Benzimidazole-containing HCV NS5A inhibitors: Effect of 4-substituted pyrrolidines in balancing genotype 1a and 1b potency

James A. Henderson, Darius Bilimoria, Monica Bubenik, Caroline Cadilhac, Kevin M. Cottrell, Evelyne Dietrich, Francois Denis, Nigel Ewing, Guy Falardeau, Simon Giroux, Ronald Grey Jr., Lucille L'Heureux, Bingcan Liu, Nagraj Mani, Mark Morris, Olivier Nicolas, Oswy Z. Pereira, Carl Poisson, B. Govinda Rao, T. Jagadeeswar Reddy, Subajini Selliah, Rebecca S. Shawgo, Louis Vaillancourt, Jian Wang, Constantin G. Yannopoulos, Nathalie Chauret, Francoise Berlioz-Seux, Laval C. Chan, Sanjoy K. Das, Anne-Laure Grillot, Youssef L. Bennani, John P. Maxwell

PII: S0960-894X(14)01353-5
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.12.045
Reference: BMCL 22290

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 24 October 2014
Revised Date: 10 December 2014
Accepted Date: 12 December 2014

Please cite this article as: Henderson, J.A., Bilimoria, D., Bubenik, M., Cadilhac, C., Cottrell, K.M., Dietrich, E., Denis, F., Ewing, N., Falardeau, G., Giroux, S., Grey, R. Jr., L'Heureux, L., Liu, B., Mani, N., Morris, M., Nicolas, O., Pereira, O.Z., Poisson, C., Govinda Rao, B., Jagadeeswar Reddy, T., Selliah, S., Shawgo, R.S., Vaillancourt, L., Wang, J., Yannopoulos, C.G., Chauret, N., Berlioz-Seux, F., Chan, L.C., Das, S.K., Grillot, A-L., Bennani, Y.L., Maxwell, J.P., Benzimidazole-containing HCV NS5A inhibitors: Effect of 4-substituted pyrrolidines in balancing genotype 1a and 1b potency, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.12.045

Benzimidazole-containing HCV NS5A inhibitors: Effect of 4-substituted pyrrolidines in balancing genotype 1a and 1b potency

James A. Henderson[*,a], Darius Bilimoria[b], Monica Bubenik[b], Caroline Cadilhac[b], Kevin M. Cottrell[a], Evelyne Dietrich[b], Francois Denis[b], Nigel Ewing[a], Guy Falardeau[b], Simon Giroux[a], Ronald Grey, Jr.[a], Lucille L'Heureux[b], Bingcan Liu[b], Nagraj Mani[a], Mark Morris[a], Olivier Nicolas[b], Oswy Z. Pereira[b], Carl Poisson[b], B. Govinda Rao[a], T. Jagadeeswar Reddy[b], Subajini Selliah[b], Rebecca S. Shawgo[a], Louis Vaillancourt[b], Jian Wang[a], Constantin G. Yannopoulos[b], Nathalie Chauret[b], Francoise Berlioz-Seux[a], Laval C. Chan[b], Sanjoy K. Das[b,+], Anne-Laure Grillot[a], Youssef L. Bennani[a], and John P. Maxwell[a]

[a] *Vertex Pharmaceuticals Incorporated, 50 Northern Avenue, Boston, Massachusetts 02210, USA*
[b] *Vertex Pharmaceuticals Canada Incorporated, 275 Boul. Armand-Frappier, Laval, QC, H7V 4A7, Canada*
[+] *Present address: AngioChem, 201 President-Kennedy Ave., Suite PK-2880, Montreal, QC H2X 3Y7, Canada*

Abstract

The treatment of HCV with highly efficacious, well-tolerated, interferon-free regimens is a compelling clinical goal. Trials employing combinations of direct-acting antivirals that include NS5A inhibitors have shown significant promise in meeting this challenge. Herein, we describe our efforts to identify inhibitors of NS5A and report on the discovery of benzimidazole-containing analogs with subnanomolar potency against genotype 1a and 1b replicons. Our SAR exploration of 4-substituted pyrrolidines revealed that the subtle inclusion of a 4-methyl group could profoundly increase genotype 1a potency in multiple scaffold classes.

Letter

Hepatitis C virus (HCV) affects an estimated 130-170 million individuals worldwide and is the leading cause of liver transplant and hepatocellular carcinoma.[1] Once only treatable with poorly tolerated and moderately efficacious regimens of pegylated interferon alpha (PEG-IFN-α) and ribavirin (RBV), chronic HCV infection is now being cured with high rates in clinical trials using IFN-free combinations of direct-acting antivirals (DAAs). The search for DAAs interfering with HCV replication has included targeting of the non-structural (NS) viral proteins NS2, NS3•4A, NS4B, NS5B and NS5A.[2-4]

* Corresponding author. Tel.: +1 617 961 7605
E-mail address: james_henderson@vrtx.com (J. A. Henderson)

In clinical monotherapy studies, NS5A inhibitors have proven to produce the most rapid viral load declines of any HCV antiviral class.[5] NS5A is a large phosphoprotein (49 kDa) with no apparent enzymatic activity or homologues in prokaryotes and eukaryotes. Although the mechanistic role of NS5A in the HCV replication cycle currently is unknown, it is thought to play important roles in viral replication, virion assembly and secretion from infected cells.[6] The elucidation of NS5A's mechanistic role remains an active area of investigation.[7] Several NS5A inhibitors are currently in various stages of development,[8] including daclatasvir (BMS-790052, Figure 1), which achieved the first clinical proof of concept in this inhibitor class by displaying an impressive $3\log_{10}$ reduction of HCV RNA in 24 h with a single 10 mg oral dose (genotypes 1a and 1b).[9]

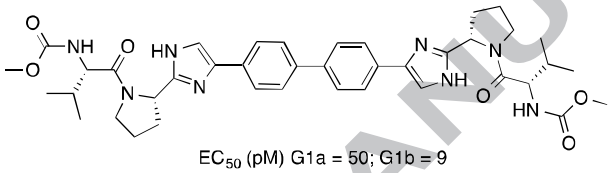


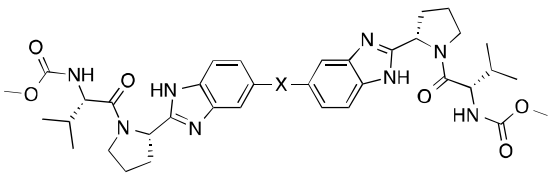
Figure 1. Structure of daclatasvir (BMS-790052)

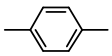
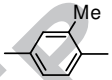
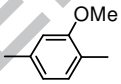
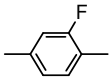
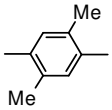
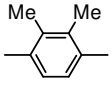
Inspired by literature leads and internal screening, our early medicinal chemistry efforts to identify NS5A inhibitors with balanced genotype 1a (G1a) and 1b (G1b) potency focused on benzimidazole-containing scaffolds (Table 1).[10,11] Directly linked *bis*-benzimidazole **1** showed low nanomolar G1b potency and no activity against G1a, whereas mono-alkyne inhibitor **2** exhibited a potency increase in both genotypes, suggesting a minimum distance requirement between the two benzimidazoles. Reduction of the alkyne linker to flexible ethyl analog **3** resulted in a 3-fold potency reduction in G1b and a 25-fold loss for G1a, suggesting the need for a rigid linker. Increasing the linker length by introduction of a *bis*-alkyne (**4**) had no affect on G1a and G1b replicon activity. Replacement of the alkynes with a more lipophilic phenyl ring **5** improved G1a potency 30-fold (EC_{50} = 550 pM). The 1,3-linked analog **6** was inactive but the 2,5-substituted thiophene analog **7** was equipotent to **5**, indicating that up to ~30^{o} deviations from linearity are tolerated in these molecules. Mono substitution appeared to be tolerated as observed with analogs **8-10** and electronic factors did not play a significant role as methyl, fluoro and

methoxy exhibited similar potencies to unsubstituted phenyl **5**, and dimethylphenyl linkers **11** and **12** also had similar potency to phenyl analog **5**.

Table 1

Activity of *bis*-benzimidazole analogs



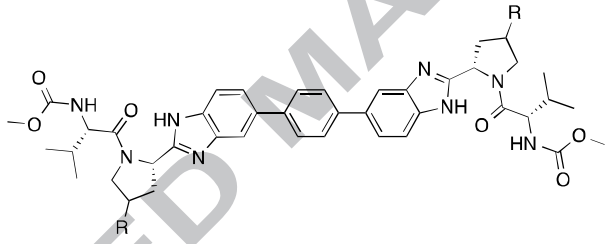
Compd	X	G1a EC_{50} (nM)	G1b EC_{50} (nM)	CC_{50} (μM)
1	None	>2000	26	>20
2		28	0.022	>20
3		688	0.076	>20
4		18	0.024	13
5		0.55	0.012	6
6		378	20	6
7		0.94	0.01	6
8		0.84	0.006	3
9		0.36	0.013	6
10		0.84	0.012	6
11		0.9	0.006	5
12		2.9	0.01	7

Our goal of identifying a NS5A inhibitor with balanced G1a and 1b potency necessitated further optimization of the benzimidazole-phenyl-benzimidazole scaffold

(e.g., compound **5**) and ultimately led to the investigation of substituted pyrrolidines. Activity profiles from our exploration of 4-substituted pyrrolidines (**13-23**) are compared to unsubstituted pyrrolidine **5** in Table 2. Modifications at the 4-position for compounds **13-23** had modest effects on G1b potency, yet a profound effect was observed with respect to G1a potency. While mono-fluorinated pyrrolidines **13** and **14** were less potent than **5**, geminal difluoride **15** was equipotent to **5** in G1a activity. A marked drop in activity for both genotypes was observed with 4*R*-hydroxyl **16**, presumably due to intolerance of polar functionality at this position given that activity returned with the corresponding methyl ether **17**. The 4*S*-methyl ether **18** demonstrated an unanticipated 10-fold increase in G1a activity and further exploration revealed 4-methyl pyrrolidines **19** and **20** were 20-fold more potent (G1a EC_{50} = 26 pM and 28 pM, respectively) than **5**, providing analogs with exceptional G1a/G1b

Table 2

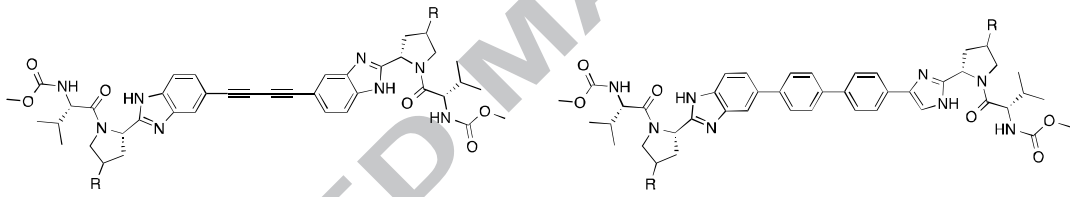
Activity of analogs containing 4-substituted pyrrolidines



Compd	R	G1a EC_{50} (nM)	G1b EC_{50} (nM)	CC_{50} (μM)
5	H	0.546	0.012	6
13	*(S)*-F	4.9	0.082	11
14	*(R)*-F	1.1	0.018	14
15	*gem*-di-F	0.5	0.004	13
16	*(R)*-OH	15	0.1	>20
17	*(R)*-OMe	1.22	0.034	6
18	*(S)*-OMe	0.053	0.031	>20
19	*(R)*-Me	0.028	0.007	14
20	*(S)*-Me	0.026	0.037	>20
21	*(S)*-CF_3	0.030	0.005	13
22	*(S)*-Et	0.085	0.019	>20
23	*gem*-di-Me	0.03	0.011	>20

potency ratios. The 4*S*-trifluoromethyl analog **21** displayed similar G1a activity to **20**, while 4*S*-ethyl **22** showed a 3-fold drop in activity. Substitution of the 4-position of the pyrrolidines with geminal dimethyl (**23)** also resulted in potent G1a and 1b activity.

Gratifyingly, the incorporation of 4-methyl substituted pyrrolidines into additional scaffolds was also effective in boosting G1a potency (Figure 2). A dramatic 1000-fold increase in G1a potency is observed when diyne-linked *bis*-benzimidazole **4** (G1a EC_{50} = 18 nM) is compared to 4*S*-methyl pyrrolidine-containing analog **24** (G1a EC_{50} = 18 pM). In order to investigate the methyl effect[12] in an analog where scaffold symmetry had been broken, we replaced one of the benzimidazoles of **5** with a phenyl-imidazole unit to provide compound **25**. Incorporation of 4*S*-methyl pyrrolidines into **25** (G1a EC_{50} = 139 pM) resulted in a 35-fold increase in G1a activity for **26** (G1a EC_{50} = 4 pM), providing a single digit picomolar inhibitor of NS5A G1a and 1b (CC_{50} = >20 μM).[13,14]



4: R = H EC_{50} G1a = 18 nm ; G1b = 0.024 nm
24: R = (*S*)-Me EC_{50} G1a = 0.018 nm ; G1b = 0.024 nm
25: R = H EC_{50} G1a = 0.139 nm ; G1b = 0.013 nm
26: R = (*S*)-Me EC_{50} G1a = 0.004 nm ; G1b = 0.005 nm

Figure 2: Activity of 4(*S*)-Me pyrrolidine-containing analogs

With excellent G1a and 1b potencies in hand, the ADME properties of **26** were investigated. Metabolic stability in both rat and human hepatocytes was high, with > 90% remaining after incubation at 5 μM for 60 minutes. The cellular permeability as measured in the Caco-2 cell line was good, and evaluation of PGP efflux potential in the human Mdr1 overexpressing cell line MDCK-Mdr1 indicated **26** was not a PGP efflux substrate (ER = 1.8). Compound **26** was moderately bound to plasma proteins in vitro (98.6% human, 91.7% rat), displayed no hERG channel inhibition (IC_{50} > 40 μM), and did not inhibit a panel of CYP enzymes (IC_{50} > 30 μM across tested isoforms). The in vivo pharmacokinetic profile of **26** in rats indicated

low clearance (Cl = 9.0 mL/min/kg) and moderate bioavailability (F = 26%) after dosing as a 3 mg/kg solution in 30% aqueous PEG-400. Key pharmacokinetic data for compound **26** are included in Table 3.

Table 3

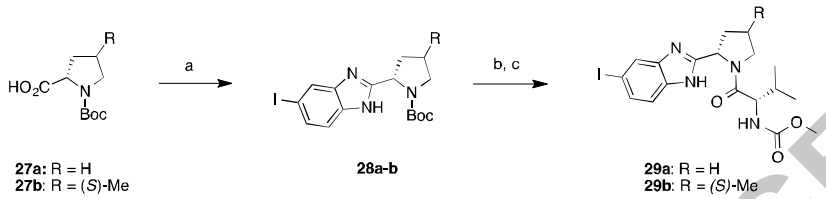
Selected in vitro and in vivo DMPK data for compound **26.**

Selected In vitro Data		**Selected In vivo Data**	
CYP Isoform Inhibition	IC_{50} (μM)	IV Rat PK	
3A4, 2C9, 2D6, 2C19, 1A2	> 30	Cl (mL/min/kg)	9.0
hERG Channel	IC_{50} (μM)	$T_{1/2}$(h)	2.0
Planar-, QPatch	> 30	V_{ss} (L/kg)	1.4
Plasma Protein Binding	% Bound		
Human	98.6	PO Rat PK	
Rat	91.7	$AUC_{0\text{-}8h}$(μg h/mL)	2.08
Permeability, Efflux		$T_{1/2}$ (h)	3.5
Caco-2, A-B (10^{-6}cm/sec)	4.1	C_{max} (ng/mL)	0.03
Mdr1, Efflux Ratio	1.8	% F	26

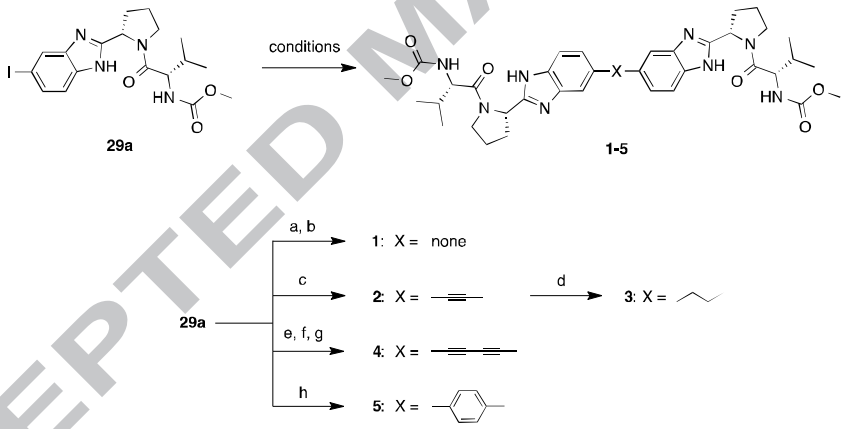
The benzimidazole-containing NS5A inhibitors described in this letter were prepared by the methods outlined in Schemes 1-3. The syntheses of iodo-benzimidazole building blocks, represented by **29a**, were accomplished in a straightforward manner by coupling of 4-iodo-benzene-1,2-diamine with *N*-Boc-L-prolines, followed by amidation of the deprotected pyrrolidine with (*S*)-2-((methoxycarbonyl)-amino)-3-methylbutanoic acid under standard conditions (Scheme 1). Symmetrical *bis*-benzimidazole analogs **1-5** were prepared from iodo-benzimidazole building block **29a** (or the corresponding pinacolboronate derivative) by a series of Sonagashira and Suzuki dimerization couplings (Scheme 2). The preparation of nonsymmetrical benzimidazole/imidazole-containing analogs **25** and **26** was ultimately accomplished by the union of boronates **31a-b** with iodo-benzimidazoles **29a-b** under Suzuki conditions (Scheme 3).

In summary, we have described the identification of symmetrical and nonsymmetrical benzimidazole-containing NS5A inhibitors with subnanomolar potency against both genotype 1a and 1b replicons. In the *bis*-benzimidazole series, several linkers designed to explore aspects of varied geometry, length and polarity were prepared, with most exhibiting excellent potency against the genotype 1b HCV replicon. A major breakthrough in genotype 1a potency was realized when the incorporation of 4-substituted pyrrolidines into these scaffolds was explored.

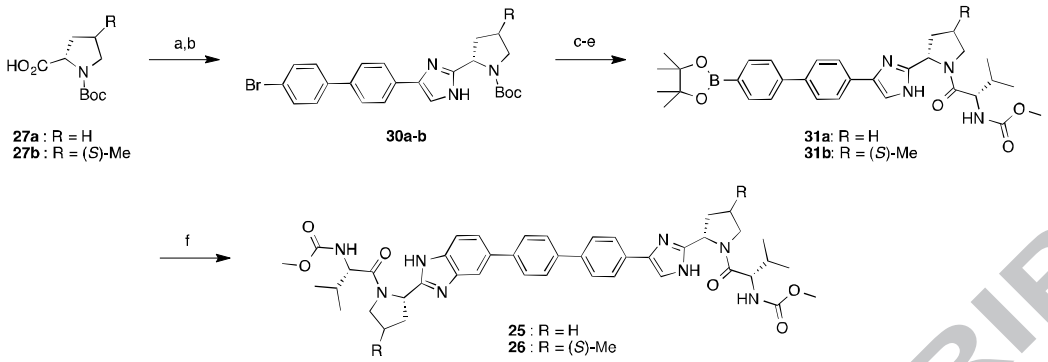
Specifically, it was demonstrated that incorporation of 4-methyl proline into both symmetrical and nonsymmetrical scaffolds has a profound effect on increasing G1a potency, delivering analogs with balanced G1a and 1b potency and good rodent PK profiles.



Scheme 1: Synthesis of building blocks **29a-b**. Reagents and conditions: (a) 4-iodo-benzene-1,2-diamine, HATU, DIPEA, THF, 0 °C; (b) TFA, DCM, rt; (c) (*S*)-2-((methoxycarbonyl)-amino)-3-methylbutanoic acid, HATU, DIPEA, DMF, rt.



Scheme 2: Synthesis of analogs **1-5**. Reagents and conditions: (a) bis(pinacolato)diboron, $PdCl_2$dppf, KOAc, DMF, 85 °C; (b) **29a** (1.0 eq), $Pd(PPh_3)_4$, Na_2CO_3 (aq), iPrOH, 80 °C; (c) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, DBU, H_2O, CH_3CN, 60 °C; (d) 10 wt % Pd/C, H_2, EtOH (e) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, Et_3N, MeCN, rt; (f) K_2CO_3, MeOH, rt; (g) $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, rt; (h) 1,4-benzenediboronic acid (0.5 eq), $PdCl_2$dppf (10 mol%), Na_2CO_3, MeCN, H_2O, 85 °C.



Scheme 3: Synthesis of analogs **25 and 26**. Reagents and conditions: (a) 2-bromo-1-[4-(4-bromophenyl)phenyl]ethanone, DIPEA, MeCN, rt; (b) ammonium acetate, PhMe, 100 °C; (c) TFA, DCM, rt; (d) (*S*)-2-((methoxycarbonyl)-amino)-3-methylbutanoic acid, HATU, DIPEA, DMF, rt; (e) bis(pinacolato)diboron, $PdCl_2$dppf, KOAc, DMF, 85 °C; (f) **29a** or **29b** (1.0 eq), $PdCl_2$dppf (10 mol%), Na_2CO_3, MeCN, H_2O, 85 °C.

Acknowledgments

The authors would like to thank Mark N. Namchuk, Ann D. Kwong, and Jean Bédard for insightful discussions.

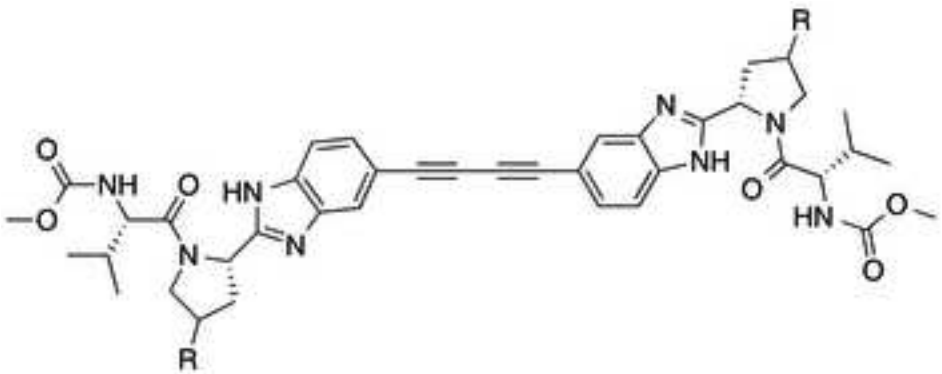
References and notes

1. For recent reviews, see: (a) Thomas, D. L. *Nat. Med.* **2013**, *19*, 850-858, (b) Scheel, T. K. H.; Rice, C. M. *Nat. Med.* **2013**, *19*, 837-849.
2. Liang, T. J.; Ghany, M. G. *N. Engl. J. Med*. **2013**, *368*, 1907-1917.
3. Dabbouseh, N. M.; Jensen, D. M. *Nat. Rev. Gastroenterol. Hepatol*. **2013**, *10*, 268-276.
4. Aghemo, A.; De Francesco, R. *Hepatology* **2013**, *58*, 428-438.
5. Belema, M.; Lopez, O. D.; Bender, J. A.; Romine, J. L.; St. Laurent, D. R.; Langley, D. R.; Lemm, J. A.; O'Boyle, D. R., II; Sun, J.-H.; Wang, C.; Fridell, R. A.; Meanwell, N. A. *J. Med. Chem.* **2014**, *57*, 1643-1672.
6. Guedj, J.; Dahari, H.; Rong, L.; Sansone, N. D.; Nettles, R. E.; Cotler, S. J.; Layden, T. J.; Uprichard, S. L.; Perelson, A. S. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 3991-3996.
7. (a) Reghellin, V.; Donnici, L.; Fenu, S.; Berno, V.; Calabrese, V.; Pagani, M.; Abrignani, S.; Peri, F.; De Francesco, R.; Neddermann, P. *Antimicrob. Agents Chemother.* **2014**, doi:10.1128/AAC.03293-14; (b) Mani, N.; Yuzhakov, A.;

Yuzhakov, O.; Coll, J. T.; Black, J.; Saxena, K.; Fulghum, J. R.; Lippke, J. A.; Govinda Rao, B.; Rijnbrand, R.; Kwong, A. D. *J. Virol.* **2014**, doi: 10.1128/JVI.01677-14. (c) for an editorial review, see: Elazar, M.; Glenn, J. S. *Gastroenterology* **2014**, *147*, 273-277.

8. On October 10th, 2014, the FDA approved Gilead Sciences' Harvoni® (sofosbuvir and ledipasvir) for the treatment of chronic HCV genotype 1 infection. http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm418365.htm
9. (a) Belema, M.; Meanwell, N. A. *J. Med. Chem.* **2014**, *57*, 5057-5071. (b) Gao, M.; Nettles, R. E.; Belema, M.; Snyder, L. B.; Nguyen, V. N.; Fridell, R. A.; Serrano-Wu, M. H.; Langley, D. R.; Sun, J.-H; O'Boyle, D.R., II; Lemm, J. A.; Wang, C.; Knipe, J. O.; Chien, C.; Colonno, R. J.; Grasela, D. M.; Meanwell, N. A.; Hamman, L.G. *Nature* **2010**, *465*, 96-100.
10. Henderson, J. A.; Maxwell, J.; Vaillancourt, L.; Morris, M.; Grey, R., Jr.; Giroux, S.; Kong, L. C. C.; Das, S. K.; Liu, B.; Poisson, C.; Cadilhac, C.; Bubenik, M.; Reddy, T. J.; Falardeau, G.; Yannopoulos, C.; Wang, J.; Pereira, O. Z.; Bennani, Y. L.; Pierce, A. C.; Bhisetti, G. R.; Cottrell, K. M.; Morone, V. Patent US 8,765,731 B2**.**
11. For details of the NS5A replicon assays used in this letter, see: Giroux, S.; Xu, J.; Reddy, T. J.; Morris, M.; Cottrell, K. M.; Cadilhac, C.; Henderson, J. A.; Nicolas, O.; Bilimora, D.; Denis, F.; Mani, N.; Ewing, N.; Shawgo, R.; L'Heureux, L.; Selliah, S.; Chan, L.; Chauret, N.; Berlioz-Seux, F.; Namchuk, M. N.; Grillot, A-L.; Bennani, Y. L.; Das, S. K.; Maxwell, J. P. *ACS Med. Chem Lett.* **2014**, *5*, 240-243.
12. Schönherr, H.; Cernak, T. *Angew. Chem. Int. Ed.* **2013,** *52,* 2-14.
13. Compound **26** was screened against recombinant replicon cell lines harboring these mutations (fold shift from wt activity): 1a Y93H, 8000-fold; 1a Q30R, 42-fold; 1a M28T, 55-fold; and 1b Y93H, 100-fold.
14. CC_{50} in Huh-7 ET cells were determined using a [^{3}H]-thymidine incorporation assay from 12 concentrations of the test compound in duplicate, and data were considered accurate when the CC_{50} of the positive control was within 40% of the mean value.

Graphical Abstract



1000-fold boost in potency

R = H EC_{50} G1a = 18 nM

R = (*S*)-Me EC_{50} G1a = 0.018 nM