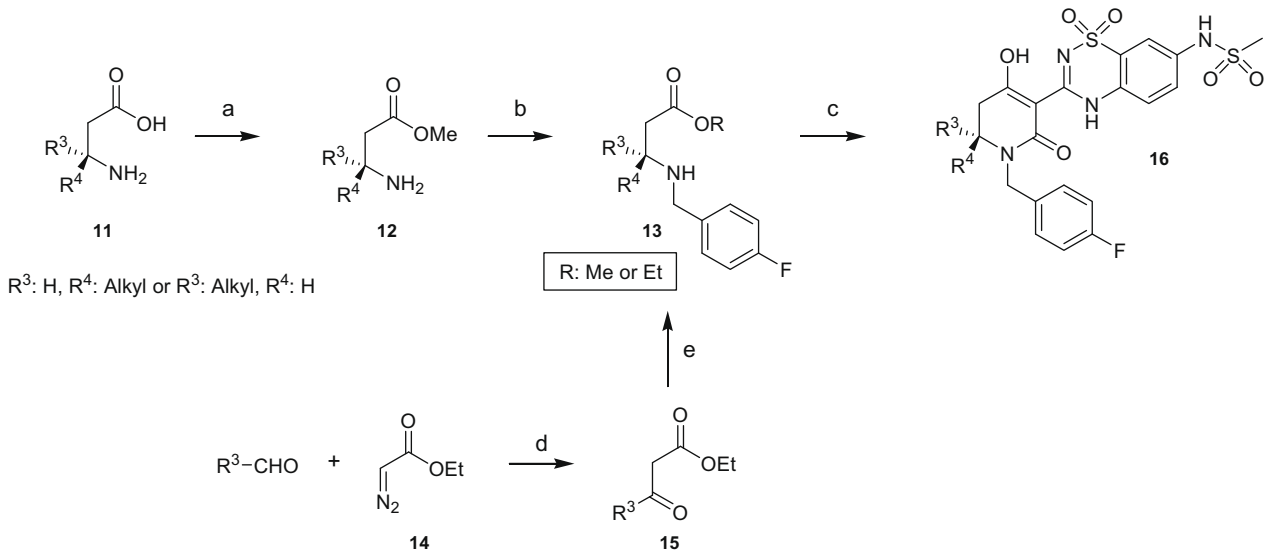
Scheme 1. Reagents and conditions: (a) (i) NaH, THF, 25 °C, 20 min, (ii) R^2X, 25 °C, 3 h, 76–98%; (b) DIBAL (2 equiv), CH_2Cl_2, −78 °C, 4 h, 8–89%; (c) (i) R^5NH_2, MeOH or EtOH, (ii) $NaCNBH_3$ or $NaBH_4$, AcOH, 25–60 °C, 4–16 h, 33–90%; (d) (i) aldehydes or ketones, MeOH, (ii) $NaCNBH_3$, NaOAc, 25 °C, 4–16 h, 10–79%; (e) (i) KHMDS, THF, −78 °C, 15 min, (ii) R_2X, −78 °C for 6 h then warm to 25 °C, 36–40%; (f) (i) EDCI, NMM, DMF, 25 °C, 1 h, (ii) NaOEt, EtOH, 70 °C, 1 h, 30–70%.

hydride (DIBAL) to afford the racemic β-formylesters **5**. Reductive amination of intermediates **5** with primary amines afforded the desired 2,2-disubstituted β-aminoesters **6**. Alternatively, reductive alkylation of commercially available β-amino esters **7** with aldehydes or ketones followed by potassium hexamethyldisilazane (KHMDS) mediated alkylation at the 2-position afforded the racemic mono-alkylated-β-aminoesters **6**. Coupling of intermediates **6** to acid **9**[9] using standard conditions afforded the corresponding amide intermediates, which, upon treatment with sodium ethoxide, cyclized to yield the desired final products **10**.

Synthetic routes to produce 6-mono-alkylated pyridinones are shown in Scheme 2. Esterification of a variety of commercially available β-substituted-β-amino acids **11**, in either racemic or optically pure form (ee >95%), followed by reductive alkylation with 4-fluoro-benzaldehyde yielded the desired 3-alkyl-*N*-4-fluoro-benzyl-β-amino esters **13**.[10] Alternatively, treatment of aldehydes with diazo-ethylacetate **14** in the presence of stannous chloride afforded β-ketoesters **15**.[11] Reductive amination of **15** with 4-fluoro-benzylamine led to the racemic 3,3′-dialkyl-*N*-4-fluoro-benzyl-β-amino esters **13**. Coupling of intermediates **13** to acid **9**, followed by cyclization, proceeded as described above to afford the final products **16**.

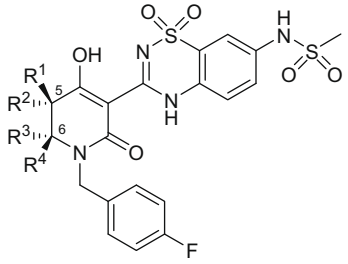
Table 1 details the structure activity relationships (SAR) obtained for compounds with substitutions at the 5- and 6-positions of the pyridinone ring system bearing a 4-fluoro-benzyl R^5 substituent. The major conclusions of this study are summarized below. Mono-substitution at the 5-position led to active, yet relatively weak NS5B inhibitors compared to the bicyclic analog **1**. Addition of a methyl group to produce the 5,5′-di-substituted pyridinones caused little change in potency (cf. **20** with **24**). Similarly, 5-spirocyclic derivatives (**26**–**28**) also offered no obvious advantage. Substitution at the 6-position afforded several compounds with improved NS5B inhibition properties relative to the 5-alkyl substituted analogs. Substitution of the 6-position with ethyl and isopropyl groups afforded the most potent inhibitors in Table 1. Interestingly, compound **29** exhibited >4-fold improvement in biochemical potency (**1b**) and 10-fold improvement in antiviral potency as compared to the corresponding enantiomer (**30**).

The stability of these compounds was assayed using human liver microsomes (HLM). The HLM $T_{1/2}$ results are summarized in Table 1. The majority of compounds described above exhibited long HLM half-lives (>60 min); except when an isoamyl group was incorporated at either the 5- or 6-position. Similarly short



Scheme 2. Reagents and conditions: (a) $TMSCHN_2$, 1:1 MeOH/benzene, 0–25 °C, 1 h, 90–98%; (b) (i) 4-fluoro-benzaldehyde, MeOH or EtOH; (ii) $NaCNBH_3$ or $NaBH_4$, AcOH, 25–60 °C, 4–18 h, 50%; (c) (i) compound **9**, EDCI, NMM, DMF, 25 °C, 1 h, (ii) NaOEt, EtOH, 70 °C, 1 h, 22–36%; (d) $SnCl_2$, CH_2Cl_2, 25 °C, 1 h, 10–55%; (e) (i) 4-fluoro-benzylamine, MeOH or EtOH, (ii) $NaCNBH_3$ or $NaBH_4$, AcOH, 25–60 °C, 4–16 h, 47–55%.

Table 1
Exploration of various mono and di-alkylated pyridinones



Compd[a]	R^1	R^2	R^3	R^4	IC_{50} **1a**[b] (μM)	IC_{50} **1b**[c] (μM)	EC_{50} **1b**[c] (μM)	CC_{50}[c] (μM)	HLM $T_{1/2}$[c] (min)
1			Figure 1		<0.025	<0.01	0.016	>100	>60
17	H	H	H	H	0.18	0.2	3.9	>33	>60
18	H	CH_2–(4-F)Ph	H	H	ND[d]	0.26	3.6	>33	>60
19	H	$CH_2CH{=}C(CH_3)_2$	H	H	1.4	0.13	0.67	>33	6.9
20[e]	H	$CH_2CH_2CH(CH_3)_2$	H	H	ND	0.22	0.49	>33	6.5
21	CH_3	CH_3	H	H	0.085	0.067	1.3	>33	>60
22	CH_3	CH_2CH_3	H	H	0.045	0.032	0.16	>1	>60
23	CH_3	CH_2-cy-Propyl	H	H	0.1	0.099	0.2	>33	>60
24	CH_3	$CH_2CH_2CH(CH_3)_2$	H	H	ND	0.24	0.77	>33	5.7
25	CH_3	Ph	H	H	ND	1.1	ND	ND	ND
26		$-CH_2CH_2-$	H	H	0.037	0.025	0.55	>1	>60
27		$-CH_2CH_2CH_2-$	H	H	0.12	0.042	0.77	>33	>60
28		$-CH_2CH_2CH_2CH_2-$	H	H	0.22	0.091	2.1	>33	28
29[f]	H	H	H	CH_2CH_3	<0.025	<0.010	0.083	>1	>60
30[f]	H	H	CH_2CH_3	H	0.042	0.043	0.97	ND	>60
31[f]	H	H	H	$CH(CH_3)_2$	0.026	<0.010	0.045	>1	>60
32[f]	H	H	H	$C(CH_3)_3$	0.042	0.063	0.81	>10	>60
33	H	H	$CH_2CH(CH_3)_2$	H	ND	0.016	2.9	ND	>60
34	H	H	$CH_2CH_2CH(CH_3)_2$	H	ND	0.15	5.5	ND	7.6
35	H	H	$CH(CH_2CH_3)_2$	H	0.061	0.07	0.31	>1	59
36[f]	H	H	H	Ph	0.17	0.078	0.66	>10	>60
37	H	H	(4-F)Ph	H	0.1	0.09	0.52	>10	>60
38	H	H	$-CH_2CH_2CH_2CH_2-$		0.15	0.028	0.84	>33	>60

[a] All compounds were prepared in racemic form unless otherwise indicated.
[b] See Ref. 12b for assay conditions and error.
[c] See Ref. 12a for assay conditions and error.
[d] ND = not determined.
[E] Synthesized via hydrogenation of compound **19**.
[f] Single enantiomer.

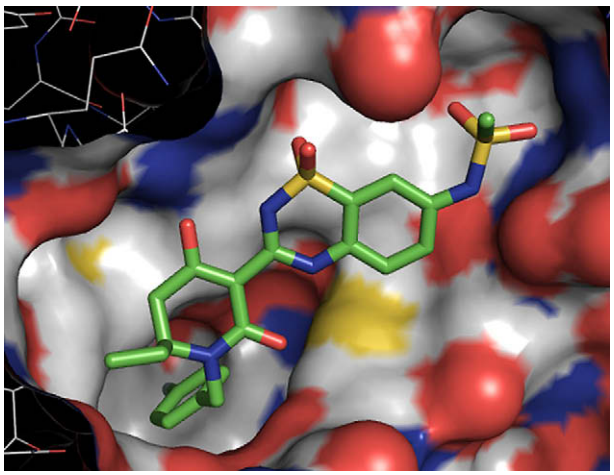


Figure 2. Co-crystal X-ray structure of compound **29** bound to the NS5B protein.

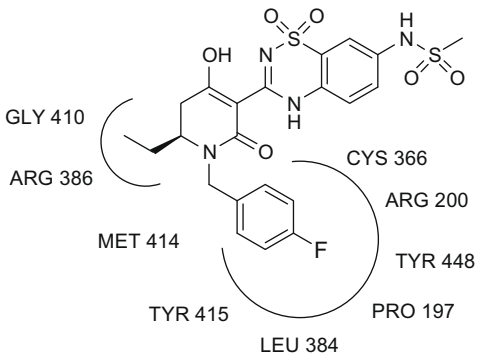


Figure 3. Schematic representation of the interactions between compound **29** and the NS5B protein.

half-lives were also observed for the alkene analog (**19**) and the cyclobutyl spirocyclic derivative (**28**).

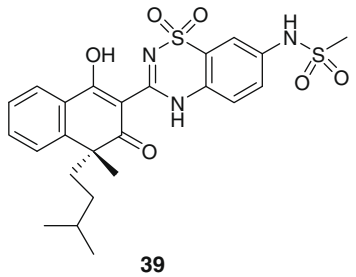
The X-ray crystal structure of compound **29** bound to the NS5B protein is shown in Figure 2.[13] A schematic diagram depicting enzyme residues near the inhibitor is also provided (Fig. 3). The 4-fluoro-benzyl fragment bound in a deep hydrophobic pocket comprised of NS5B residues Pro-197, Arg-200, Leu-384, Cys-366, Met-414, Tyr-415 and Tyr-448. In contrast, the R^4 ethyl substituent bound in a shallow cleft comprised of Gly-410 and Met-414. Based on these observations, we assumed a similar binding mode for all of the 4-fluoro-benzyl containing inhibitors in Table 1.

Given the expected rotational freedom along the bond connecting the benzothiadiazine and dihydropyridinone moieties of **3**, we hypothesized that depending on the substitution pattern chosen, an NS5B binding mode similar to that exhibited by a previously described series of 1-hydroxy-4,4-dialkyl-3-oxo-3,4-dihydronaphthalenes (structure **39**, Fig. 4)[14] could be achieved. Such modifications would effectively 'flip' the orientation of the dihydropyridinone ring relative to that established for the 4-fluoro-benzyl containing molecules of Table 1 (e.g., structure **40**, Figure 4). Accordingly, we designed a second series of 5,5′-di-alkylated pyridinones containing R^1 and R^2 substituents known from the 1-hydroxy-4,4-dialkyl-3-oxo-3,4-dihydronaphthalene series to afford potent NS5B inhibitors.[14] In order to further 'force' the molecules into a flipped conformation, we also incorporated cyclic aliphatic R^5 substituents that we had previously shown to lead to less favorable interactions in the deep hydrophobic pocket occupied by the 4-fluoro-benzyl group of **29**.[7] Table 2 summarizes the SAR obtained for this second series of 5,5′-di-alkylated pyridinones.

Overall, the 'flipped' series strategy led to the synthesis of active, yet only moderately potent NS5B inhibitors relative to compound **1**. Of the compounds listed in Table 2, inhibitors containing an isoamyl R^2 moiety exhibited the best enzymatic and antiviral potency compared to all others shown. Interestingly, *tert*-butyl-ethyl R^2 groups led to a severe loss in anti-NS5B potency relative to the isoamyl analogs (cf. **49** with **54**). Increasing the R^5 cyclic aliphatic ring size from cyclopropyl to cyclohexyl had relatively little impact on the NS5B inhibition properties as shown by compounds **41**, **44**, **49** and **57**, where the R^2 substituent was held fixed as an isoamyl moiety. Replacement of the cyclohexyl R^5 substituent with a phenyl group exhibited a dramatic loss in enzymatic activity (cf. **57** with **60**). Removal of the R^1 methyl (cf. **49** with **51**) had only minimal effect on overall NS5B activity whereas increasing the size of the R^1 substituent to an ethyl group (**52**) led to significantly reduced antiviral potency. All inhibitors shown in Table 2 containing a 4-fluoro-benzyl R^2 substituent displayed severe loss in potency. These results are consistent with our hypothesis that molecules depicted in Table 2 adopt an alternate NS5B binding conformation relative to those in Table 1.

Stability towards human liver microsomes (HLM) was also assessed for the inhibitors listed in Table 2. Interestingly, after exposure of the racemic analogs to HLM, analysis by chiral HPLC/LC–MS indicated one enantiomer to be much more stable than the other (**44**, **49**, **57** and **60**).[15] No attempt was made to assign the absolute stereochemistry of the more stable enantiomer. Chiral analysis was not performed during the HLM assessment of **45**, **46**, **47**, **48**, **52**, **53**, **54**, and **58**. However, a measurement of % compound remaining vs. time after exposure of these racemic analogs to HLM indicated a sharp drop in concentration followed by a gradual decline. This data again suggested the possibility of one enantiomer being much more stable than the other. Double experimental decay curve fitting was applied to both the rapid and gradual decay phases of the plot. We interpreted the resulting half-lives as the actual half-lives corresponding to each enantiomer and the results are reported in Table 2.

To further confirm our earlier hypothesis that the compounds described in Table 2 would likely adopt a 'flipped' conformation



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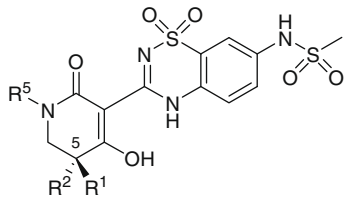
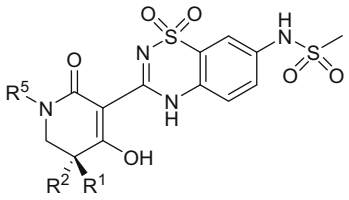


40

Figure 4. HCV polymerase inhibitors containing a quaternary carbon center.

Table 2
Exploration of the 'flipped' series of NS5B inhibitors



Compd	R^5	R^1	R^2	IC_{50} **1a**[a] (μM)	IC_{50} **1b**[b] (μM)	EC_{50} **1b**[b] (μM)	CC_{50}[b] (μM)	HLM $T_{1/2}$[b] (min)
1	Figure 1			<0.025	<0.01	0.016	>100	>60
41	cy-Propyl	CH_3	$CH_2CH_2CH(CH_3)_2$	0.068	0.073	0.097	>33	5.5
42	cy-Propyl	CH_3	$CH_2CH_2C(CH_3)_3$	0.075	0.15	0.33	>10	10
43	cy-Propyl	CH_3	CH_2–(4-F)Ph	ND[c]	0.39	2.1	>33	ND
44	cy-Butyl	CH_3	$CH_2CH_2CH(CH_3)_2$	0.044	0.023	0.014	>10	>60[d]/2.2[e]
45	cy-Butyl	CH_3	CH_2CH_2-cy-Propyl	<0.025	0.028	0.15	>1	>60[f]/5.1[g]
46	cy-Butyl	CH_3	$CH_2CH_2C(CH_3)_3$	0.17	0.041	0.46	>1	>60[f]/3[g]
47	cy-Butyl	CH_3	$CH_2CH_2CH_3$	0.11	0.063	8.5	ND	>60[f]/2.8[g]
48	cy-Butyl	CH_3	$CH_2CH_2CH_2CH_3$	0.049	0.03	>10	ND	>60[f]/3[g]
49	cy-Pentyl	CH_3	$CH_2CH_2CH(CH_3)_2$	0.061	0.047	0.045	>33	>60[d]/2.6[e]
50	cy-Pentyl	H	$CH_2CH{=}C(CH_3)_2$	0.65	0.11	0.24	>33	35
51[h]	cy-Pentyl	H	$CH_2CH_2CH(CH_3)_2$	0.12	0.056	0.043	>33	32
52	cy-Pentyl	CH_2CH_3	$CH_2CH_2CH(CH_3)_2$	0.048	0.084	0.3	>1	>60[f]/3[g]
53	cy-Pentyl	CH_3	CH_2CH_2-cy-Propyl	0.057	0.1	0.17	>10	>60[f]/3.7[g]
54	cy-Pentyl	CH_3	$CH_2CH_2C(CH_3)_3$	ND	0.25	0.31	>10	>60[f]/1.6[g]
55	cy-Pentyl	CH_3	$CH_2C{\equiv}CH$	ND	0.96	2.9	>33	43
56	cy-Pentyl	CH_3	CH_2–(4-F)Ph	ND	1	14	>33	ND
57	cy-Hexyl	CH_3	$CH_2CH_2CH(CH_3)_2$	0.17	0.02	0.025	>33	>60[d]/3.6[e]
58	cy-Hexyl	CH_3	$CH_2CH_2C(CH_3)_3$	ND	0.91	1.4	>10	>60[f]/1.4[g]
59	cy-Hexyl	CH_3	CH_2–(4-F)Ph	ND	2	ND	ND	ND
60	Ph	CH_3	$CH_2CH_2CH(CH_3)_2$	ND	3.4	ND	ND	>60[d]/2.3[e]

[a] See Ref. 12b for assay conditions and error.
[b] See Ref. 12a for assay conditions and error.
[c] ND = not determined.
[d] Chiral analysis: peak 1.
[e] Chiral analysis: peak 2.
[f] Double experimental decay curve fitting—gradual decay phase.
[g] Double experimental decay curve fitting—rapid decay phase.
[h] Synthesized via hydrogenation of compound **50**.

with respect to those in Table 1, we obtained a co-crystal structure of compound **49** bound to the NS5B protein (Fig. 5). A schematic diagram depicting enzyme residues is also provided in Figure 6. As predicted, the pyridinone ring of **49** was observed to be "flipped" relative to the orientation noted for compound **29** with the R^2 isoamyl fragment of the former molecule occupying the same deep hydrophobic pocket that was filled by the latter's 4-fluoro-benzyl substituent.

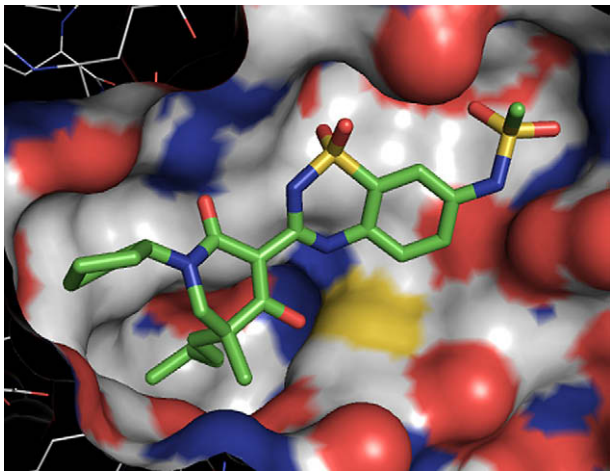


Figure 5. Co-crystal X-ray structure of compound **49** bound to the NS5B protein.

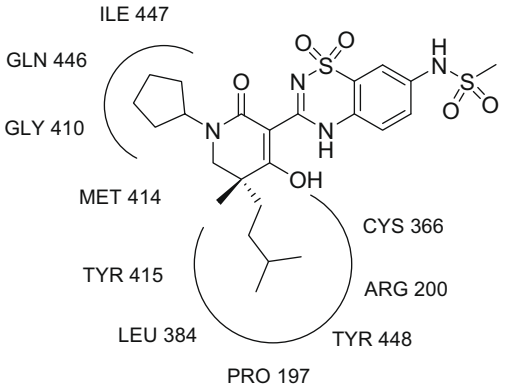


Figure 6. Schematic representation of the interactions between compound **49** and the NS5B protein.

Table 3 details the in vitro and in vivo DMPK parameters for compound **44** compared to those observed for inhibitor **1**. As observed in the HLM stability studies, chiral analysis after treatment of **44** with cynomolgus monkey liver microsomes (MLM) indicated greater stability of one enantiomer over the other. Although oral bioavailability was determined to be 16% for compound **44**, a much faster clearance rate relative to **1** was observed (Fig. 7),[7] and as a result, **44** was no longer detectable 12 h post oral administration.

In summary, we have identified a novel series of HCV inhibitors derived from 5,5′ and 6,6′-dialkylated dihydro-pyridinone ring systems linked to a 1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazine

Table 3
In vitro and in vivo DMPK parameters for selected compounds

Compd	MLM $T_{1/2}$[a] (min)	P_{app}[a,b] ((cm/s) × 10^{-6})	F_{PO}[c] (%)	AUC_{inf}[c] (ng/h/mL) PO/IV	CL (IV)[c] (mL/min/kg)	$C_{12\ h}$ (PO)/EC_{50}[d]
1	>60	1.6	21	6041/29086	0.63	10.49
44[e]	55[f] 13[g]	2.9	16	556/3563	4.7	0[h]

[a] See Ref. 12c for assay conditions and error.
[b] Controls: P_{app} atenolol (low) = (0.2–0.6) × 10^{-6} (cm/s), P_{app} propranolol (high) = (10–15) × 10^{-6} (cm/s).
[c] Cynomolgus monkeys; dose: 1 mg/kg; formulation (for both po and iv administration): 1% DMSO, 9.9% cremophor EL in 50 mM PBS, pH 7.4.
[d] $C_{12\ h}$ (PO)/EC_{50} = plasma concentration 12 h after oral administration divided by EC_{50} (**1b**) value.
[e] Dosed in racemic form.
[f] Chiral analysis: peak 1.
[g] Chiral analysis: peak 2.
[h] Signal below lower limit of quantification.

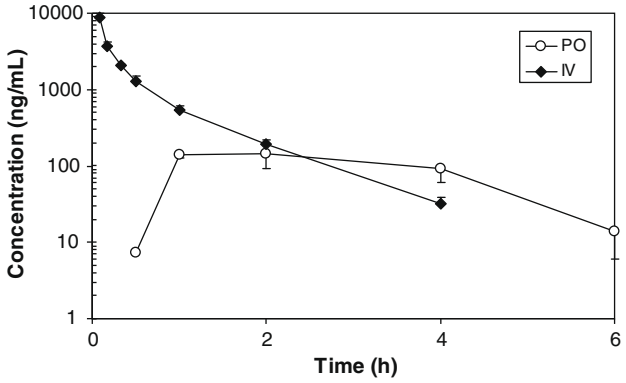


Figure 7. Average plasma concentrations of compound **44** in cynomolgus monkeys at various times after iv and po administration.

moiety. SAR studies identified a number of potent compounds in both biochemical and cellular HCV assays. Oral bioavailability was determined for compound **44** to be 16%, however fast in vivo clearance led to undetectable amounts of **44** at 12 h post dosing. Our ongoing efforts to further improve potency and PK properties around this class of NS5B polymerase inhibitors will be reported in a future Letter.

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13. Crystals of HCV NS5B polymerase (genotype 1b, strain BK, Δ21) were grown by the hanging drop method at room temperature using a well buffer of 20% PEG 4 K, 50 mM ammonium sulfate, 100 mM sodium acetate, pH 4.7 with 5 mM DTT. The crystals formed in space group $P2_12_12_1$ with approximate cell dimensions, a = 85 Å, b = 106 Å, c = 127 Å and two protein molecules in the asymmetric unit. Protein/inhibitor complexes were prepared by soaking these crystals for 3–24 h in solutions containing 15–20% DMSO, 20% glycerol, 20% PEG 4 K, 0.1 M Hepes and 10 mM $MgCl_2$ at pH 7.6 and an inhibitor concentration of 2–10 mM. Diffraction data were collected to a resolution of 2.6 Å for compound **29** and 2.15 Å for compound **49**. The crystal structures discussed in this Letter has been deposited in the Protein Databank (www.rcsb.org) with entry codes: 3IGV and 3GYN, respectively. Full structure determination details are provided in the PDB entry.
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