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# Novel HCV NS5B polymerase inhibitors derived from 4-(1′,1′-dioxo-1′,4′-dihydro-1′λ$^6$-benzo[1′,2′,4′]thiadiazin-3′-yl)-5-hydroxy-2*H*-pyridazin-3-ones. Part 5: Exploration of pyridazinones containing 6-amino-substituents

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ABSTRACT

The synthesis of 4-(1′,1′-dioxo-1′,4′-dihydro-1′λ$^6$-benzo[1′,2′,4′]thiadiazin-3′-yl)-5-hydroxy-2*H*-pyridazin-3-ones bearing 6-amino substituents 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 (microsome $t_{1/2}$, Caco-2 $P_{app}$) for many of the compounds are also disclosed, and a crystal structure of a representative inhibitor complexed with the NS5B protein is discussed.



Chronic hepatitis C virus (HCV) infection afflicts more than 170 million people worldwide and 3–4 million individuals are estimated to become newly infected each year.[1] Current HCV therapy is comprised of combinations of pegylated interferon (peg-IFN) and the nucleoside analog ribavirin (RBV). Unfortunately, response rates to this therapy are sub-optimal and are particularly low in patients infected with genotype 1 HCV.[2] In addition, treatment with peg-IFN/RBV is often associated with significant side effects including flu-like symptoms, depression, and anemia.[2,3] The relatively low response rates and the significant side effect burden of current HCV therapies necessitate the identification of more effective anti-HCV agents, especially for treatment of patients infected with genotype 1 HCV.

The HCV RNA-dependent RNA polymerase (RdRp, NS5B) is an attractive target for the development of novel HCV treatments.[4] The activity of this virally encoded enzyme is essential for HCV replication[5] and many nucleoside and non-nucleoside NS5B inhibitors have been described in the literature.[6,7] Encouragingly, several such molecules have also exhibited antiviral activity in HCV infected patients.[8] Most of the non-nucleoside NS5B inhibitors bind to one of three locations on the enzyme's surface that are distinct from the active site.[7] Among these, the Anadys NS5B inhibitor development efforts have focused on compounds which bind to the 'palm' site of the NS5B protein.

We previously disclosed the identification of a novel series of non-nucleoside NS5B inhibitors containing a 1,1-dioxo-1,4-dihydro-1λ$^6$-benzo[1,2,4]thiadiazine moiety linked to a substituted 5-hydroxy-3(2*H*)-pyridazinone (exemplified by structure **1**, Fig. 1).[9–11] Many such compounds exhibit potent NS5B inhibition activities and often display sub-micromolar antiviral properties when tested against an HCV replicon in cell culture. Unfortunately, these molecules also frequently exhibit poor pharmacokinetic properties following oral administration to animals.[9c,9d] In an effort to improve these undesirable pharmacokinetic characteristics, we sought to diversify the 5-hydroxy-3(2*H*)-pyridazinone pharmacophore by the inclusion of a 6-amino-substituent into the inhibitor design (structure **2**, Fig. 1).[12] Below, we describe the preparation of such compounds along with an initial assessment of their biological properties and structure–activity relationships.

The desired 6-aminopyridazinones (**2**) were prepared using two related synthetic methods (Scheme 1).[13] Each of these methods involved derivatization of an appropriately substituted 6-amino-5-hydroxy-3(2*H*)-pyridazinone intermediate bearing an ethyl ester

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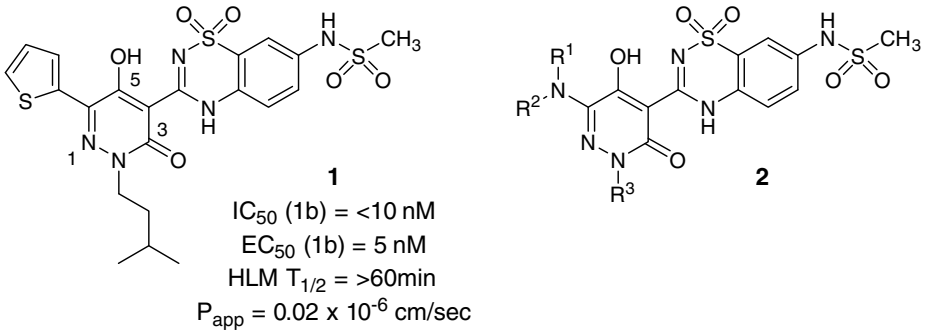
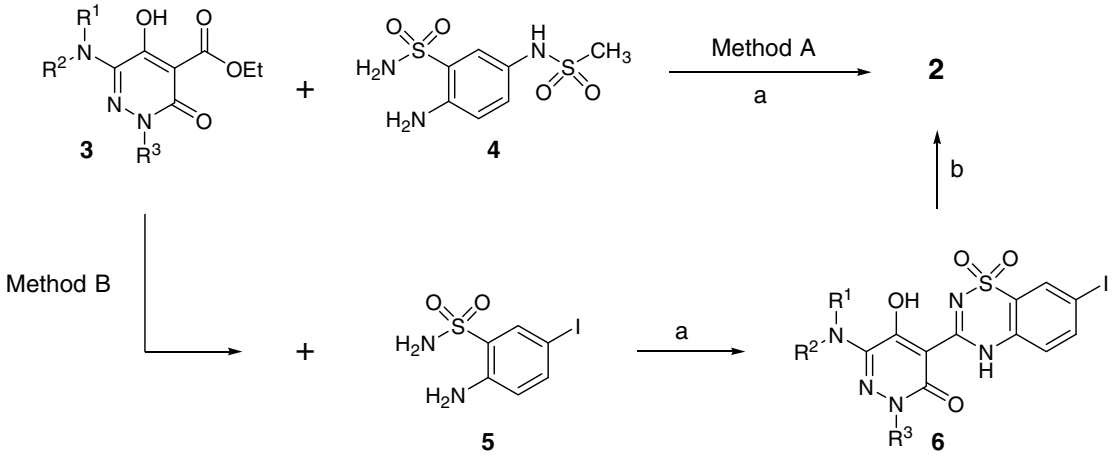



**Figure 1.** 5-Hydroxy-3(2*H*)-pyridazinone derivatives as HCV NS5B polymerase inhibitors.




**Scheme 1.** General methods for the synthesis of aminopyridazinone inhibitors (**2**). (a) Pyridine, 110 °C, 2–8 h; then add 2 equiv DBU, 110 °C, 2–12 h, 10–30%. (b) 0.05 equiv CuI, 0.20 equiv sarcosine, 2.5 equiv $K_3PO_4$, 1.2 equiv $H_2NSO_2CH_3$, DMF, 100 °C, 20–36 h, 10–20% (step b).

moiety at the 4-position (structure **3**).[14] The most straightforward synthesis of **2** (Method A) entailed thermal condensation of **3** with 2-amino-5-methanesulfonylaminobenzenesulfonamide (**4**)[15] in pyridine to form an amide intermediate (not shown), followed by addition of DBU and continued heating to effect benzothiadiazine formation. For reasons that we do not currently understand, this direct synthesis failed to provide significant quantities of **2** when applied to several aminopyridazinone substrates **3**. Accordingly, we developed an alternate synthesis of compounds **2** that was employed for these problematic cases.

This alternate preparation (Method B, Scheme 1) involved thermal condensation of iodo compound **5**[15c,16] in the benzothiadiazine-forming derivatization of **3** to give **6**. Copper-mediated coupling of the iodide present in **6** with methanesulfonamide then provided **2** in moderate overall yield.[17]

The structure–activity relationships associated with the aminopyridazinone-containing NS5B inhibitors under study are depicted in Table 1. Many of these molecules incorporate an isoamyl ($CH_2CH_2i$Pr) substituent at the pyridazinone 2-position ($R^3$ in structure **2**) that was known from our earlier studies to impart potent NS5B inhibition properties to pyridazinone-containing compounds.[9] For example, a molecule that contained such an $R^3$ fragment and a diethylamino moiety at the 6-position on the pyridazinone ring displayed sub-micromolar levels of both NS5B inhibition activity and antiviral potency (**2a**). Several related compounds bearing *N*-methyl-*N*-alkyl-aminopyridazinone 6-substituents were also examined, and the corresponding NS5B inhibition properties were observed to improve somewhat for those containing bulkier alkyl groups (cf. **2b** with **2c** and **2d**). An even more dramatic improvement in both anti-NS5B and antiviral activity was realized by replacing the diethylamino moiety present in the lead molecule with a pyrrolidine ring (cf. **2e** with **2a**). However, incorporating a piperidine moiety at this location did not afford similar enhancements (cf. **2f** with **2a**). These results parallel the SAR trends previously observed during our variation of alkyl and aryl pyridazinone 6-substituents in which compounds bearing 5-membered cyclic structures were more potent NS5B inhibitors than analogs containing 6-membered moieties.[9]

Interestingly, addition of a methyl group adjacent to the ring nitrogen in compounds **2e** and **2f** had opposite effects on inhibitor potency. Derivatization of the former compound in this manner resulted in a worsening of anti-NS5B and antiviral properties (cf. **2e** with **2g**), while similar modification of the latter molecule slightly improved NS5B inhibition activity (cf. **2f** with **2h**). Incorporation of a morpholine moiety in lieu of the piperidine ring in the inhibitor design provided a considerable improvement in anti-NS5B properties (cf. **2i** with **2f**). However, this alteration was detrimental to antiviral activity in cell culture, as **2i** was considerably weaker in the replicon assay than several other compounds with equivalent NS5B inhibition properties (cf. **2i** with **2e**, **2j**, and **2k**). The large $EC_{50}/IC_{50}$ ratio exhibited by **2i** is likely due to the compound's poor cell membrane permeability properties. Consistent with this hypothesis, **2i** exhibited a Caco-2 $P_{app}$ value that was significantly lower (less permeable) than all of the other 6-aminopyridazinones examined in this work (Table 1).

Several additional NS5B inhibitors were prepared which combined optimal 6-amino substituents with non-isoamyl $R^3$ moieties that were also known from our previous SAR studies to impart potent NS5B inhibition properties to the compounds that contained them.[9] For example, a molecule incorporating an $R^3$ *tert*-butyl ethyl

**Table 1**
Structure–activity relationships of aminopyridazinone NS5B inhibitors (**2**)

| Compound[a] | Synth. method | $R^1$ | $R^2$ | $R^3$ | **1a** $IC_{50}$[b] (μM) | **1b** $IC_{50}$[c] (μM) | $EC_{50}$[d] (μM) | $CC_{50}$[d] (μM) | HLM $t_{1/2}$[e] (min) | Caco-2 $P_{app}$[f] ($10^{-6}$ cm/s) |
|---|---|---|---|---|---|---|---|---|---|---|
| **2a** | A | Et | Et | $CH_2CH_2i$Pr | ND[g] | 0.43 | 2.2 | >33 | 13 | 0.30 |
| **2b** | B | Pr | Me | $CH_2CH_2i$Pr | 0.50 | 0.14 | 0.58 | > 1.0 | 8 | 0.12 |
| **2c** | B | *i*Pr | Me | $CH_2CH_2i$Pr | 0.47 | 0.038 | 0.65 | > 1.0 | 4 | 0.77 |
| **2d** | A | *t*Bu | Me | $CH_2CH_2i$Pr | ND | 0.047 | 0.50 | >10 | 4 | ND |
| **2e** | A | Pyrrolidine | | $CH_2CH_2i$Pr | <0.025 | 0.014 | 0.017 | >1.0 | 10 | 0.32 |
| **2f** | A | Piperidine | | $CH_2CH_2i$Pr | ND | 0.35 | 1.0 | >33 | 21 | ND |
| **2g**[h] | A | 2-Me-Pyrrolidine | | $CH_2CH_2i$Pr | ND | 0.047 | 0.10 | >10 | 9 | ND |
| **2h**[h] | A | 2-Me-Piperidine | | $CH_2CH_2i$Pr | ND | 0.17 | 1.1 | >10 | 5 | ND |
| **2i** | B | Morpholine | | $CH_2CH_2i$Pr | 0.073 | 0.015 | 0.62 | > 33 | >60 (69) | 0.021 |
| **2j** | A | Pyrrolidine | | $CH_2CH_2t$Bu | 0.086 | 0.029 | 0.011 | > 10 | 14 | 0.37 |
| **2k** | A | Pyrrolidine | | $CH_2CH_2$CyPr | 0.038 | 0.035 | 0.19 | >1.0 | 9 | 0.22 |
| **2l** | A | *t*Bu | Me | $CH_2CH_2t$Bu | ND | 0.20 | 0.20 | >33 | 6 | ND |
| **2m** | A | Pyrrolidine | | Ph | ND | 0.11 | >10 | >10 | >60 (90) | ND |

[a] See Ref. 13.
[b] Genotype 1a NS5B polymerase. See Ref. 9b for assay method and experimental error.
[c] Genotype 1b NS5B polymerase. See Ref. 9a for assay method and experimental error.
[d] Genotype 1b HCV replicon activity and associated cytotoxicity. See Ref. 9a for assay method and experimental error. $CC_{50}$ limit determined at the highest concentration of the compound tested in the assay.
[e] Half-life in presence of human liver microsomes. For values ⩾60 min, % remaining at 60 min is given in parentheses. All compounds were tested at 1 μM. See Ref. 9a for assay method and experimental error.
[f] See Ref. 9c for assay method and experimental error. Controls: $P_{app}$ Atenolol (low) = 0.3 (cm/sec) × $10^{-6}$, $P_{app}$ Propranolol (high) = 10 (cm/s) × $10^{-6}$.
[g] ND, not determined.
[h] Racemic.

($CH_2CH_2t$Bu) fragment and a pyrrolidine substituent at the 2-position of the pyridazinone ring displayed excellent NS5B inhibition activity and potent antiviral properties in cell culture (**2j**, Table 1). Similarly potent anti-NS5B characteristics were noted for a compound which contained an $R^3$ cyclopropyl ethyl moiety ($CH_2CH_2$CyPr) in lieu of the *tert*-butyl ethyl fragment (**2k**). However, the antiviral activity exhibited by the latter molecule was significantly worse than that displayed by the former inhibitor. This difference may be due to differing cell membrane permeability properties of the compounds in question, although the corresponding Caco-2 $P_{app}$ values were similar for both molecules. Somewhat surprisingly, combination of the *tert*-butyl ethyl $R^3$ fragment with a *N*-methyl-*N*-*tert*-butyl-aminopyridazinone 6-substituent resulted in somewhat poorer NS5B inhibition activity relative to the corresponding $R^3$ isoamyl analog (cf. **2l** with **2d**). However, the two compounds displayed similar antiviral properties in the cell-based assay. While the precise reasons for these activity trends are not currently understood, these results (along with those observed above for compounds **2g** and **2h**) suggest that NS5B recognition by this class of inhibitors is highly sensitive to subtle structural variations within both the $R^3$ moiety and the 6-aminopyridazinone substituent. Accordingly, a molecule incorporating a phenyl $R^3$ fragment (**2m**) displayed reduced NS5B inhibition activity and drastically attenuated antiviral properties relative to the most potent compounds described in this work.

The stability of the aminopyridazinone-containing compounds toward human liver microsomes (HLM) was also assessed to better predict their potential for metabolic transformation in vivo. As shown in Table 1, the majority of the molecules tested displayed relatively short HLM half-lives (<25 min) that were considerably attenuated relative to the long half-life exhibited by compound **1** (Fig. 1). These results suggest that incorporation of 6-amino-substituents into the pyridazinone-containing NS5B inhibitor series introduces at least one metabolically labile site into the inhibitor design. One exception to this trend was compound **2i** which displayed a long half-life in the HLM assay. As described above, however, this molecule also exhibited reduced Caco-2 permeability and antiviral properties relative to the other aminopyridazinone-containing inhibitors. The other exception (compound **2m**) displayed even poorer activity in the replicon cell-based assay. These observations highlight the difficulty of improving the HLM stability of this inhibitor series while simultaneously retaining good potency, permeability, and/or absorption characteristics.

The X-ray crystal structure of compound **2e** bound to the NS5B protein is shown in Figure 2.[18] A schematic diagram depicting enzyme residues near the inhibitor as well as protein–ligand hydrogen bonding interactions is also provided (Fig. 3). As was observed in previous pyridazinone-NS5B crystal structures,[9a–c] the $R^3$ isoamyl fragment of **2e** 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 pyrrolidine ring present in **2e** bound to a much more shallow cleft near the surface of the enzyme that was made up of residues Gly-410, Met-414, and Gln-446. The 5-hydroxy group on the pyridazinone ring formed hydrogen bonds with the backbone amide NH of Tyr-448 and a structural water molecule that interacted with the backbone NH of Gly-449. In addition, one of the oxygen atoms present in the benzothiadiazine ring system of **2e** and one of the methylsulfonamide oxygen atoms each formed a hydrogen bond with a second structural water molecule (itself hydrogen-bonded to the side

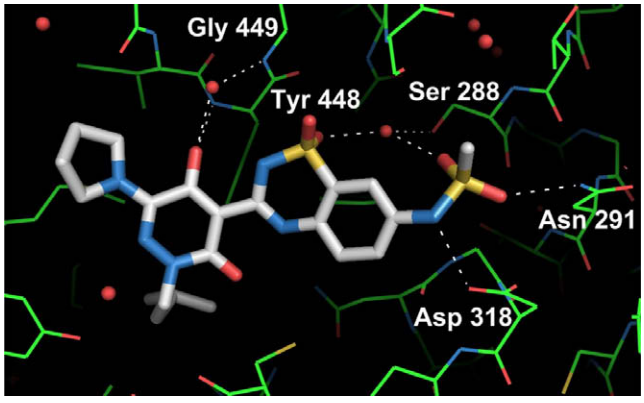



**Figure 2.** Co-crystal X-ray structure of compound **2e** bound to the NS5B protein (1.9 Å).[18]

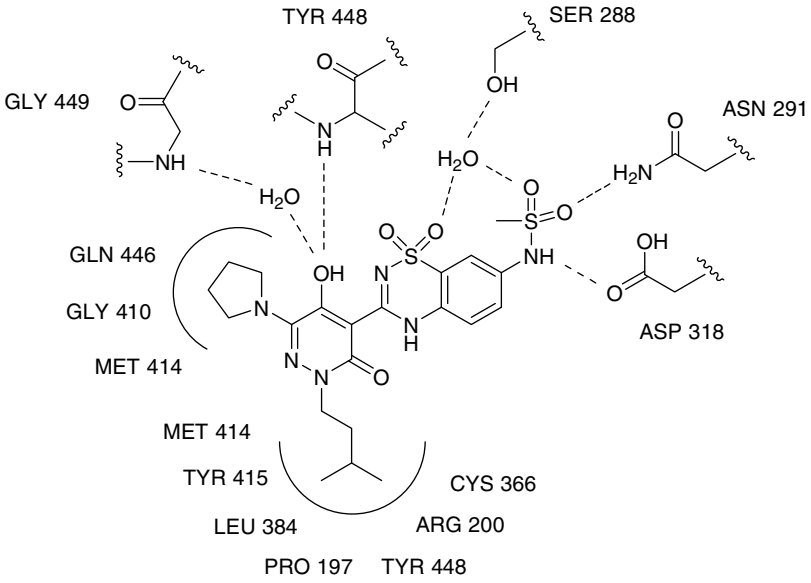



**Figure 3.** Schematic diagram of compound **2e** bound in the NS5B palm site. Hydrogen bonds are represented as dashed lines, and the residues which make up the enzyme-binding subsites are depicted.[19]

chain of Ser-288). Hydrogen bonds were also noted between the other methylsulfonamide oxygen atom and the side chain of Asn-291 as well as between the methylsulfonamide NH moiety and the side chain of Asp-318. Similar hydrogen bonding interactions were observed in co-crystal structures of other benzothiadiazine-containing NS5B inhibitors with NS5B,[9a–c] and these likely contribute to the potent polymerase inhibition properties exhibited by many of the compounds described in this work.

In summary, we synthesized a new class of 4-(1′,1′-dioxo-1′,4′-dihydro-1′$\lambda^6$-benzo[1′,2′,4′]thiadiazin-3′-yl)-5-hydroxy-2*H*-pyridazin-3-ones bearing 6-amino substituents as potent inhibitors of the HCV RNA-dependent RNA polymerase (NS5B). Many of these agents also display antiviral activity in cell culture experiments. However, in vitro DMPK data suggest that it may be difficult to combine potent antiviral activity, good metabolic stability, and favorable permeability/absorption characteristics in this class of NS5B inhibitors. Further optimization of the benzothiadiazine-containing compounds will be described in future communications.

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## References and notes

1. (a) Tellinghuisen, T. L.; Evans, M. J.; von Hahn, T.; You, S.; Rice, C. M. *J. Virol.* **2007**, *81*, 8853; (b) Moradpour, D.; Penin, F.; Rice, C. M. *Nat. Rev. Microbiol.* **2007**, *5*, 453; (c) Major, M. E., Rehermann, B., Feinstone, S. M., Hepatitis C Viruses. In Fields Virology, 4th ed.; Knipe, D. M.; Howley, P. M.; Griffin, D. E.; Lamb, R. A.; Martin, M. A.; Roizmann, B.; Straus, S. E. Eds.; vol. 1, Lippincott, Williams & Wilkins: Philadelphia, 2001; Vol. 1, p 1127.
2. Hoofnagle, J. H.; Seeff, L. B. N. *Engl. J. Med.* **2007**, *355*, 2444.
3. Fried, M. W. *Hepatology* **2002**, *36*, S237.
4. For reviews of the biological properties and functions of the NS5B enzyme, see: (a) Frick, D. N. *Curr. Org. Chem.* **2004**, *8*, 223; (b) Wu, J. Z.; Hong, Z. *Curr. Drug. Targets Infect. Disord.* **2003**, *3*, 207; (c) Hagedorn, C. H.; van Beers, E. H.; De Staercke, C. *Curr. Top. Microbiol. Immunol.* **2000**, *242*, 225.
5. Kolykhalov, A. A.; Agapov, E. V.; Blight, K. J.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *Science* **1997**, *277*, 570.
6. For reviews describing nucleoside NS5B inhibitors, see: (a) Koch, U.; Narjes, F. *Curr. Top. Med. Chem.* **2007**, *7*, 1302; (b) Carroll, S. S.; Olsen, D. B. *Infect. Disord. Drug Targets* **2006**, *6*, 17.
7. For reviews describing non-nucleoside NS5B inhibitors, see: (a) Beaulieu, P. L. *Curr. Opin. Invest. New Drugs* **2007**, *8*, 614; (b) Ref. 6a.; (c) Koch, U.; Narjes, F. *Infect. Disord. Drug Targets* **2006**, *6*, 31.
8. (a) Sorbera, L. A.; Castaner, J.; Leeson, P. A. *Drugs Future* **2006**, *31*, 320; (b) Villano, S.; Chandra, P.; Raible, D., et al., Digestive Disease Week 2006, Los Angeles, CA, May 20–25, 2006; (c) Schmeltz A.; Dunne, M.; 47th ICAAC, September 17–20, 2007; (d) Pharmasset press release, January 7, 2008.
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. M.; Tsan, M.; Showalter, R. E.; Patel, R.; Li, B.; Zhao, Q.; Han, Q.; Hermann, T.; Kissinger, C. R.; 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. M.; Tsan, M.; Averill, A.; Showalter, R. E.; Patel, R.; Han, Q.; Zhao, Q.; Hermann, T.; Kissinger, C. R.; 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**, 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**, 3421.
10. The structures of all compounds described in this work are arbitrarily drawn in a single tautomeric form.
11. For other examples of benzothiadiazine-containing NS5B inhibitors, see: (a) Hutchinson, D. K.; Rosenberg, T.; Klein, L. L.; Bosse, T. D.; Larson, D. P.; He, W.; Jiang, W. W.; Kati, W. M.; Kohlbrenner, W. E.; Liu, Y.; Masse, S. V.; Middleton, T.; Molla, A.; Montgomery, D. A.; Beno, D. W. A.; Stewart, K. D.; Stoll, V. S.; Kempf, D. *J. Bioorg. Med. Chem. Lett.* **2008**, *18*, 3887; (b) 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; (c) Krueger, A. C.; Madigan, D. L.; Green, B. E.; Hutchinson, D. K.; Jiang, W. W.; Kati, W. M.; Liu, Y.; Maring, C. J.; Masse, S. V.; McDaniel, K. F.; Middleton, T. R.; Mo, H.; Molla, A.; Montgomery, D. A.; Ng, T. I.; Kempf, D. *J. Bioorg. Med. Chem. Lett.* **2007**, *17*, 2289; (d) Tedesco, R.; Shaw, A. N.; Bambal, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Dhanak, D.; Fitch, D. M.; Gates, A.; Gerhardt, W. G.; Halegoua, D. L.; Han, C.; Hofmann, G. A.; Johnston, V. K.; Kaura, A. C.; Liu, N.; Keenan, R. M.; Goerke, J. L.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N.; Duffy, K. J. *J. Med. Chem.* **2006**, *49*, 971; (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.
12. The research described in this work was conducted prior to our obtaining data suggesting that pyridazinone PK improvements could be achieved through the reduction of compound polar surface area. See Refs. 9c and 9d for additional details regarding this analysis.
13. The structures of all new compounds described in this work were consistent with $^1$H NMR and LC–MS analysis data ($\geqslant$95% HPLC purity).
14. For the synthesis of the 6-amino-5-hydroxy-3(2*H*)-pyridazinone intermediates required to prepare the compounds described in this work, see: Dragovich, P. S.; Blazel, J. K.; Dao, K.; Ellis, D. A.; Li, L.-S.; Murphy, D. E.; Ruebsam, F.; Tran, C. V.; Zhou, Y. *Synthesis* **2008**, 610.

15. (a) Dragovich, P. S.; Murphy, D. E.; Tran, C. V.; Ruebsam, F. *Synth. Commun.* **2008**, *38*, 1909; (b) Ref. 11c; (c) Hutchinson, D. K., et al., United States Patent Application #: 2005/0107364; (d) Goldfarb, A. R.; Berk, B. *J. Am. Chem. Soc.* **1943**, *65*, 738.
16. Sun, Z.; Li, L.-S.; Webber S. E.; Ruebsam, F.; Tran, M.T.; Dragovich P. S.; Kucera, D.J.; Murphy, D.E.; Tran CV WO2007150001, 2007.
17. Deng, W.; Liu, L.; Zhang, C.; Liu, M.; Guo, Q.-X. *Tetrahedron Lett.* **2005**, *46*, 7295.
18. 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 containing two protein molecules in the asymmetric unit. Protein–inhibitor complexes were prepared by soaking these NS5B crystals for 3–24 h in solutions containing 15–20% DMSO, 20% glycerol, 20% PEG 4 K, 0.1 M HEPES, 10 mM $MgCl_2$ at pH 7.6 and inhibitors at concentrations of 2–10 mM. Diffraction data were collected to a resolution of 1.9 Å for compound **2e**. This crystal structure has been deposited in the Protein Databank (www.rcsb.org) with entry code: 3E51. Full details of the structure determination are given in the PDB entry.
19. The O atom at the 5-position of the pyridazinone in Figure 3 was arbitrarily drawn in the protonated form. The precise extent of the protonation was not determined.