Bioorganic & Medicinal Chemistry Letters 22 (2012) 713–717

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

II. Novel HCV NS5B polymerase inhibitors: Discovery of indole C2 acyl sulfonamides

Gopinadhan N. Anilkumar*, Oleg Selyutin, Stuart B. Rosenblum, Qingbei Zeng, Yueheng Jiang, Tin-Yau Chan, Haiyan Pu, Li Wang, Frank Bennett, Kevin X. Chen, Charles A. Lesburg, Jose Duca, Stephen Gavalas, Yuhua Huang, Patrick Pinto, Mousumi Sannigrahi, Francisco Velazquez, Srikanth Venkatraman, Bancha Vibulbhan, Sony Agrawal, Eric Ferrari, Chuan-kui Jiang, H.-C. Huang, Neng-Yang Shih, F. George Njoroge, Joseph A. Kozlowski

Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

ARTICLE INFO

Article history:
Received 31 August 2011
Revised 10 October 2011
Accepted 11 October 2011
Available online 20 October 2011

Keywords:
HCV
NS5B Polymerase

ABSTRACT

Development of SAR at the C2 position of indole lead **1**, a palm site inhibitor of HCV NS5B polymerase (NS5B IC_{50} = 0.053 μM, replicon EC_{50} = 4.8 μM), is described. Initial screening identified an acyl sulfonamide moiety as an isostere for the C2 carboxylic acid group. Further SAR investigation resulted in identification of acyl sufonamide analog **7q** (NS5B IC_{50} = 0.039 μM, replicon EC_{50} = 0.011 μM) with >100-fold improved replicon activity.

Hepatitis C virus (HCV) is a major health concern affecting over 170 million individuals worldwide[1a] and is a leading cause of liver transplants and chronic liver cirrhosis, leading to death from liver disease in the United States.[1b,c] The current standard of care is treatment with a combination of subcutaneous pegylated interferon administration with oral dosing of the cytotoxic nucleoside drug ribavirin.[2] The response rate is >75% for HCV patients with genotypes 2 and 3 after a 24 week treatment regimen, while genotype 1 patients have a response rate of less than 50% after 48 weeks of treatment.[3] Recent FDA approval of Victrelis (boceprevir) has invigorated interest in small molecule inhibitors of HCV.[4] With a clear opportunity to improve clinical outcomes, and given the side effects associated with the current standard of care, it is valuable to discover potent inhibitors of HCV replication that will improve outcomes and shorten treatment duration.

The HCV NS5B protein is an RNA-dependent RNA polymerase critical for the synthesis of progeny viral genomes. The crystal structure of HCV NS5B displayed an overall subdomain architecture similar to other members of the Poll family,[5] with a deep active site cavity, located at the top of the 'palm' subdomain, and sealed at its base by a unique β-loop. Furthermore, there was an unexpected interaction identified between the tip of the 'fingers' subdomain and the 'thumb' subdomain to encircle the presumed nucleoside triphosphate substrate entry trajectory.

Sequence variation analysis suggests that residues lining the active site cavity ('palm site') are more conserved than in other regions, making the palm site an attractive target for inhibition of the viral polymerase. Clinical efficacy has been demonstrated with non-nucleoside inhibitors binding at the palm, thumb, and finger-loop subdomains.[6]

In a previous Letter,[7] we described discovery of a novel class of indole C2 carboxylic acids as non-nucleoside palm site inhibitors of HCV NS5B polymerase exemplified by structure **1** (Fig. 1). The unique feature of this class of inhibitors was the bidentate hydrogen bonding interactions with the polypeptide backbone of Tyr-448 and Ile-447. These dual interactions anchor the inhibitors to the palm site cavity. The lead compounds in this series exhibited good NS5B inhibition activities and moderate antiviral properties in the cell based replicon assay. The lead inhibitors all contained an indole C2 carboxylic acid moiety, which did not make direct interaction with the protein as revealed by X-ray structure. Several potential sites for interactions with protein backbone prompted investigation of elaborated carboxylic acid isosteres. Probable acyl-glucuronide conjugation of an acid functionality was an added incentive to look for suitable functionality at C2 position.

During the initial screening of C2 functionalities, a C5 chloro group was chosen since this was well tolerated in the previous SAR explorations. At the N1 position of the indole either an aminopyridylmethyl group or fluoro substituted benzyl moieties were chosen, as these substitutions exhibited the best activity profile in earlier studies. The C-linked pyridone was essential at the indole

* Corresponding author.
E-mail address: gopinadhan.anilkumar@Merck.com (G.N. Anilkumar).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.10.041

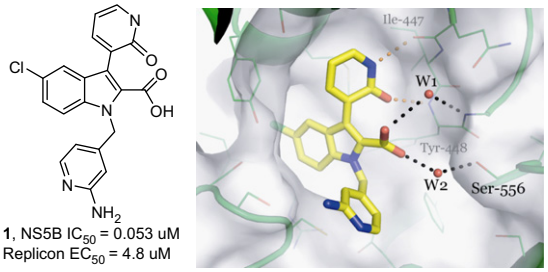
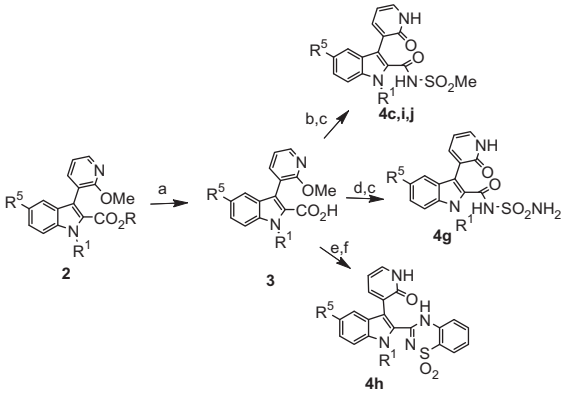


Figure 1. X-ray structure of complex between HCV NS5B and lead indole acid **1** bound in the palm site. The compound (yellow) makes two hydrogen bond interactions (orange) with the backbone carbonyl oxygen and amide nitrogen of Ile-447 and Tyr-448, respectively. In addition to these main chain atoms, side chain atoms within 5 Å of the compound are shown as thin sticks. The C2 acid group interactions with the protein indirectly via two water molecules labeled W1 and W2. Hydrogen bond interactions are shown as dotted lines.

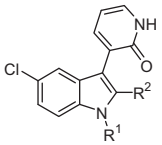
C3 position and anchors the inhibitors to the palm site of the enzyme as detailed above. Synthesis of most of the analogs at the C2 position was accomplished as outlined in Scheme 1. Intermediate **2** was prepared as described previously.[7] Hydrolysis of the C2 ester under basic conditions afforded the advanced intermediate **3** in nearly quantitative yields. Activation with carbonyl diimidazole and subsequent treatment with alkyl sulfonamides and DBU afforded C2 acyl sufonamide intermediates. Final synthetic manipulation to yield analogs **4c,i,j** involved the demethylation of the methoxy pyridine moiety by refluxing with 4 N HCl in dioxane. Similarly, treatment with sulfonyl urea instead of an alkyl sulfonamide and subsequent demethylation reaction afforded C2 acyl sulfamide analog **4g**. For the synthesis of cyclic thiadiazine analog **4h**, the activated acyl imidazole intermediate was treated with *o*-amino phenyl sulfonamide to afford the corresponding phenyl acyl sulfonamide intermediate. Subsequent cyclization and demethylation with 4 N HCl afforded C2 thiadiazine analog **4h** in modest yield.

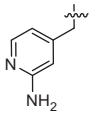
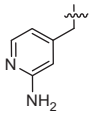
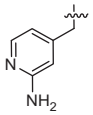
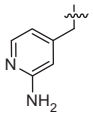
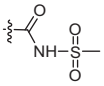
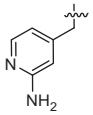
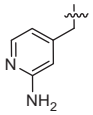
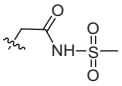
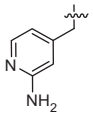
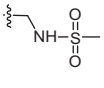
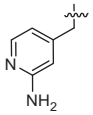
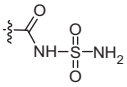
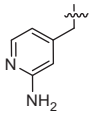
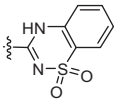
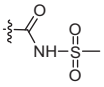
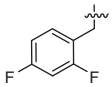
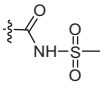
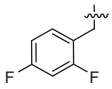
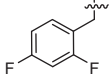
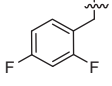
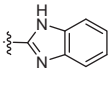
The initial screening of the C2 position with cyano (**4a**), and ester (**4b**) moieties showed very weak binding affinities (IC_{50} >20 μM) compared to the carboxylic acid analog **1** (IC_{50} = 0.053 μM) suggesting the need for a H-bond donor (Table 1). Attempts to prepare the C2 primary amide from either **4a** or **4b** failed, as it was readily hydrolyzed to the acid **1**. Replacement of the C2 acid functionality with an acyl sulfonamide provided **4c** with enhanced binding affinity of IC_{50} = 0.018 μM and significantly improved replicon activity of EC_{50} = 0.3 μM (>10-fold). Extending the acid and acyl sulfonamide



Scheme 1. General synthesis of C2 analogs. Reagents and conditions: (a) aq 1 N LiOH, THF, reflux; (b) CDI, Me-SO_2NH_2, DBU, THF, reflux; (c) 4 N HCl/dioxane, 90 °C; (d) CDI, $NH_2SO_2NH_2$, DBU, THF, reflux; (e) CDI, *o*-NH_2-$PhCONH_2$, DBU, THF, reflux; (f) 4 N HCl/dioxane, MeOH, 90 °C.

Table 1
Variation of C2 substitutions



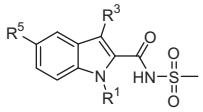
Compds	R^1	R^2	IC_{50}[8] (μM)	EC_{50}[9] (μM)
1			0.053	4.8
4a			>20	8.3
4b			>20	NA
4c			0.018	0.3
4d			7	NA
4e			2.2	NA
4f			>20	NA
4g			0.029	2.2
4h			0.012	0.6
4i			0.033	0.23
4j			0.041	2.2
4k			3.0	NA
4l			2.7	NA
4m			0.57	8

moieties with a methylene linker to enable the inhibitor to achieve additional interactions in this region was not successful. All corresponding analogs (**4d, 4e**) showed diminished enzyme activities. Moreover, the sulfonamide functionality without the carbonyl linker at C2 showed weak activity (**4f**). These results pointed toward the critical importance of a sp2 center at the C2 position for activity. The acyl sulfamide analog **4g** showed good binding activity with a reduced cell based activity (EC_{50} = 2.2 μM). We also explored heterocyclic modifications containing a sulfonyl group which could present the groups in a different spatial orientation. The benzothiadiazine analog **4h** showed excellent enzyme activity while maintaining sub-micromolar replicon activity (IC_{50} = 0.012 μM, EC_{50} = 0.6 μM). Since fluoro substituted benzyl groups were well tolerated at the N1 indole position, additional C2 variations were targeted in this series. The C2 acyl sulfonamide analogs with 2-fluorobenzyl (**4i**, EC_{50} = 0.23 μM) and 2,4-difluorobenzyl groups (**4j**, EC_{50} = 2.2 μM) at N1, showed comparable enzyme activities while the former displayed better replicon activity. Reverse sulfonamides (**4k, 4l**) and benzimidazole (**4m**) substitutions were among the many functionalities at the C2 position that were not tolerated. The initial C2 SAR clearly indicated that the methyl acyl sulfonamide group was the most preferred moiety at this position. The fact that acyl sulfonamide can also act like a tether to extend new functional groups further into in this area prompted exploration of substituted sulfonamides.

In our earlier publication we have identified other heterocycles such as C-linked and N-linked pyrimidine diones at the indole C3 position which make similar interactions with the protein backbone and showed a comparable activity profile to the pyridone.[7] It was appropriate to benchmark the C2 acyl sulfonamides with C3 pyridone to the C3 pyrimidinedione analogs (Table 2). In comparison to pyridone derivative **4c**, the C-linked pyrimidinedione analog **5a** showed a comparable enzyme activity but a significantly reduced cell based activity (EC_{50} = 0.3 μM vs EC_{50} = 3.0 μM). Similarly the N-linked bicyclic pyrimidinedione derivative **5c** also showed a comparable enzyme activity, but a reduced replicon activity to the corresponding pyridone analog **5b** (EC_{50} = 0.12 μM vs EC_{50} = 0.7 μM). Thus the C-linked pyridone was chosen as the optimal C3 functionality for further SAR development with a goal of achieving another log reduction in the replicon activity.

Further SAR exploration with a variety of C2 acyl sulfonamide analogs is represented in Table 3. The syntheses of these analogs were similar to the methyl sulfonamide analog, **4c** shown in Scheme 1. In general alkyl and aryl variations were tolerated at the C2 acyl sulfonamide moiety. None of these variations (**6a–g**) showed a significant improvement in replicon activity including the extended alkyl analogs with substitutions (**6h–i**). The fact that phenyl analog **6c** showed comparable activity to methyl analog **4c** was encouraging (EC_{50} = 0.4 μM vs EC_{50} = 0.3 μM). We hypothesized phenyl would be a suitable tether to explore functionalities which could make some new interactions with the protein backbone.

Table 2
Comparison of the C3 heterocycles in C2 acyl sulfonamide series



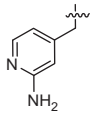
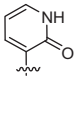
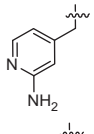
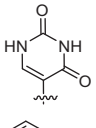
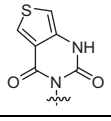
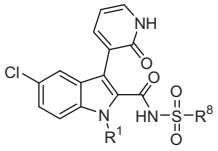
Compds	R^5	R^1	R^3	IC_{50}[8] (μM)	EC_{50}[9] (μM)
4c	Cl			0.018	0.3
5a	Cl			0.027	3.0
5b	CF_3			0.006	0.12
5c	CF_3			0.015	0.7

Table 3
Variation of C2 acyl sulfonamides



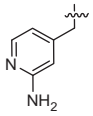
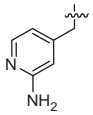
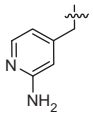
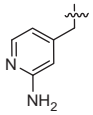
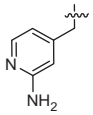
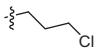
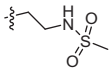
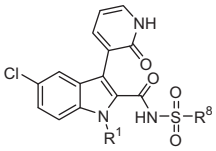
Compds	R^1	R^8	IC_{50}[8] (μM)	EC_{50}[9] (μM)
4c		Me	0.018	0.3
6a		Et	0.018	0.6
6b			0.031	1.0
6c		Ph	0.016	0.4
6d		Bn	0.024	1.2
6e			0.005	0.27
6f			0.013	0.4
6g			0.028	1.4
6h			0.008	2.8
6i			0.005	0.47

Table 4
Substitutions of C2 phenyl acyl sulfonamides



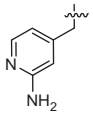
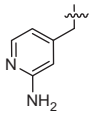
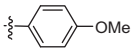
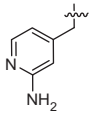
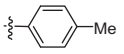
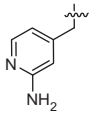
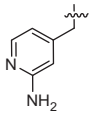
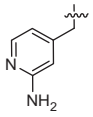
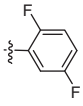
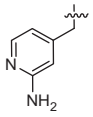
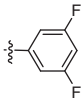
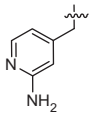
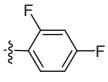
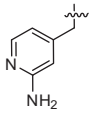
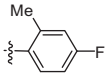
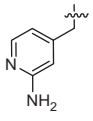
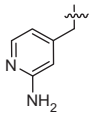
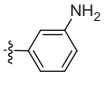
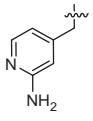
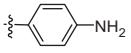
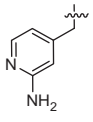
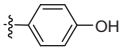
Compds	R^1	R^8	IC_{50}[8] (μM)	EC_{50}[9] (μM)
6c			0.016	0.4
7a			0.022	0.5
7b			0.019	0.6
7c			0.031	0.8
7d			0.043	1.5
7e			0.028	0.7
7f			0.026	0.7
7g			0.049	1.6
7h			0.034	1.9
7i			0.028	1.7
7j			0.036	1.0
7k			0.035	0.4
7l			0.024	0.1

Table 4 (*continued*)

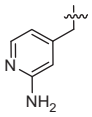
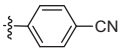
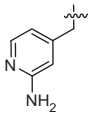
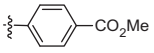
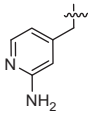
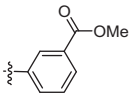
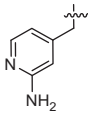
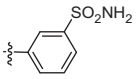
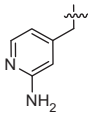
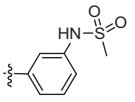
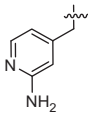
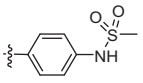
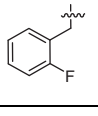
Compds	R^1	R^8	IC_{50}[8] (μM)	EC_{50}[9] (μM)
7m			0.036	1.7
7n			0.032	1.1
7o			0.027	0.3
7p			0.034	1.9
7q			0.039	0.011
7r			0.02	0.5
7s			0.018	0.02

Table 4 represents a summary of selected SAR efforts in the phenyl acyl sulfonamide substitutions. Differentially substituted phenyl acyl sulfonamides were synthesized using a similar route as before (Scheme 1). Various lipophilic mono substitutions on the phenyl ring such as methoxy, methyl, fluoro and chloro showed no improvement in the replicon activity (**7a–d**, EC_{50} >0.5 μM). Additionally the disubstituted phenyl ring as exemplified in **7e–h**, also resulted in diminished replicon activities (EC_{50} >0.7 μM) compared to parent phenyl analog **6c**. Since lipophilic substitutions in general were not desirable, we turned our attention to polar substitutions which are more likely to make additional interactions

Figure 2. X-ray structure of complex between HCV NS5B and extended acyl sulfonamide **7q** bound in the palm site. As in the previous complex, the compound is shown in yellow and hydrogen bonds interactions are shown as dotted lines. Side chain atoms within 5 Å of the compound are shown as thin sticks. Water molecule W1 remains in the identical position as observed in the complex with **1**, and compound **7q** makes indirect interactions with the protein via water W3. There are direct compound/protein interactions with the side chain atoms of Asp-318 and Asn-291.[10,11]

Table 5
PK profile of lead HCV NS5B inhibitors

Compds	IC_{50}[7] (μM)	EC_{50}[8] (μM)	Rat AUC[c] (nM h)
4c	0.018	0.3	740
4i	0.033	0.23	11,400
7q	0.039	0.011	0
7s	0.018	0.02	14

[c] PO, Sprague-Dawly rat, 10 mpk, 6 h, 0.4% MC.

and also improve cell permeability. Among the different amino substituted analogs, the *ortho* and *meta* substitutions (**7i** and **7j**, EC_{50} >1 μM) showed decreased replicon activity compared to the *para* amino analog (**7k**, EC_{50} = 0.4 μM). Interestingly the corresponding *para* hydroxy derivative showed a four fold improvement in the replicon activity (**7l**, EC_{50} = 0.1 μM). Other hydrogen bond acceptor groups such as cyano and methyl ester at this position were not tolerated for replicon activity. Surprisingly the *meta* methyl ester compound **7o** (EC_{50} = 0.3 μM) showed appreciably improved replicon activity compared to *para* isomer **7n** (EC_{50} = 1.1 μM). This prompted further exploration of the *meta* position with similar functionalities. The sulfonamide analog **7p** showed weak cell based activity. Unexpectedly a reverse methyl sulfonamide analog **7q** (EC_{50} = 0.011 μM) showed another log improvement in the replicon activity (2 log improvement compared to the aniline **7j**), thus providing highly potent HCV inhibitor for further evaluation. The importance of *meta* substitution was further confirmed by loss of activity of corresponding *para* isomer **7r**. Other optimized N1 substitutions such as fluoro benzyl also showed excellent replicon activity in this series as exemplified by **7s** (EC_{50} = 0.02 μM).

The X-ray structure of the most potent analog **7q** with NS5B showed that it resides in the expected location in the palm site (Fig. 2). Previously observed hydrogen bonding interactions with the backbone of Ile-447 and Tyr-448 were maintained. Furthermore, the extended C2 acyl sulfonamide group displaced one of the two water molecules observed in the C2 carboxylic acid structure which are part of the hydrogen bonding network between the compound and the protein. The compound (**7q**) forms a new indirect protein interaction via an additional ordered water molecule. The phenyl ring of **7q** stacks in a face-to-face manner with the N1 aminopyridine group. The terminal methyl sulfonamide moiety makes a series of polar interactions with both backbone and side chain atoms within a preexisting polar sub pocket.

After the SAR for replicon activity at C2 position was established, attention was turned to the pharmacokinetics of the lead acyl sulfonamide series (Table 5). Initial methyl sulfonamide analog **4c** demonstrated moderate exposure in a rat when orally dosed (AUC = 740 nM h, 10 mpk, 6 h, MC). The change of the N1 substitution to a fluoro benzyl, compound **4i**, had a profound improvement in the oral exposure (AUC = 11,400 nM h, 10 mpk, 6 h, MC).[12] Unfortunately the most active extended sulfonamide analogs **4q** and **4s** showed very low oral exposure irrespective of the N1 substitutions presumably due to high PSA and high molecular weight.[13]

In summary, modifications at the C2 position of indole **1**, led to the discovery of acyl methyl sulfonamide moiety as an isosteric replacement for the carboxylic acid functionality, with approximately one log improvement in the replicon activity. While tolerated, most of the acyl sulfonamide substitutions did not enhance replicon activity further. Appropriate polar substitutions in the extended phenyl acyl sulfonamide series such as **7q** (EC_{50} = 0.01 μM) and **7s** (EC_{50} = 0.02 μM) showed an additional one log improvement in the replicon activity. This work gave rise to highly potent HCV inhibitors suitable for further development. Further optimization in this series to identify a HCV NS5B inhibitor with improved pharmacokinetic properties will be reported in separate publications.

References

1. (a) Cohen, J. *Science* **1999**, *285*, 26; (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. Eng. J. Med.* **1999**, *341*, 556; (c) Cuthbert, J. A. *Clin. Microbiol. Rev.* **1994**, *7*, 505; (d) Monto, A.; Wright, T. L. *Semin. Oncol.* **2001**, 441.
2. (a) Neumann, A. U.; Lam, N. P.; Dahari, H.; Gretch, D. R.; Wiley, T. E.; Layden, T. J.; Perelson, A. S. *Science* **1998**, *282*, 103; (b) Rosen, H. R.; Gretch, D. R. *Mol. Med. Today* **1999**, *5*, 393; (c) Di Bisceglie, A. M.; McHutchison, J.; Rice, C. M. *Hepatology* **2002**, *35*, 224.
3. Zeuzem, S.; Berg, T.; Moeller, B.; Hinrichsen, H.; Mauss, S.; Wedemeyer, H.; Sarrazin, C.; Hueppe, D.; Zehnter, E.; Manns, M. P. *J. Viral Hepat.* **2009**, *16*, 75.
4. Venkatraman, S.; Bogen, S.; Arasappan, A.; Bennet, F.; Chen, K.; Jao, E.; Liu, Y.-T.; Lovey, R.; Hendreta, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, A.; Agrawal, S.; Ingravallo, P.; Pichardo, J.; Kong, R.; Baroudy, B.; Malcolm, B.; Guo, Z.; Prongay, A.; Madison, V.; Broske, L.; Cui, X.; Cheng, K.-C.; Hsieh, T. Y.; Brisson, J.-M.; Prelusky, D.; Korfmacher, W.; White, R.; Bogdanowich-Knipp, S.; Pavlovsky, A.; Bradley, P.; Saksena, A. K.; Ganguly, A.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. *J. Med. Chem.* **2006**, *49*, 6074.
5. (a) Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. C. *Nat. Struct. Biol.* **1999**, *6*, 937; (b) Bressanelli, S.; Tomei, L.; Roussel, A.; Incitti, I.; Vitale, R. L.; Mathieu, M.; De Francesco, R.; Rey, F. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13034.
6. (a) Beaulieu, P. L. *Expert Opin. Ther. Patents* **2009**, *19*, 145; (b) Ago, H.; Adachi, H. T.; Yoshida, A.; Yamamoto, A.; Habuka, N.; Yatsunami, K.; Miyano, M. *Structure* **1999**, *7*, 1417.
7. Anilkumar, G. N.; Lesburg, C. A.; Selyutin, O.; Rosenblum, S. R.; 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. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5336.
8. HCV NS5B polymerase activity was measured in a radiolabeled nucleotide incorporation assay as described [Cheng 2010], in a reaction buffer containing 20 mM HEPES (pH 7.3), 7.5 mM DTT, 20 units/mL RNasIN, 0.1 μM GTP, ATP and UTP, 60 μCi/ml [^{33}P]-CTP supplemented to 20 nM CTP, 10 mM $MgCl_2$, 60 mM NaCl, 100 μg/ml BSA, 100 nM heteropolymer RNA template, 0.25 mM trinucleotide initiator and 30 nM NS5B (Δ21) enzyme. Reaction was allowed to proceed for 150 min at room temperature and terminated by EDTA. The reaction mixture was washed on Millipore DE81 filter plate and the incorporated labeled CTP quantitated by Packard TopCount. Compound IC_{50} values were calculated from experiments with 10 serial twofold dilutions of the inhibitor in duplicate.
9. To measure cell-based anti-HCV activity, replicon cells (1b-Con1) were seeded at 5000 cells/well in 96-well plates one day prior to inhibitor treatment. Various concentrations of an inhibitor in DMSO were added to the replicon cells, with the final concentration of DMSO at 0.5% and fetal bovine serum at 5% in the assay media. Cells were harvested 3 days post dosing. The replicon RNA level was measured using real-time RT-PCR (Taqman assay) with GAPDH RNA as endogenous control. EC_{50} values were calculated from experiments with 10 serial twofold dilutions of the inhibitor in duplicate.
10. Figures 1and 2 were generated using the program PYMOL (The PYMOL Molecular Graphics System, Version 1.2r1, Schrödinger, LLC).
11. Crystal structures of HCV NS5B in complex with **1** and **7q** have been deposited in the Protein Data Bank with accession numbers 3U4O, 3U4R, respectively.
12. *C*log*P* and PSA measurements of compounds **4c** (*C*log*P* = 1.43, polar surface area = 138 Å^2) and **4i** (*C*log*P* = 3.39, polar surface area = 100 Å^2).
13. *C*log*P* and PSA measurements of compounds **7q** (*C*log*P* = 2.25, polar surface area = 185 Å^2) and **7s** (*C*log*P* = 4.2, polar surface area = 148 Å^2).