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Hexahydro-pyrrolo- and hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-ones as potent inhibitors of HCV NS5B polymerase

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Hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-one

ABSTRACT

Hexahydro-pyrrolo- and hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-one analogs were discovered as a novel class of inhibitors of genotype 1 HCV NS5B polymerase. Among these, compound 4c displayed potent inhibitory activities in biochemical and replicon assays (IC_{50} (1b) <10 nM; EC_{50} (1b) = 34 nM) as well as good stability towards human liver microsomes (HLM $t_{1/2}$ = 59 min).

Hepatitis C virus (HCV), a blood-borne pathogen belonging to the *Flaviviridae* family of viruses,[1] is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. The disease affects an estimated 170 million individuals worldwide and 4 million people in the US, with 3–4 million people newly infected each year.[2] Despite major efforts by research groups in academia and the pharmaceutical industry, there is currently no vaccine available to prevent hepatitis C, nor a HCV-specific antiviral agent approved for treatment of chronic hepatitis C. The current standard of care is a combination of pegylated interferon (IFN) with ribavirin.[3]

However, the current HCV therapy suffers from inadequate sustained viral response rates, in particular for patients infected with genotype 1 HCV, along with significant side-effects, resulting in a continuing medical need for improved treatments.[4] Our research has been focused on identifying novel non-nucleoside inhibitors of the HCV NS5B protein, a virally encoded RNA-dependent RNA polymerase (RdRp), the activity of which is critical for the replication of the virus.[5] We focused our attention on the palm binding site, one of several inhibitor binding pockets distinct from the active site.[6] Several series of NS5B inhibitors have been reported to bind at the palm binding site.[7] More specifically, we have previously reported that compounds containing the pyridazin-2-one motif, as exemplified by compound **1** (Fig. 1[8]) exhibit potent inhibitory activity against NS5B with IC_{50} (1b) values of <0.01 μM.[9] However, many of these compounds displayed limited oral bioavailability in animals that was likely due to poor intestinal absorption. We believe that this is related to the high polar surface area (PSA) of these compounds, which is outside the normal range typically correlated with good absorption (<140 Å^2).[9c,9d,10] Accordingly, we explored other palm-binding NS5B inhibitors with reduced PSA values relative to compound **1** (e.g., **2**[11] and **3**[12]) in the hope of improving both intestinal permeability and oral bioavailability. Unfortunately, these alternate inhibitor series did not display significant improvements in these two biological parameters.

Here we disclose a new series of palm-binding NS5B inhibitors that also possess lower PSA values relative to compound **1**. The described compounds (**4**, Fig. 1) incorporate hexahydro-pyrrolo- and hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-ones into their design, the former of which are formally derived from **3** by saturation of the fused pyrrole ring. Our analysis of the co-crystal structure of **3b** bound to the NS5B protein[12] suggested that the saturated, and

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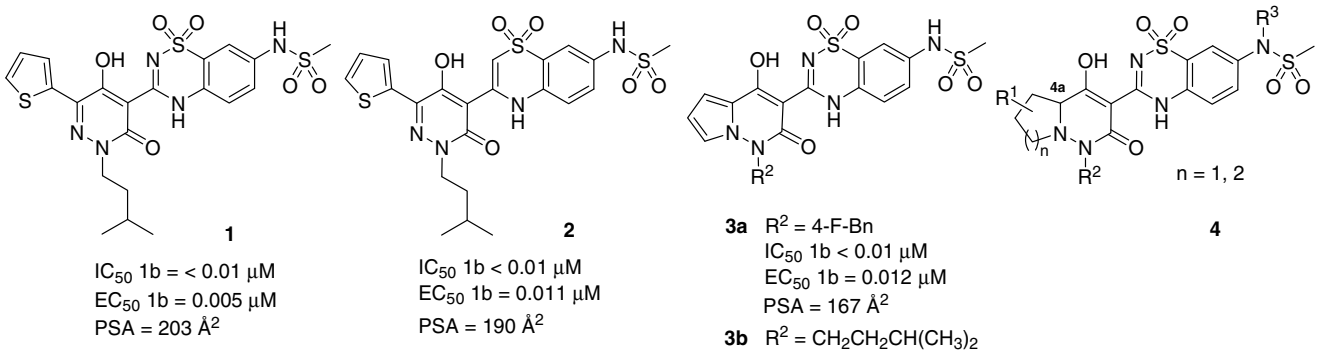


Figure 1. HCV NS5B polymerase inhibitors.

thus probably slightly puckered, ring system could be accommodated in the palm binding site.

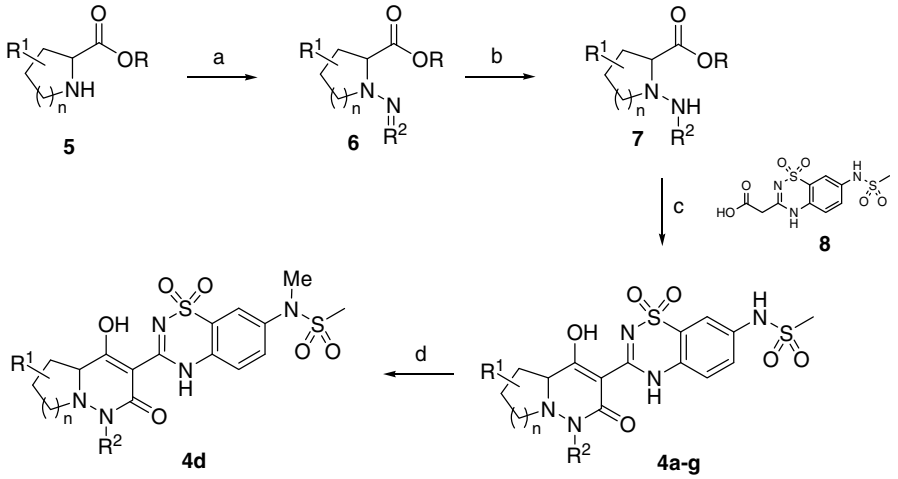
Compounds **4** were synthesized following the route shown in Scheme 1. Commercially available cyclic α-amino esters **5** were *N*-aminated using freshly prepared 1-oxa-2-aza-spiro[2.5]octane[13] and the intermediates were directly treated with aldehydes to yield the imines **6**. Reduction with sodium cyanoborohydride afforded the corresponding *N*-substituted hydrazines **7**, which were coupled with acid **8**[14] and subsequently cyclized in the presence of sodium ethoxide to give the desired compounds **4**. These compounds could be further treated with iodomethane to yield analogs that were *N*-methylated at the R^2 sulfonamide moiety as shown, for example **4d**.

Table 1 details the structure–activity relationships (SAR) obtained for compounds **4**, focusing on their biochemical potencies against HCV genotype 1b, activities against the HCV genotype 1b subgenomic replicon in tissue culture, cytotoxicity, and stability against human liver microsomes (HLM).

We began our SAR exploration by synthesizing **4a** as the direct analog of compound **3a**. Compound **4a** was a potent inhibitor in the enzymatic assay thus confirming our hypothesis that a saturated ring system would be tolerated in the palm binding site. Since the activity of **4a** in the replicon assay was relatively modest, we went on to explore further structural variations in R^2 with the goal of improving this parameter. However, we were surprised to note a different trend for the SAR around the R^2 moiety as compared to that observed for the corresponding unsaturated molecules of general structure **3**.[12] While incorporating the 4-fluorobenzyl and isoamyl groups in R^2 previously led to excellent replicon potencies (see Fig. 1), the activities of such derivatives in the present series were disappointing (**4a** and **4b**). Interestingly, the replicon activities could be improved by introducing a *tert*-butylethyl group into the inhibitor design (**4c**) leading to a compound that was roughly equipotent to **3a**. Our comparison of the co-crystal structures from this (Fig. 2) with the previously reported series did not reveal any significant structural changes in the R^2 binding pocket that could explain these differences.

In our earlier work, we observed that the methanesulfonamide group appended to the benzothiadiazine ring was crucial for achieving potent activities in the biochemical and replicon assays.[9c,9d,12] As such, it came as no surprise that *N*-methylation of this moiety (R^3 = Me) resulted in substantial increases of both IC_{50} and EC_{50} values as seen in **4d**.

We also explored variations on the left-hand ring system including introduction of a methyl group at the C-4a bridgehead (**4e**). The additional methyl group had a detrimental effect on the potencies as well as on the compound's stability toward HLM. Expanding the ring size from 5- to 6-membered led to the hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-one analogs **4f** and **4g**, which were well tolerated in the enzymatic assay, but led to diminished activities against the replicon, the reasons for which are currently not understood. Overall, with the exception of compound **4e**, all compounds under study exhibited good stability toward HLM (see Table 1).



Scheme 1. Reagents and conditions: (a) i—1-oxa-2-aza-spiro[2.5]octane, toluene, 80 °C, 16 h; ii—RCHO, MeOH, 45 °C (39–97% over two steps); (b) $NaCNBH_3$, MeOH, 25 °C, 2–22 h (68–89%); (c) i—**8**, DCC, DCM/DMF, 25 °C, 2–16 h or EDC, NMM, DMF, 25 °C, 2–16 h; ii—NaOEt, EtOH, 60 °C, 3–16 h (8–81% over two steps); (d) MeI, K_2CO_3, DMF, 25 °C, 18 h (38%).

Table 1
SAR of hexahydro-pyrrolo- and hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-one analogs **4**

Compound[a]	*n*	R^1	R^2	R^3	IC_{50} (1b) [μM][b]	EC_{50} (1b) [μM][b]	CC_{50} (GAPDH) [μM][b]	HLM $t_{1/2}$ [min][b,c]
3a	Figure 1	H	4-F-Bn	H	<0.01	0.012	>1	>60 (86%)
4a	1	H	4-F-Bn	H	<0.01	0.19	>1	>60 (87%)
4b	1	H	$CH_2CH_2CH(CH_3)_2$	H	0.012	0.16	>1	>60 (65%)
4c	1	H	$CH_2CH_2C(CH_3)_3$	H	<0.01	0.034	>1	59
4d	1	H	$CH_2CH_2C(CH_3)_3$	Me	0.16	3.6	>33	>60
4e	1	4a-Me	$CH_2CH_2C(CH_3)_3$	H	0.18	1.1	>33	4.9
4f	2	H	$CH_2CH_2C(CH_3)_3$	H	0.041	0.11	>33	52
4g	2	H	4-F-Bn	H	0.016	0.3	>1	>60 (102%)

[a] All compounds were obtained from racemic starting materials.
[b] See Ref. 9a for assay conditions.
[c] For values >60 min, % remaining at 60 min is given in parentheses. All compounds were tested at 1 μM.

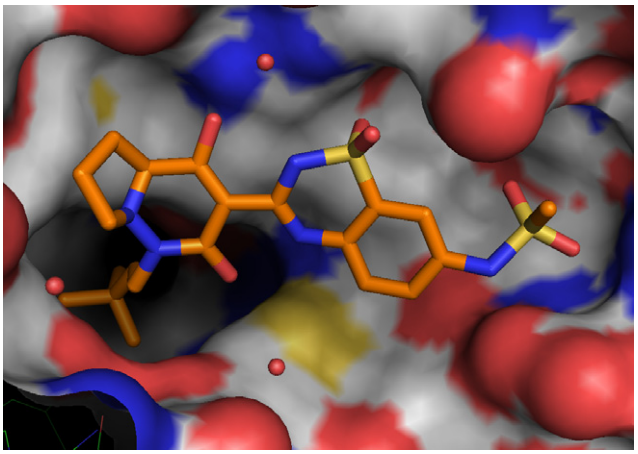


Figure 2. Co-crystal structure of compound **4c** bound to the NS5B protein (2.3 Å).

Figure 2 shows the co-crystal structure of **4c** bound to the palm binding site of NS5B.[15] Figure 3 schematically details the interactions between compound **4c** and the NS5B protein as observed in the co-crystal structure. As previously reported,[9c,12] the sulfonamide substituent on the benzothiadiazine ring forms several H-bonds with the NS5B protein.

These include an interaction between the sulfonamide NH and the side chain of Asp318, as well as a H-bond between one sulfonamide oxygen and the side chain of Asn291. The other sulfonamide oxygen forms a H-bond with a key structural water molecule. Interrupting this network of interactions in the compounds under study leads to a loss of activity as evident in compound **4d**, where the methyl group presumably prevents the formation of some of these H-bonds with the NS5B protein.

In addition to defining the above protein–ligand interactions, we also wished to determine the impact (if any) that introduction of the sp^3 hybridized center at C-4a had on inhibitor binding to NS5B.

An overlay of the co-crystal structures obtained for compound **4c** and the related pyrrolo-[1,2-*b*]pyridazin-2-one compound **3b**[12] bound to the NS5B protein illustrates the different binding geometries of the two series (Fig. 4). While the majority of the compounds' atoms overlay very well, the saturated ring in **4c** (adopting a puckered conformation) clearly protrudes above and below the plane defined by the pyrrolo-[1,2-*b*]pyridazin-2-one ring of **3b**. Since the R^2 substituents in these two compounds are slightly different (isoamyl vs. *tert*-butylethyl), it is difficult to compare the binding conformation in the R^2 sub-pocket. To the best of our knowledge, this is the first structural analysis of a benzothiadiazine-containing NS5B inhibitor bearing a sp^3 hybridized center at the bridgehead carbon (here at C-4a).

Table 2 shows the calculated physicochemical parameters and in vitro DMPK data for a selected number of compounds. Formal saturation of the fused pyrrole ring system present in compounds **3** did not alter polar surface area (PSA) which we had concluded to be one of the parameters causing poor permeability in the previously reported series. With the exception of the *N*-methylated analog **4d** where PSA was reduced to 156 $Å^2$, the PSA values associated with all other compounds were calculated as 165 Å.[2]

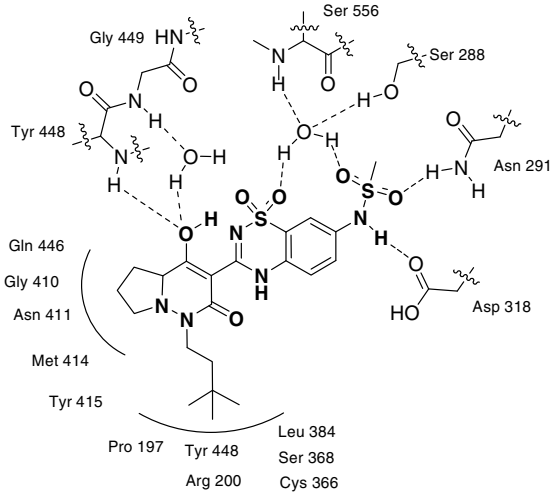


Figure 3. Schematic representation of the interactions between compound **4c** and the NS5B protein. Hydrogen bonds are represented as dashed lines, and the residues which make up the enzyme binding sub-sites are shown.

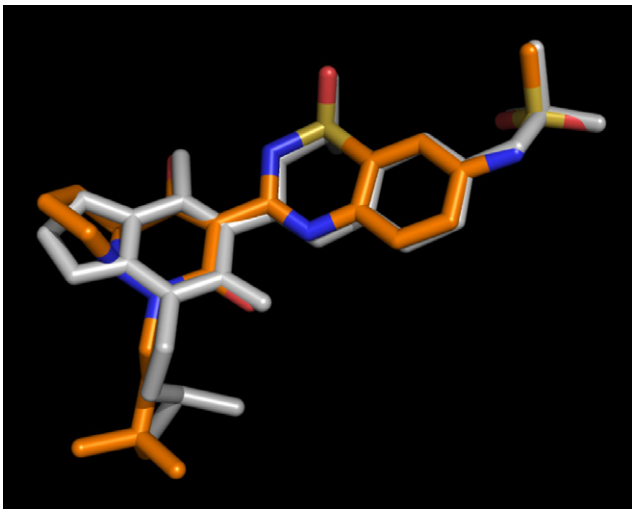


Figure 4. Overlay of co-crystal structures of compound **4c** (orange, 2.3 Å) with compound **3b** (gray, 2.1 Å) bound to the NS5B protein.

Table 2
Correlation of calculated physicochemical parameters and in vitro DMPK data of selected analogs **4**

Compound	$c\log P$[a]	MLM $t_{1/2}$ [min][b,c]	P_{app} [(cm/s) × 10^{-6}][b,d]
3a	0.66	>60 (90%)	0.10
4a	0.53	>60 (94%)	0.20
4b	0.57	63	0.20
4c	0.92	7.5	0.44
4d	0.99	17	2.0
4e	1.4	10	0.60
4f	1.49	18	0.31
4g	1.09	>60 (100%)	0.10

[a] Calculated using ACD/Labs, version 10.0, Advanced Chemistry Development, Inc., Toronto ON, Canada, www.acdlabs.com, 2006.
[b] See Ref. 9c for assay conditions.
[c] For MLM (monkey liver microsome) half-lives >60 min, % remaining at 60 min is given in parentheses. All compounds were tested at 1 μM.
[d] Controls: P_{app} Atenolol (low) = 0.4 (cm/s) × 10^{-6}, P_{app} Propranolol (high) = 10 (cm/s) × 10^{-6}.

While the calculated log*P* values indicated an increase in lipophilicity, in particular for the hexahydro-1*H*-pyrido[1,2-*b*]pyridazin-2-one analogs **4f** and **4g**, the gain did not translate into improved intestinal permeability as judged by the Caco-2 data.

Consistent with our previous conclusions, only the weakly active *N*-methylated analog **4d**, which has a lower PSA value relative to the other inhibitors, displayed any significant permeability improvement. Overall, all compounds under study displayed good solubility in a rapid biochemical assessment (>100 μM) but many showed only low to modest stability toward monkey liver microsomes (MLM). Compound **4c**, the only inhibitor that combined high potencies in the biochemical and replicon assays with good HLM half-life, was evaluated in vivo in cynomolgus monkeys in which we found the corresponding oral bioavailability to be poor (F_{po} = 7%; AUC_{inf} (PO/IV) = 344/5178 ng/h/mL).[16] Although the compound was very unstable toward MLM, the experimental fraction absorbed for this inhibitor (FA_{po} = 8%, calculated based on the measured oral bioavailability (F_{po}%) and clearance of the compound in cynomolgus monkeys)[17] suggested that poor intestinal permeability, rather than extensive metabolism, was the likely cause of the low in vivo exposures.

In summary, we describe a novel series of non-nucleoside inhibitors of genotype 1b HCV NS5B polymerase (**4**) that were formally derived via saturation of the fused left-hand ring system previously described (**3**). While this modification led to a number of very potent compounds in the biochemical and replicon assays, the data also indicated that permeability is still too low for the compounds in this series to be effectively absorbed. Our ongoing efforts to further improve the PK properties of the benzothiadiazine-containing NS5B inhibitors will be reported in a future communication.

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References and notes

1. Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. (a) Kim, W. R. *Hepatology* **2002**, *36*, 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. *N. Engl. J. Med.* **1999**, *341*, 556; (c) Alberti, A.; Benvegnu, L. *J. Hepatol.* **2003**, S104. Suppl. 1.
3. (a) Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. *Science* **1972**, *177*, 705; (b) Smith, R. A.; Kirkpatrick, W.. In *Ribavirin, a Broad Spectrum Antiviral Agent*; Academic Press: New York, 1980; Vol. xiii,. 237 (c) De Clercq, E. *Adv. Virus Res.* **1993**, *42*, 1.
4. Hoofnagle, J. H.; Seeff, L. B. *N. Eng. J. Med.* **2007**, *355*, 2444.
5. Kolykhalov, A. A.; Agapov, E. V.; Blight, K. J.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *Science* **1997**, *277*, 570.
6. Koch, U.; Narjes, F. *Curr. Top. Med. Chem.* **2007**, *7*, 1302.
7. (a) Slater, M. J.; Amphlett, E. M.; Andrews, D. M.; Bravi, G.; Burton, G.; Cheasty, A. G.; Corfield, J. A.; Ellis, M. R.; Fenwick, R. H.; Fernandes, S.; Guidetti, R.; Haigh, D.; Hartley, C. D.; Howes, P. D.; Jackson, D. L.; Jarvest, R. L.; Lovegrove, V. L. H.; Medhurst, K. J.; Parry, N. R.; Price, H.; Shah, P.; Singh, O. M. P.; Stocker, R.; Thommes, P.; Wilkinson, C.; Wonacott, A. *J. Med. Chem.* **2007**, *50*, 897; (b) Dhanak, D.; Duffy, K. J.; Johnston, V. K.; Lin-Goerke, J.; Darcy, M.; Shaw, A. N.; Gu, B.; Silverman, C.; Gates, A. T.; Nonnemacher, M. R.; Earnshaw, D. L.; Casper, D. J.; Kaura, A.; Baker, A.; Greenwood, C.; Gutshall, L. L.; Maley, D.; DelVecchio, A.; Macarron, R.; Hofmann, G. A.; Alnoah, Z.; Cheng, H.-Y.; Chan, G.; Khandekar, S.; Keenan, R. M.; Sarisky, R. T. *J. Biol. Chem.* **2002**, *277*, 38322; (c) Evans, K. A.; Chai, D.; Graybill, T. L.; Burton, G.; Sarisky, R. T.; Lin-Goerke, J.; Johnston, V. K.; Rivero, R. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2205; d Blake, J. F.; Fell, J. B.; Fischer, J. P.; Hendricks, R. T.; Spencer, S. R.; Stengel, P. J. WO2006117306, **2006**.; (e) Pratt, J. K.; Donner, P.; McDaniel, K. F.; Maring, C. J.; Kati, W. M.; Mo, H.; Middleton, T.; Liu, Y.; Ng, T.; Xie, Q.; Zhang, R.; Montgomery, D.; Molla, A.; Kempf, D. J.; Kohlbrenner, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1577; f Hutchinson, D. K. et al. U.S. Patent US2005107364, **2005**.; (g) Bosse, T. D.; Larson, D. P.; Wagner, R.; Hutchinson, D. K.; Rockway, T. W.; Kati, W. M.; Liu, Y.; Masse, S.; Middleton, T.; Mo, H.; Montgomery, D.; Jiang, W.; Koev, G.; Kempf, D. J.; Molla, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 568.
8. All structures are arbitrarily drawn as one of several possible tautomers.
9. (a) Zhou, Y.; Webber, S. E.; Murphy, D. E.; Li, L.-S.; Dragovich, P. S.; Tran, C. V.; Sun, Z.; Ruebsam, F.; Shah, A.; Tsan, M.; Showalter, R.; Patel, R.; Li, B.; Zhao, Q.; Han, Q.; Hermann, T.; Kissinger, C.; LeBrun, L.; Sergeeva, M. V.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1413; (b) Zhou, Y.; Li, L.-S.; Dragovich, P. S.; Murphy, D. E.; Tran, C. V.; Ruebsam, F.; Webber, S. E.; Shah, A.; Tsan, M.; Averill, A.; Showalter, R.; Patel, R.; Han, Q.; Zhao, Q.; Hermann, T.; Kissinger, C.; LeBrun, L.; Sergeeva, M. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1419; (c) Li, L.-S.; Zhou, Y.; Murphy, D. E.; Stankovic, N.; Zhao, J.; Dragovich, P. S.; Bertolini, T.; Sun, Z.; Ayida, B.; Tran, C. V.; Ruebsam, F.; Webber, S. E.; Shah, A. M.; Tsan, M.; Showalter, R. E.; Patel, R.; LeBrun, L. A.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Kamran, R.; Brooks, J.; Sergeeva, M. V.; Kirkovsky, L.; Zhao, Q.; Kissinger, C. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3446; (d) Sergeeva, M. V.; Zhou, Y.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Okamoto, E.; Kirkovsky, L.; Kamran, R.; LeBrun, L. A.; Tsan, M.; Patel, R.; Shah, A. M.; Lardy, M.; Gobbi, A.; Li, L.-S.; Zhao, J.; Bertolini, T.; Stankovic, N.; Sun, Z.; Murphy, D. E.; Webber, S. E.; Dragovich, P. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3421.
10. (a) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615; (b) Palm, K.; Stenberg, P.; Luthman, K.; Artursson, P. *Pharm. Res.* **1997**, *14*, 568.
11. Ellis, D. A.; Blazel, J. K.; Webber, S. E.; Tran, C. V.; Dragovich, P. S.; Sun, Z.; Ruebsam, F.; McGuire, H. M.; Xiang, A. X.; Zhao, J.; Li, L.-S.; Zhou, Y.; Han, Q.; Kissinger, C. R.; Showalter, R. E.; Lardy, M.; Shah, A. M.; Tsan, M.; Patel, R.; LeBrun, L. A.; Kamran, R.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Sergeeva, M. V.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4628.
12. Ruebsam, F.; Webber, S. E.; Tran, M. T.; Tran, C. V.; Murphy, D. E.; Zhao, J.; Dragovich, P. S.; Kim, S. H.; Li, L.-S.; Zhou, Y.; Han, Q.; Kissinger, C. R.; Showalter, R. E.; Lardy, M.; Shah, A. M.; Tsan, M.; Patel, R.; LeBrun, L. A.; Kamran, R.; Sergeeva, M. V.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3616.
13. Schmitz, E.; Ohme, R.; Schramm, S.; Striegler, H.; Heyne, H.-U.; Rusche, J. *J. Prakt. Chem.* **1977**, *319*, 195.
14. Ruebsam, F.; Tran, M. T.; Webber, S. E.; Dragovich, P. S.; Li, L.-S.; Murphy, D. E.; Kucera, D. J.; Sun, Z.; Tran, C. V. PCT International patent application **2007**, WO 2007150001 A1.
15. 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 4K, 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 = 86 Å, b = 106 Å, c = 126 Å and two protein molecules in the asymmetric unit. Protein/inhibitor complexes were prepared by soaking these crystals for 3 to 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 2.3 Å for compound **4c**. The crystal structure discussed in this paper has been deposited in the Protein Databank (www.rcsb.org) with entry code: 3CVK. Full structure determination details are provided in the PDB entry.
16. 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.
17. Davies, B.; Morris, T. *Pharm. Res.* **1993**, *10*, 193. $FA_{po} = F_{po}/(1\text{-}CL/Q)$ where Q = 44 mL/min/kg (hepatic blood flow in cynomolgus monkey) and CL = 3.3 mL/min/kg (clearance in cynomolgus monkey after IV administration).