



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

HCV NS5B polymerase inhibitors 1: Synthesis and in vitro activity of 2-(1,1-dioxo-2H-[1,2,4]benzothiadiazin-3-yl)-1-hydroxynaphthalene derivatives

Guangyi Wang^{a,*}, Yanzen He^b, Jun Sun^b, Debasis Das^b, Mougang Hu^a, Jianhua Huang^a, Donald Ruhrmund^a, Lisa Hooi^a, Shawn Misialek^a, P. T. Ravi Rajagopalan^a, Antitsa Stoycheva^a, Brad O. Buckman^a, Karl Kossen^a, Scott D. Seiwert^a, Leonid Beigelman^{a,*}

^a Discovery Research, InterMune, Inc., Brisbane, CA 94005, USA

^b Chemistry Department, WuXi AppTec Co., Ltd, Shanghai 200131, PR China

ARTICLE INFO

Article history:

Received 6 March 2009

Revised 5 May 2009

Accepted 6 May 2009

Available online 23 May 2009

Keywords:

Benzothiadiazine

HCV NS5B polymerase inhibitors

ABSTRACT

2-(1,1-Dioxo-2H-[1,2,4]benzothiadiazin-3-yl)-1-hydroxynaphthalene derivatives as potential anti-HCV drugs targeting NS5B polymerase have been investigated. Their synthesis, HCV NS5B polymerase inhibition and replicon activity are discussed.

© 2009 Elsevier Ltd. All rights reserved.

WHO estimates that about 3% of the world's population has been infected with HCV and that some 170 million are chronic carriers at risk of developing liver cirrhosis and/or liver cancer.¹ Hepatitis C is recognized as a major cause of end-stage liver disease as well as the leading cause of liver transplantations in the developed world.² Thus far, there is no universally effective therapy for all HCV genotypes. The current treatment for patients infected with genotype 1 HCV is 48 weeks of pegylated interferon- α (Peg-IFN- α) and ribavirin (RBV), and the success rate for achieving a sustained viral response for genotype 1 patients in the US, Europe and Japan is ~40%.³ The long duration of treatment is difficult for patients to tolerate due to side effects associated with Peg-IFN- α and RBV that include flu-like symptoms, fatigue, depression, gastrointestinal symptoms, pulmonary effects and others.³ These limitations have led to intense interest in the discovery and development of novel compounds that target the viral and host proteins.

HCV NS5B polymerase is an RNA dependent RNA polymerase that resides at the C-terminal domain of a polypeptide of several structural and nonstructural proteins and contains the catalytic machinery responsible for synthesis and replication of the viral RNA.⁴ NS5B is essential for viral replication and has been clinically validated.⁶ Along with HCV protease NS3/4A, NS5B is recognized as the most viable protein target for HCV drug discovery.^{5–7} Two classes of NS5B inhibitors have been well developed: active site inhib-

itors such as nucleoside or nucleotide inhibitors that mimic natural polymerase substrates and allosteric inhibitors that bind to less conserved sites outside the active site and impair the enzyme's catalytic efficiency.

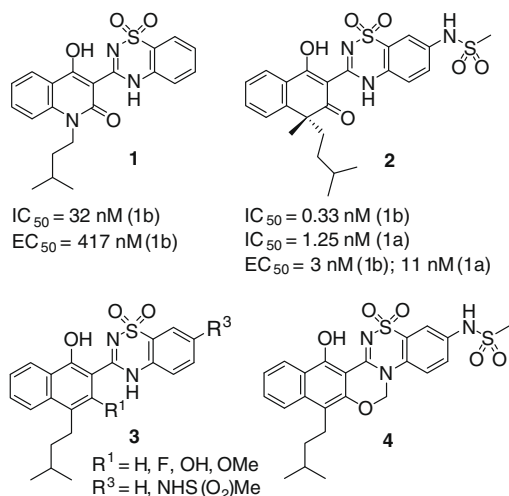
Recently, several classes of non-nucleoside allosteric NS5B inhibitors^{5–7} have been reported, some of which achieved low nanomolar inhibition in both enzyme and replicon assays. Among the most potent inhibitors are 1,1-dioxo-2H-benzothiadiazine compounds, of which compound **1** shown below was one of the first benzothiadiazine compounds found active against HCV NS5B polymerase.⁸ Compound **1** binds in a site that is located in the palm domain of the polymerase in the proximity of the active site (see Ref. 8 for a crystal structure of a benzothiadiazine compound bound to NS5B). Recent work from another institution identified the low nanomolar benzothiadiazine inhibitor **2**,^{9,10} which exhibited a good DMPK profile achieving high plasma and liver concentration and showed anti-HCV activity in chimpanzee model.¹¹

In search of potent allosteric inhibitors of HCV polymerase NS5B at InterMune, we explored several series of benzothiadiazine compounds. In this report we describe synthesis and in vitro anti-HCV activities of compounds **3**, **4** and their analogs.

Target compound **3a** was synthesized as shown in Scheme 1. Compound **5** was alkylated with isopentyl iodide, followed by alkaline hydrolysis, to give the acid **6**. Treatment of **6** with thionyl chloride and subsequent reaction of the resulting acyl chloride with diethyl malonate yielded **7**. Cyclization of **7** in methanesulfonic acid afforded the naphthalene derivative **8** in good yield. Methylation of **8** with TMS-diazomethane, followed by hydrolysis, afforded

* Corresponding authors.

E-mail addresses: gyiwang@yahoo.com, gwang@intermune.com (G. Wang), beigelman@intermune.com (L. Beigelman).

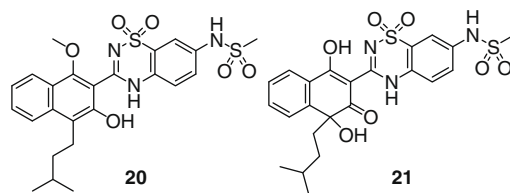


the acid **10**. Attempts to use **8** as starting material for condensation with 2-aminobenzenesulfonamide under various conditions yielded a complicated mixture, probably due to the instability of the hydroxyl groups at high temperature. Condensation of **9** with 2-aminobenzenesulfonamide did not proceed under various conditions, presumably owing to the unreactive nature of the carboxylic ester. It appeared that a more reactive derivative of **9** was required for a successful condensation. After treatment of **10** with thionyl chloride, the resulting acyl chloride was condensed with 2-aminobenzenesulfonamide in the presence of DMAP and triethylamine. Interestingly, the acyl chloride resulting from **10** did not react with 2-amino group of 2-aminobenzenesulfonamide, instead, the acyl chloride was coupled with the sulfonamide amino group to give **11**. Treatment of **11** in aqueous potassium hydroxide at up to 140 °C failed to produce the desired benzothiadiazine **12**. Finally, **12** was obtained by heating **11** neat at 200 °C. Demethylation of **12** with boron tribromide afforded the desired benzothiadiazine **3a**.

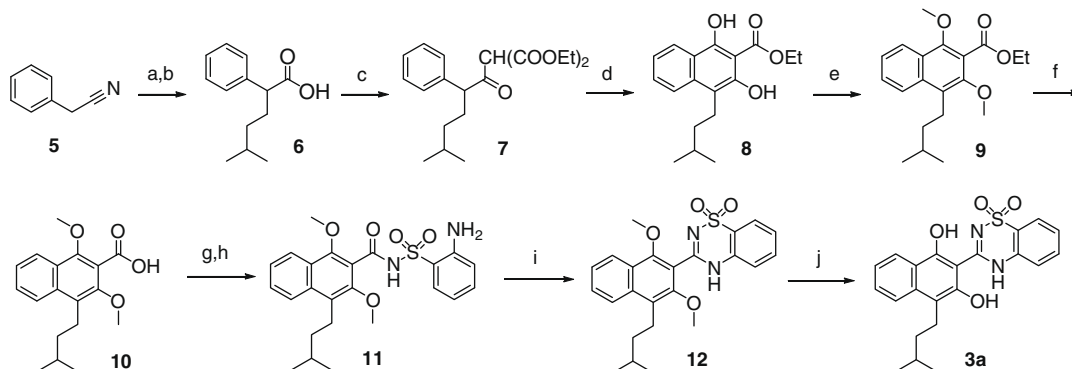
The naphthalene-2-carboxylic acids **14** and **16** as the intermediates for **3b–e** were synthesized according to Scheme 2. Compound **8** was selectively methylated and subsequently triflated. The methylation site was determined by NOESY NMR experiment of the monomethoxy product, where the methoxy protons had a correlation with a naphthalene proton (H-8). The resulting triflate **13** was subjected to reduction and hydrolysis to give the acid **14**. Compound **13** was also converted to the amino derivative **15** via the

amination and subsequent hydrogenolysis. Sandmeyer-type reaction of the amine **15**, followed by hydrolysis, afforded the acid **16**.

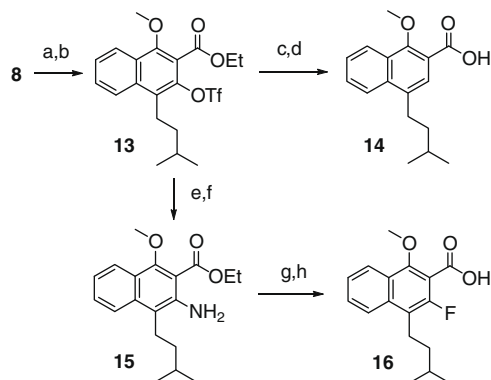
The desired benzothiadiazine compounds **3b–g** were prepared by the general procedure as shown in Scheme 3. Condensation of the acids **10**, **14** and **16** with the 2-aminobenzenesulfonamides **17** and **18**⁹ in polyphosphoric acid trimethylsilyl ester (PPSE)¹² gave the benzothiadiazines **19a–e**, respectively, in 50–65% yields. While the preparation of the benzothiadiazines **3b–f** and **20** was readily achieved by demethylation of **19a–e** under mild conditions, removal of both methyl groups of **19e** for preparation of **3g** required a large excess of BBr₃ and 2–3 days at 38 °C under argon flow. Compounds **3a** and **3g** having two hydroxy groups could be oxidized in the air, particularly **3g** which was much more unstable than **3a**. In preparation of **3g**, a fast flash chromatographic purification on silica gel with EtOAc/DCM gave the desired compound, but with significant loss in yield while **3a** was obtained in good yield by flash chromatography. When a solution of **3g** in methanol and water (1:1) was in an open flask for 10 days, **3g** almost completely disappeared and a major product was formed. This product was purified and assigned by MS and NMR as the racemic **21**.¹³ Although oxidative mechanism remains unclear at this time, it is certain that the oxidant source is air. Compounds **3f** and **20** as a mixture prepared from **19e** were readily separated by flash chromatography on silica gel. The regiochemical assignment of **3f** and **20** was determined by NOESY NMR: the methoxy protons in **20** correlated with a naphthalene proton (H-8) and had no correlation with the alpha-methylene while the methoxy protons in **3f** had a correlation with alpha-methylene protons and no correlation with a naphthalene proton (H-8). In contrast to **3g**, compounds **3f** and **20** having only one hydroxy group were stable and remained intact at room temperature after several months.



To find out whether a constrained conformation of the naphthalene-benzothiadiazine compounds would enhance NS5B binding, compound **4** was designed as a rigid analog of **3**. Synthesis of **4** and its analogs is shown in Scheme 4. A mixture of **3f** and **20** was treated with benzoyl chloride to protect both hydroxy and methylsulfonamide functions. After separation by chromatogra-



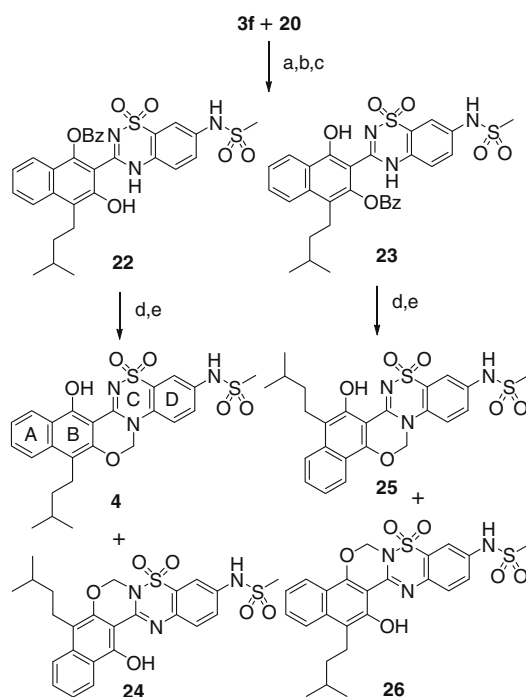
Scheme 1. Reagents and conditions: (a) *i*-pent-1, NaH, 0 °C, 30 min, 69%; (b) NaOH, H₂O, EtOCH₂CH₂OH, reflux, overnight, 93%; (c) (i) SOCl₂, ClCH₂CH₂Cl, reflux, 2 h; (ii) diethyl malonate, MgCl₂, TEA, CH₃CN, rt, overnight; (d) CH₃SO₃H, 30 °C, overnight, 67% (three steps); (e) TMS-diazomethane, DIPEA, THF, MeOH, 30 °C, overnight, 93%; (f) NaOH, H₂O, 1,4-dioxane, reflux, 2 days; (g) SOCl₂, ClCH₂CH₂Cl, 60 °C, 5 h; (h) 2-aminobenzenesulfonamide, DMAP, TEA, DMF, 45 °C, 2 h, 37% (three steps); (i) neat, 200 °C, 1.5 h, 40%; (j) BBr₃, ClCH₂CH₂Cl, 45 °C, 24 h, 73%.



Scheme 2. Reagents and conditions: (a) TMS–diazomethane, MeOH, rt, 4 h, 35%; (b) TiF_2O , pyridine, rt, 3 h, 46%; (c) $\text{Pd}(\text{Ac})_2$, PPh_3 , TEA, HCOOH , DMF, 65 °C, 24 h, 93%; (d) NaOH, EtOH, H_2O , rt; (e) benzylamine, $\text{Pd}(\text{Ac})_2$, BINAP, Cs_2CO_3 , toluene, 80 °C, 20 h, 78%; (f) Pd/C , EtOH, rt, 30 h, 87%; (g) BF_3 -etherate, $t\text{-BuONO}$, 0 °C, 2 h, 18%; (h) NaOH, EtOH, H_2O , quant.

phy, the two benzoyl-protected products were subjected to demethylation with BBr_3 and subsequent selective removal of benzoyl group from the sulfonamide both under mild conditions to give **22** and **23**, respectively. Cyclization of **22** and **23** and subsequent removal of benzoyl group afforded two pairs (**4** and **24**, **25** and **26**) of compounds, respectively. The structural assignments for each pair of compounds are determined by NOESY NMR. For the pair of **4** and **24**, the isomer in which the methylene protons correlate with H5 of the D ring in NOESY NMR was assigned as **4** and the other as **24**. Similarly, for the pair of **25** and **26**, the isomer in which there was such a correlation in NOESY NMR was assigned as **25** and the other as **26**.

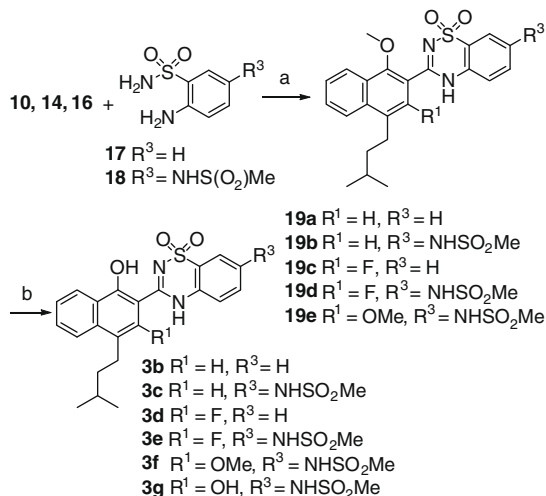
Benzothiadiazine compounds **3a–g**, **4**, **19a–b**, **19e**, **20**, **21** and **24–26** were tested in HCV NS5B genotype 1b polymerase and replicon assays.^{14,15} As can be seen from Table 1, compounds **3c**, **3e** and **19b** with methylsulfonamide moiety (IC_{50} : 0.15, 0.031 and 0.048 μM) were significantly more potent than **3b**, **3d** and **19a** without the moiety (IC_{50} : 8.4, 0.11, and 27 μM) in the NS5B enzyme inhibition assay. In the cellular assay the compounds **3c** and **3e** with the methylsulfonamide moiety (EC_{50} : 4.3 and 2.7 μM , both CC_{50} : >100 μM) exhibited not only higher potency, but also higher selectivity window over **3b** and **3d** (EC_{50} : 26 and



Scheme 4. Reagents and conditions: (a) BzCl , DAMP, pyridine, rt, overnight, 82%; (b) BBr_3 , DCM, –10 °C, 1 h, 29–48%; (c) NH_3 , MeOH, H_2O , rt, 1 h, 83–96%; (d) CH_2Br_2 , Cs_2CO_3 , DMF, 145 °C, overnight, 21–29%; (e) NH_3 , MeOH, H_2O , rt, 39–71%.

19 μM ; CC_{50} : 37 and 12 μM). The enhanced potency and decreased cytotoxicity achieved from the methylsulfonamide moiety at R^3 position were likely due to both enhanced binding and selectivity to NS5B. The replacement of the C1-hydroxy group on the naphthalene ring of **3** by methoxy group seems tolerated, as demonstrated by **19b**, **20** and **19e** (IC_{50} : 0.048, 0.005 and 0.63 μM) despite previous studies showed that the corresponding hydroxy functionality of quinolinone–benzothiadiazine scaffold was essential for the binding to the enzyme.⁸ Fluoro and methoxy group at R^1 position led to more potent inhibition than proton at R^1 position as revealed by IC_{50} values for **3f**, **3e** and **3c** (0.037 and 0.031 μM vs 0.15 μM). The introduction of the constrain within the scaffold proved to be detrimental, as shown by the weak activities of **4** and **24–26**.

In the initial in vitro evaluation, compounds **3a** and **3g** showed promising activity in both NS5B and replicon assays, with IC_{50}



Scheme 3. Reagents and conditions: (a) PPSE, 160 °C, 1–3 h; (b) 2 equiv BBr_3 , $\text{ClCH}_2\text{CH}_2\text{Cl}$, argon, –10 °C, 1 h for **3b–f**; 10 equiv BBr_3 , 38 °C, 2–3 days for **3g**.

Table 1

Inhibition of HCV NS5B polymerase genotype 1b and replicon by benzothiadiazine compounds

Compound	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
3b	8.4	26	37
3c	0.15	4.3	>100
3d	0.11	19	12
3e	0.031	2.7	>100
3f	0.037	2.9	120
19a	27	9.5	13
19b	0.048	21	51
19e	0.63	>100	40
20	0.005	1.1	110
21	<0.005	0.019	>100
4	8.7	>100	>100
24	0.49	8.4	33
25	0.53	34	>100
26	1.3	12	35

values of 44 nM (**3a**) and less than 5 nM (**3g**) and EC₅₀ values of 6.1 μM (**3a**) and 22 nM (**3g**). However, it was found later that **3a** and **3g** were not stable under the assay conditions although **3a** was much more stable than **3g**. A stability study demonstrated that **3g** in phosphate buffer (pH 7.4) was partially converted to hydroxylated derivative **21**. Therefore, the inhibitory effects observed for **3g** could result from both **3g** and **21**. Unlike **3a** and **3g**, compound **21** and other compounds in Table 1 showed good stability. Compound **21** also exhibited excellent activities in both NS5B and replicon assay, with IC₅₀ < 5 nM and EC₅₀ = 19 nM. Although **21** has a structural similarity to **2**, the hydroxyl group may provide additional advantages concerning chemical and biological properties.

In summary, a series of substituted naphthalene-benzothiadiazine compounds have been synthesized. Several compounds showed potent NS5B inhibition while compound **21** exhibited both potent NS5B and replicon activity. The discovery of **21** as a potent NS5B inhibitor provides a lead for further optimization.

References and notes

- WHO, Hepatitis C. Incidence/Epidemiology under Section Surveillance and Control. 2002. <http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html>.
- Shepard, C. W.; Finelli, L.; Alter, M. J. *Lancet Infect. Dis.* **2005**, *5*, 558.
- Manns, M.; Wedemeyer, H.; Cornberg, M. *Gut* **2006**, *55*, 1350.
- Lindenbach, B. D.; Rice, C. M. *Nature* **2005**, *436*, 933.
- Beaulieu, P. L. *Curr. Opin. Invest. Drugs* **2007**, *8*, 614.
- Rönn, R.; Sandström, A. *Curr. Top. Med. Chem.* **2008**, *8*, 533.
- Tramontano, E. *Mini-Rev. Med. Chem.* **2008**, *8*, 1298.
- 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.; Lin-Goerke, J.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N.; Duffy, K. J. *J. Med. Chem.* **2006**, *49*, 971.
- 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.
- Krueger, A. C.; Madigan, D. L.; Jiang, W. W.; Kati, W. M.; Liu, Y.; Maring, C. J.; Masse, S.; McDaniel, K. F.; Middleton, T.; Mo, H.; Molla, A.; Montgomery, D.; Pratt, J. K.; Rockway, T. W.; Zhang, R.; Kempf, D. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3367.
- Chen, C.-M.; He, Y.; Lu, L.; Lim, H. B.; Tripathi, R. L.; Middleton, T.; Hernandez, L. E.; Beno, D. W. A.; Long, M. A.; Kati, W. M.; Bosse, T. D.; Larson, D. P.; Wagner, R.; Lanford, R. E.; Kohlbrenner, W. E.; Kempf, D. J.; Pilot-Matias, T. J.; Molla, A. *Antimicrob. Agents Chemother.* **2007**, *51*, 4290.
- Imai, Y.; Mochizuki, A.; Kakimoto, M. *Synthesis* **1983**, *10*, 851.
- Thanks to this manuscript reviewer's comments, the chemical synthesis of compound **21** via a different route was found in a patent application (WO2006093801, e.g., 2F), but without activity data.
- NS5B assay: A modified assay based on a published method (McKercher, G.; Beaulieu, P. L.; Lamarre, D.; LaPlante, S.; Lefebvre, S.; Pellerin, C.; Thauvette, L.; Kukolj, G. *Nucleic Acids Res.* **2004**, *32*, 422) was used. Assays were performed at room temperature in 96-well (white, round bottom) plates in 20 mM Tris, pH 7.5 buffer containing 5 mM MgCl₂, 5 mM KCl, 1 mM EDTA, 2 mM DTT, 0.01% BSA. Appropriate serial dilutions of inhibitors in DMSO were prepared and added to 5 nM NS5bΔ21 (genotype 1b, J4 strain) enzyme in above buffer. After 5 min of incubation, reactions were initiated by the addition of a buffered substrate mix containing 250 nM 5'-biotinylated-rU₁₂ RNA primer, 1 μg/mL poly-rA RNA template, 1 μM UTP and 0.625 μCi 5,6-³H-UTP. Total reaction volumes were 100 μL with 5% DMSO (v/v). The reaction was stopped after 2 h by adding 20 μL of 164 μg/mL yeast RNA and 10 mg/mL streptavidin PVT SPA beads in 0.5 M EDTA, pH 8.0. After 30 min, 80 μL of 5 M CsCl was added and incubated for 1 h. Plates were then read using a Wallac MicroBeta reader. Inhibition data was plotted and fit to a 4-parameter logistic equation to extract IC₅₀ values. Z prime values under these conditions were >0.6.
- HCV replicon assay (EC₅₀, μM): A modified assay based on a published method (Vrolijk, J. M.; Kaul, A.; Hansen, B. E.; Lohmann, V.; Haagmans, B. L.; Schalm, S. W.; Bartenschlager, R. *J. Virol. Methods* **2003**, *110*, 201) was used. Exponentially growing Huh-7 cells stably transfected with luc/neo ET replicon were maintained in DMEM media supplemented with 10% fetal calf serum and seeded at a density of 5 × 10³ cells/well in white 96-well plates. Compounds were dissolved in DMSO, diluted in DMSO in a serial fashion to create an appropriate range of concentrations, and added to cells approximately 24 h after plating. The final DMSO concentration in the cell plate was 1%. After 46–50 h exposure, the media was discarded from the assay plate and the cell monolayers were lysed by addition of 100 μL of either BrightGLO (Promega) or ATPlite reagent (PerkinElmer) with incubation at 20 °C for 2 min on an orbital shaker. Following incubation, luminescence was assessed on a SpectraMax M5 plate reader (Molecular Devices). Plots of luminescence versus log compound concentration were fit to a 4-parameter logistic equation to determine EC₅₀ and CC₅₀ values.