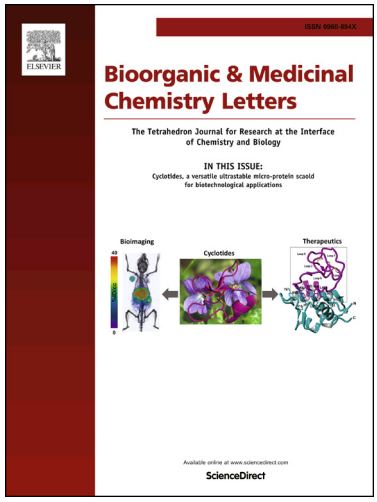

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PII: S0960-894X(18)30039-8
DOI: https://doi.org/10.1016/j.bmcl.2018.01.029
Reference: BMCL 25555

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 27 September 2017
Revised Date: 12 January 2018
Accepted Date: 17 January 2018

Please cite this article as: Zhong, M., Peng, E., Huang, N., Huang, Q., Huq, A., Lau, M., Colonno, R., Li, L., Discovery of novel potent HCV NS5B polymerase non-nucleoside inhibitors bearing a fused benzofuran scaffold, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.01.029

Discovery of novel potent HCV NS5B polymerase non-nucleoside inhibitors bearing a fused benzofuran scaffold

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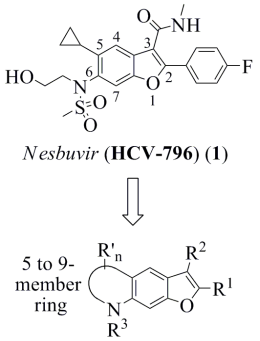
This is where the receipt/accepted dates will go; Received Month XX, 2000; Accepted Month XX, 2000 [BMCL RECEIPT]

Abstract— This letter describes the discovery of a fused benzofuran scaffold viable for preparing a series of novel potent HCV NS5B polymerase non-nucleoside inhibitors. Designed on the basis of the functionalized benzofuran derivative *nesbuvir* (**HCV-796**), these compounds presumably bind similarly to the allosteric binding site in the "palm" domain of HCV NS5B protein. SAR of each potential hydrogen-bonding interaction site of this novel scaffold is discussed along with some preliminary genotypic profile and PK data of several advanced compounds. [BMCL ABSTRACT]

It was estimated that more than 170 million people worldwide have been suffering from chronic infections with various genotypes of Hepatitis C virus (HCV). Chronic HCV infection has been regarded as one of the main causes for chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, and the related liver transplant and death.[1-4] Following an extensive effort in HCV drug discovery over the past decade, a number of efficacious all-oral combination regimens[5-12] have recently been approved by the US FDA to cure HCV infections.

Among the various classes of HCV drugs utilized in highly effective combination regimens are HCV NS5B polymerase non-nucleoside inhibitors.[13] For example, *Viekira Pak*® is composed of co-formulated *ombitasvir* (HCV NS5A inhibitor, 12.5 mg/tablet), *paritaprevir* (HCV NS3/4A protease inhibitor, 75 mg/tablet) and *ritonavir* (CYP3A inhibitor, 50 mg/tablet) (two tablets and once daily), and *dasabuvir* (HCV NS5B polymerase non-nucleoside inhibitor, 250 mg/tablet, single tablet, and twice daily), which is prescribed for treating patients with genotype 1 HCV infection.[8a] Recently, *Viekira XR*™, a once-daily extended-release co-formulation of the active ingredients in *Viekira Pak*®, was approved by the US FDA.[8c]

As part of our HCV drug discovery effort, we were interested in discovering and developing a novel potent HCV NS5B polymerase non-nucleoside inhibitor, which could be used in combination with an HCV NS5A inhibitor (e.g. *ravidasvir*®)[14] and an HCV NS3/4A protease inhibitor as a new all-oral combination regimen.



Nesbuvir (**HCV-796**) (**1**)

Figure 1. Chemical structures of *nesbuvir* (**HCV-796**) (**1**) and a fused benzofuran scaffold

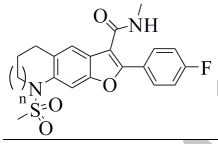
Nesbuvir (**HCV-796**) (**1**, Figure 1) is a potent HCV NS5B polymerase non-nucleoside inhibitor[15] that advanced to a Phase II human clinical trial in combination with pegylated interferon alfa-2b (pegINF α2b) in the presence of *ribavirin*. This combination regimen demonstrated good efficacy in genotype 1a (gt-1a) infected subjects; however, its further development was suspended due to unexpected elevations of liver enzymes.[16]

On the basis of the reported co-crystal structure,[17] *nesbuvir* binds to the allosteric binding site in the "palm" domain of the HCV NS5B polymerase protein, where the –NH– and the –C(O)– of the amide moiety at the C3 position show H-bond interactions with Ser365 and Arg200, respectively. Also, the –C(O)– has H-bond interaction with Cys316, the $–S(O)_2–$ shows H-bond interaction with Arg200 and Met414, and the cyclopropyl residue displays hydrophobic interaction with the protein. The –OH seems not to exhibit direct interaction with the protein. Presumably, it helps improve the intrinsic solubility.

With the understanding of the co-crystal structural information, we employed a macrocyclization drug design strategy[18] (Figure 1) to the structure of *nesbuvir* (**1**), which led to the discovery of a series of novel potent HCV NS5B polymerase non-nucleoside inhibitors bearing a fused benzofuran scaffold. Herein, we report the SAR of each potential H-bonding interaction region of this new scaffold and some preliminary genotypic profile and rat PK data of several advanced compounds.

All of the fused benzofuran derivatives were prepared by following our previously disclosed protocols.[19] A panel of wild-type (gt-1a and gt-1b) and gt-1a variant (L31V+Y93H) HCV replicons were employed as the primary cell-based assays to evaluate all of the compounds.[20] These replicon stable cell lines were generated in naïve Huh-7 cells. As expected, these HCV NS5B polymerase non-nucleoside inhibitors exhibit strong potency against the gt-1a variant (L31V+Y93H) replicon, in which the potency of HCV NS5A inhibitors bearing a dimeric bis-imidazole motif are completely diminished, demonstrating the complementary effect of these two classes of HCV inhibitors.

Table 1. SAR of the ring size in the fused benzofuran analogs (**2a-e**)

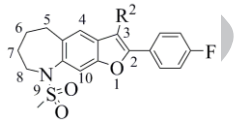


Entry	Compd.	n	CLogP[a]	HCV gt-1b EC_{50} (μM)
1	**1**		2.16	0.012 0.009[b]
2	**2a**	0	2.52	0.304
3	**2b**	1	2.93	0.451
4	**2c**	2	3.49	0.017
5	**2d**	3	4.05	0.005
6	**2e**	4	4.61	0.026

[a]Calculated values using ChemBioOffice 2014 developed by CambridgeSoft. CLogP refers to the calculated logarithm of a compound's partition coefficient between n-octanol and water.
[b]Reported value, see ref. 15a

In view that the cyclopropyl moiety in *nesbuvir* (**1**) (Figure 1) mainly has hydrophobic interaction with the protein and the $–CH_2CH_2OH$ moiety presumably serves as a solubilizing group, we thought that linking the –N– of the sulfonamide residue to the C5 in central benzene ring with an aliphatic ring could retain hydrophobic interaction to achieve the desired potency. As shown in Table 1, when a five-member ring is employed, the resulting compound (**2a**, entry 2) showed an EC_{50} of 0.304 μM in the gt-1b replicon. The corresponding six-member analog (**2b**, entry 3) showed slightly weaker potency. Interestingly, with the increase of the ring size from five-member to seven-, eight- and nine-member, the resulting analogs (**2c-e**, entries 4-6) showed 10 to 60-fold increase in potency relative to **2a** in the gt-1b replicon. Considering the importance of lipophilicity to the physicochemical property of a compound, the seven-member ring was chosen as the backbone for further chemical modifications.

Table 2. SAR of R^2 in the fused benzofuran analogs (**2c** and **2f-q**)



Entry	Compd.	R^2	HCV inhibition EC_{50} (μM)[a]	
			gt-1a	gt-1b
1	**1**		0.024 0.024[b] 0.005[c]	0.012 0.009[c]
2	**2c**	-C(O)NHMe	0.083	0.017
3	**2f**	$-C(O)NH_2$	>2.0	-[d]
4	**2g**	$-C(O)NMe_2$	>2.0	-
5	**2h**	-C(O)NHEt	>2.0[b]	-
6	**2i**	-C(O)NHcPr	>2.0	-
7	**2j**	-C(O)NHCN	>2.0	-
8	**2k**	-C(O)NHOMe	0.090	0.031
9	**2l**	$-C(O)NHS(O)_2Me$	>2.0	-
10	**2m**	-C(O)N(OH)Me	>2.0	0.298
11	**2n**		>2.0	-
12	**2o**		0.059 0.033[b]	0.005
13	**2p**		>2.0	0.850
14	**2q**		>2.0 >2.0[b]	-

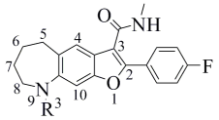
[a]The data was generated in gt-1a (L31V+Y93H)_LucNeo Luciferase and gt-1b (wild-type)_LucNeo Luciferase HCV replicons, respectively.
[b]The data was generated in gt-1a (wild-type) HCV replicon. The potency of an HCV NS5B inhibitor in gt-1a (wild-type) and gt-1a (L31V+Y93H)_LucNeo Luciferase HCV replicons is generally comparable to each other.
[c]Reported value, see ref. 15a
[d]Not determined

To understand the SAR in the R^2 region (–C(O)NHMe as R^2 in **2c**), a number of functional groups bearing

potential H-bond donor (e.g. –NH– or –OH) and acceptor (e.g. –C=O or –C=N–) were employed. As outlined in Table 2, neither primary (**2f**, entry 3) nor tertiary (**2g**, entry 4) amide is tolerated. Replacement of the –C(O)NHMe group with bulkier –C(O)NH-alkyls (**2h-i**, entries 5-6), –C(O)NHC≡N (**2j**, entry 7), or –C(O)NHS(O)$_2$Me (**2l**, entry 9) is detrimental to the potency. Also, the –C(O)NHMe cannot be switched to an –C(O)N(OH)Me residue (**2m**, entry 10). Interestingly, substitution of the –C(O)NHMe with –C(O)NHOMe afforded potent **2k** (entry 8) comparable to **2c**. Among the tested five-member heterocycles, including 4,5-dihydro-1*H*-imidazole (**2n**, entry 11), imidazole (**2o**, entry 12), [1,2,4]-triazole (**2p**, entry 13), and tetrazole (**2q**, entry 14), that retain a potential pair of H-bond donor and acceptor similar to –C(O)NHMe, **2o** demonstrated fairly improved potency in both the gt-1a and gt-1b replicons; however, lower intrinsic solubility associated with **2o** (CLogP = 4.37) relative to **2c** (CLogP = 3.49) was observed.

As shown in Table 3, in the R^3 region (–S(O)$_2$Me group as R^3 in **2c**) (entry 1), significant decrease of the potency in the gt-1a replicon was observed when the -Me group was substituted with –NH$_2$ (**2p**, entry 2), -NMe$_2$ (**2q**, entry 3), or –cPr (**2r**, entry 4), respectively. Moreover, replacement of the –S(O)$_2$Me group with –C(O)Me (**2s**, entry 5) or –C(O)OMe (**2t**, entry 6) was not tolerated.

Table 3. SAR of R^3 in the fused benzofuran analogs (**2c** and **2p-t**)



Entry	Compd.	R^3	HCV inhibition EC$_{50}$ (μM)[a]	
			gt-1a	gt-1b
1	**2c**	-S(O)$_2$Me	0.083	0.017
2	**2p**	-S(O)$_2$NH$_2$	0.719 0.605[b]	-[c]
3	**2q**	-S(O)$_2$NMe$_2$	0.518 0.480[b]	-
4	**2r**	-S(O)$_2$cPr	0.423 0.335[b]	0.073
5	**2s**	-C(O)Me	>2.0 >2.0[b]	-
6	**2t**	-C(O)OMe	0.722	0.164

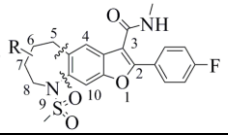
[a]The data was generated in gt-1a (L31V+Y93H)_LucNeo Luciferase and gt-1b (wild-type)_LucNeo Luciferase HCV replicons, respectively.
[b]The data was generated in gt-1a (wild-type) HCV replicon. The potency values of an HCV NS5B inhibitor in gt-1a (wild-type) and gt-1a (L31V+Y93H)_LucNeo Luciferase HCV replicons is generally comparable to each other.
[c]Not determined

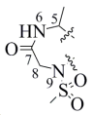
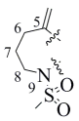
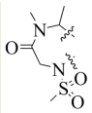
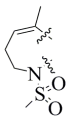
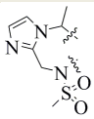
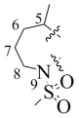
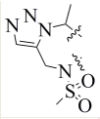
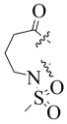
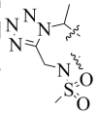
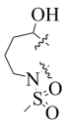
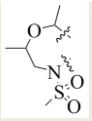
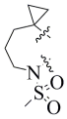
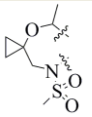
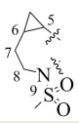
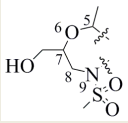
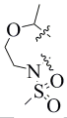
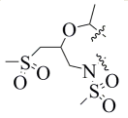
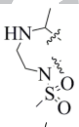
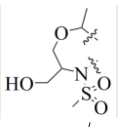
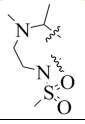
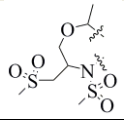
Our main effort has been expended on the modifications of the seven-member ring to understand the SAR. As shown in Table 4, a terminal vinyl residue at C5 is tolerated (**3a**, entry 2) and the corresponding internal olefinic analog **3b** (entry 3) seems to be more potent. Saturation of the double bond led to a more potent analog (±)-**3c** (entry 4) with an EC$_{50}$ of 10 nM and 3 nM in the gt-1a and gt-1b replicons, respectively. Between those two enantiomers, (5*R*)-**3c** is more potent than (5*S*)-**3c**.[21] Neither a carbonyl (**3d**, entry 5) nor hydroxyl ((±)-**3e**, entry 6) at the C5 position was well tolerated. Introduction of a cyclopropyl residue at the C5 position reasonably maintained good potency (**3f**, entry 7). Also, improved potency was observed when the cyclopropyl residue was fused to the C5 and C6 carbons ((±)-**3g**, entry 8) and (5*R*,6*R*)-**3g** is more active than (5*S*,6*S*)-**3g** in the gt-1a and gt-1b replicons.

To reduce the lipophilicity of (±)-**3c** (CLogP = 4.01) (entry 4), an oxygen atom was inserted at the C6 position, giving (±)-**3h** (CLogP = 2.38) (entry 9) with four-fold increase of the potency in the gt-1a replicon. As expected, the corresponding *S* isomer ((5*S*)-**3h,** entry 4) is the more potent enantiomer with an EC$_{50}$ of 9 nM and 3 nM in the gt-1a and gt-1b replicons, respectively. Moreover, the metabolic stability of (5*S*)-**3h** (entry 3, Table 5) in rat and human liver microsomes (LMs) was improved as compared to (±)-**3c** (entry 2, Table 5). Replacement of the –O– in (±)-**3h** with –NH– ((±)-**3i**, entry 10) resulted in a significant loss of the potency, but the potency of (±)-**3i** was fairly recovered by methylating the –NH– residue ((±)-**3j**, entry 11). Interestingly, the lactam analog of (±)-**3i** (**3k**, entry 12) demonstrated comparable potency to (±)-**3h** (entry 9) in both the gt-1a and gt-1b replicons, while the corresponding *N*-methylated analog (±)-**3l** (entry 13) is less potent. Replacement of the lactam residue in (±)-**3k** or (±)-**3l** with five-member heterocycles gave (±)-**3m** (entry 14), (±)-**3n** (entry 15), and (±)-**3o** (entry 16), respectively, with good potency. However, decreased intrinsic solubility of (±)-**3k-o** relative to (±)-**3i-j** was noted, possibly due to the increase of planarity of the molecules. Other functional groups can also be attached to the C7 position in (±)-**3h**. For example, when a methyl group was introduced, the resulting (±)-**3p** (entry 17) showed comparable potency; while a cyclopropyl moiety slightly reduced the potency ((±)-**3q**, entry 18).

Considering that (±)-**3h** has poor metabolic stability in rat and human LMs, further reduction of the lipophilicity was considered to improve it. When an HOCH$_2$– moiety was introduced to the C7 position, the resulting compound ((±)-**3r**, entry 19) exhibited an EC$_{50}$ of 52 nM and 12 nM in the gt-1a and gt-1b replicons, respectively; and more importantly, significant improvement of the metabolic stability in rat and human LMs was achieved (entries 4-5, Table 5). Among those four diastereomers of (±)-**3r**,[22] (5*S*,7*S*)-**3r** is the most potent one with an EC$_{50}$ of 9 nM and 3 nM in the gt-1a and gt-1b replicons, respectively, while (5*S*, 7*R*)-**3r** is

Table 4. SAR of the substituted 7-member ring moiety in the fused benzofuran analogs (**2c** and **3a-u**)

fused 7-member ring moiety 

Entry	Compd.	Fused ring moiety	HCV inhibition EC_{50} (μM)[a]		Entry	Compd.	Fused ring moiety	HCV inhibition EC_{50} (μM)[a]	
			gt-1a	gt-1b				gt-1a	gt-1b
1	**2c**		0.083	0.017	12	(±)-**3k** (5*S*)-**3k** (5*R*)-**3k**		0.014 0.019[b] 0.011[b] 1.795[b]	0.015 0.005 0.586
2	**3a**		0.106	0.026	13	(±)-**3l** (5*S*)-**3l** (5*R*)-**3l**		0.180[b] 0.102[b] 0.688[b]	0.017 0.013 0.059
3	**3b**		0.070	0.016	14	(±)-**3m**		0.026[b]	0.074
4	(±)-**3c** (5*R*)-**3c** (5*S*)-**3c**		0.010 0.009 0.010[b] 0.089	0.003 0.004 0.046	15	(±)-**3n** (5*S*)-**3n** (5*R*)-**3n**		0.054[b] 0.020[b] 0.617[b]	0.006 0.003 0.038
5	**3d**		0.318	0.078	16	(±)-**3o**		0.100[b]	0.011
6	(±)-**3e**		0.730	0.107	17	(±)-**3p**		0.033[b]	0.008
7	**3f**		0.118	0.020	18	(±)-**3q** (5*S*)-**3q** (5*R*)-**3q**		0.130[b] 0.093[b] >2.0[b]	0.019 0.013 0.666
8	(±)-**3g** (5*R*,6*R*)-**3g** (5*S*,6*S*)-**3g**		0.044 0.035[b] 0.025 >2.0	0.009 0.007 >2.0	19	(±)-**3r** (5*S*,7*S*)-**3r** (5*R*,7*R*)-**3r** (5*R*,7*S*)-**3r** (5*S*,7*R*)-**3r**		0.052[b] 0.009[b] >2.0[b] 0.554[b] 0.064[b]	0.012 0.003 1.473 0.069 0.013
9	(±)-**3h** (5*S*)-**3h** (5*R*)-**3h**		0.021 0.028[b] 0.009 0.624	0.011 0.003 0.686	20	(±)-**3s** (5*S*,7*S*)-**3s** (5*R*,7*R*)-**3s** (5*R*,7*S*)-**3s** (5*S*,7*R*)-**3s**		0.009[b] 0.004[b] >2.0[b] 0.100[b] 0.057[b]	0.007 0.003 1.208 0.024 0.013
10	(±)-**3i**		0.265	0.043	21	(±)-**3t**		0.040[b]	0.033
11	(±)-**3j**		0.050	0.014	22	(±)-**3u**		0.016[b]	0.019

[a]The data was generated in gt-1a (L31V+Y93H)_LucNeo Luciferase and gt-1b (wild-type)_LucNeo Luciferase HCV replicons, respectively.
[b]The data was generated in gt-1a (wild-type) HCV replicon. The potency of an HCV NS5B inhibitor in gt-1a (wild-type) and gt-1a (L31V+Y93H)_LucNeo Luciferase HCV replicons is generally comparable to each other.

reasonably potent with an EC_{50} of 64 nM and 13 nM in the gt-1a and gt-1b replicons, respectively. As expected, the corresponding (5*R*) isomers (i.e. (5*R*,7*R*)- **3r** and (5*R*,7*S*)-**3r**) do not possess comparable potency. Moreover, a $MeS(O)_2CH_2$– moiety can be attached to the C7 position, giving (±)-**3s** (entry 20) with improved potency relative to (±)-**3h**. As expected, the metabolic stability in rat and human LMs was significantly improved (entries 6-7, Table 5) and (5*S*,7*S*)-**3s** is the most potent diastereomer with an EC_{50} of 4 nM and 3

nM in the gt-1a and gt-1b replicons, respectively. Furthermore, the C8 position in (±)-**3h** can be substituted with either $HOCH_2$– or $MeS(O)_2CH_2$– to give (±)-**3t** (entry 21) or (±)-**3u** (entry 22), which has comparable potency to (±)-**3r** or (±)-**3s** in the gt-1a and gt-1b replicons, respectively.

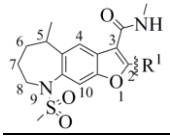
Table 5. Metabolic stability of representative compounds in rat and human liver microsomes

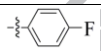
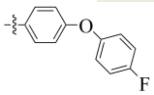
Entry	Compd.	% remaining of the parent at 60 min		CLogP[a]	tPSA[a]
		RLM[b]	HLM[b]		
1	1	84.0	53.3	2.16	95.94
2	(±)-**3c**	0.8	0.8	4.01	75.71
3	(5*S*)-**3h**	5.0	32.6	2.38	84.94
4	(±)-**3r**	68.9	85.9	2.30	105.17
5	(5*S*,7*S*)-**3r**	60.0	100	2.30	105.17
6	(±)-**3s**	99.7	100	1.30	119.08
7	(5*S*,7*S*)-**3s**	100	100	1.30	119.08

[a]Calculated values using ChemBioOffice 2014 developed by CambridgeSoft. CLogP refers to the calculated logarithm of a compound's partition coefficient between n-octanol and water. tPSA referes to total polar surface area.
[b]RLM refers to rat liver microsome and HLM refers to human liver microsome.

It is worth noting that the cytotoxicity of these potent HCV NS5B analogs in Huh-7 naïve cells is reasonably low. For example, the CC_{50} of (5*S*,7*S*)-**3r** is > 3 μM and that of (5*S*,7*S*)-**3s** is > 10 μM, which was determined by incubating a compound with the cells at rt for 24 h.

Table 6. SAR of R^1 in the fused benzofuran analogs ((±)-**3c** and (±)-**3v-y**)

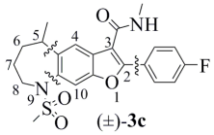


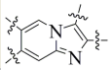
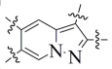
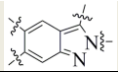
Entry	Compd.	R^1	HCV inhibition EC_{50} (μM)[a]	
			gt-1a	gt-1b
1	(±)-**3c**		0.010	0.003
2	(±)-**3v**		0.011	0.007
3	(±)-**3w**		0.015	0.008
4	(±)-**3x**		>2.0	0.056
5	(±)-**3y**		0.071	0.014

[a]The data was generated in gt-1a (L31V+Y93H)_LucNeo Luciferase and gt-1b (wild-type)_LucNeo Luciferase HCV replicons, respectively.

Meanwhile, a set of analogs of (±)-**3c**, in which the 4-F-phenyl group was replaced with varying R^1, was prepared to establish the preliminary SAR. As outlined in Table 6, when the 4-F-phenyl in (±)-**3c** (entry 1) was replaced with 2-F-phenyl ((±)-**3v**, entry 2), the gt-1a potency was retained but there was a 2-fold loss of the gt-1b potency. As expected, the 2,4-diF-phenyl analog ((±)-**3w**, entry 3) showed comparable potency to (±)-**3v**. Modulation of the conformation between R^1 and the central benzofuran moiety by substituting the 2-F in (±)-**3w** with a bulkier 2-OMe yielded a much weaker compound ((±)-**3x**, entry 4). Interestingly, the 4-F in (±)-**3c** could be replaced with a 4-F-phenoxyl residue ((±)-**3y**, entry 5) without significantly reducing the potency.

Table 7. SAR of the central substituted [6,5]-heterocycles


(±)-**3c**

Entry	Compd.	Central substituted [6,5]-heterocycle	HCV inhibition EC_{50} (μM)[a]	
			gt-1a	gt-1b
1	(±)-**3c**		0.010	0.003
2	(±)-**4**		0.801	0.182
3	(±)-**5**		0.523	0.109
4	(±)-**6**		>2.0[b]	-[c]

[a]The data was generated in gt-1a (L31V+Y93H)_LucNeo Luciferase and gt-1b (wild-type)_LucNeo Luciferase HCV replicons, respectively.
[b]The data was generated in gt-1a (wild-type) HCV replicon. The potency of an HCV NS5B inhibitor in gt-1a (wild-type) and gt-1a (L31V+Y93H)_LucNeo Luciferase HCV replicons is generally comparable to each other.
[c]Not determined

Moreover, several fused [6,5]-hetereocyclic systems were also tested to compare with the central benzofuran scaffold. As shown in Table 7, a compound bearing a substituted imidazo[1,2-a]pyridine ((±)-**4**, entry 2), pyrazolo[1,5-a]pyridine ((±)-**5**, entry 3), or 2*H*-indazole ((±)-**6**, entry 4) central core is much less potent than the corresponding benzofuran analog ((±)-**3c**, entry 1) in the gt-1a and gt-1b replicons, respectively.

In addition to testing these compounds in the wide-type gt-1a and gt-1b HCV replicons, their preliminary genotypic profiles were evaluated with wide-type gt-2a, gt-3a and gt-4a HCV replicons, respectively. These replicon stable cell lines were also generated in naïve Huh-7 cells. As summarized in Table 8, compounds (5*S*,7*S*)-**3r** and (5*S*,7*S*)-**3s** demonstrated superior overall virological profiles to *nesbuvir* (**1**). Although (±)-**3t** and (±)-**3u** have comparable gt-1a and -1b potency to (±)-**3r** and (±)-**3s**, respectively, they exhibited less attractive overall genotypic profiles.

To evaluate the pharmacokinetic of this novel series of potent HCV NS5B polymerase non-nucleoside

inhibitors, a number of compounds along with *nesbuvir* (**1**) were dosed at 1 mg/kg and 5 mg/kg in rat iv and po PK in a solution formulation of 10% NMP/10% solutol/40% PEG-400/40% saline, respectively. As shown in Table 9, *nesbuvir* (**1**) showed good PK profile with a half-life ($t_{1/2}$) of 2.73 h, clearance (*CL*) of 10.4 mL/min/kg, steady state volume distribution (V_{ss}) of 1.52 L/kg, and area under curve (AUC_{INF}) of 1610 hr*ng/mL in rat iv PK, and bioavailability (*F*%) of 73% in rat po PK (entry 1). As compared to *nesbuvir* (**1**), (5*R*)-**3c**, (5*R*)-**3h**, and (5*S*)-**3k** exhibited relatively higher clearance, shorter half life, lower exposure and poorer bioavailability (entries 2-4). Both (5*S*)-**3n** and (5*S*,7*S*)-**3r** performed comparably to *nesbuvir* (**1**) (entries 5-6). Although (±)-**3s** showed similar *iv* PK profile to *nesbuvir* (**1**), its bioavailability was much lower, presumably due to the much decreased lipophilicity (entry 7).

Table 8. Genotypic profile of HCV NS5B polymerase non-nucleoside inhibitors bearing a fused benzofuran scaffold relative to *nesbuvir* (**1**)

Compd.	HCV inhibition EC_{50} (μM)[a]				
	gt-1a	gt-1b	gt-2a	gt-3a	gt-4a
1	0.024	0.012	0.072	0.019	0.021
(±)-**3r**	0.052	0.012	0.062	0.020	0.032
(5*S*,7*S*)-**3r**	0.009	0.003	0.012	0.006	0.009
(±)-**3s**	0.009	0.007	0.064	0.017	0.017
(5*S*,7*S*)-**3s**	0.004	0.003	0.018	0.010	0.007
(±)-**3t**	0.040	0.033	0.141	0.053	0.117
(±)-**3u**	0.016	0.019	0.134	0.070	0.051

[a]The data was generated in wide-type gt-1a, gt-1b, gt-2a, gt-3a and gt-4a_LucNeo Luciferase HCV replicons.

Table 9. Rat *iv* and *po* PK of representative compounds

entry	Compd.[a]	$t_{1/2}$ (h)[b]	*CL* (mg/ml/kg)[b]	*V*ss (L/kg)[b]	AUC_{INF} (hr*ng/mL)[b]	*F* (%)[c]
1	**1**	2.73	10.4	1.52	1610	73
2	(5*R*)-**3c**	0.96	38.2	2.36	439	22
3	(5*R*)-**3h**	0.34	78.0	1.79	234	16
4	(5*S*)-**3k**	0.68	44.5	1.74	379	14
5	(5*S*)-**3n**	2.43	12.8	1.41	1301	57
6	(5*S*,7*S*)-**3r**	1.32	22.0	2.25	759	73
7	(±)-**3s**	3.62	16.0	1.51	1059	30

[a]A solution formulation of 10% NMP/10% solutol/40% PEG-400/40% saline was employed in the rat *iv* and *po* PK experiments.
[b]Rat *iv* PK was dosed at 1 mg/kg in Sprague-Dawley rats (n=3).
[c]Rat *po* PK was dosed at 5 mg/kg in Sprague-Dawley rats (n=3).

In summary, we have successfully discovered a series of novel potent HCV NS5B polymerase non-nucleoside inhibitors bearing a fused benzofuran scaffold. As compared to *nesbuvir* (**1**), some of the compounds, such as (5*S*,7*S*)-**3r** and (5*S*,7*S*)-**3s**, demonstrated better potency in the gt-1a and gt-1b replicons and superior genotypic profiles. Also, (5*S*,7*S*)-**3r** and (5*S*,7*S*)-**3s** showed reasonably good *iv* and *po* PK profile in rats. Further work will be focused on *in vitro* virological assessment of representative compounds in combination with other direct acting agents (DAAs) and the understanding of safety profile of this series of compounds as compared to *nesbuvir* (**1**), which will be discussed in due course.

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Acknowledgment. The authors thank Dr. Jason Xiang and his chemistry team at Shanghai ChemPartner for their excellent chemistry CRO services.

Supplementary material. Synthetic procedure to the preparation of (5*S*,7*S*)-**3r** and the corresponding spectroscopic data, including ^{1}H NMR, COSY, NOESY, and chiral HPLC data (PDF)

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18. Macrocyclization strategy has been successfully employed in the discovery of HCV NS3/4A protease inhibitors with improved potency, resistance and PK profile. See examples: (a). Rosenquist, Å.; Samuelsson, B.; Johansson, P.-O.; Cummings, M. D.; Lenz, O.; Raboisson, P.; Simmen, K.; Vendeville, S.; de Kock, H.; Nilsson, M.; Horvath, A.; Kalmeijer, R.; de la Rosa, G.; Beumont-Mauviel, M. *J. Med. Chem.*, **2014**, *57*, 1673–1693. (b). Jiang, Y.; Andrews, S. W.; Condroski, K. R.; Buckman, B.; Serebryany, V.; Wenglowsky, S.; Kennedy, A. L.; Madduru, M. R.; Wang, B.; Lyon, M.; Doherty, G. A.; Woodard, B. T.; Lemieux, C.; Do, M. G.; Zhang, H.; Ballard, J.; Vigers, G.; Brandhuber, B. J.; Stengel, P.; Josey, J. A.; Beigelman, L.; Blatt, L.; Seiwert, S. D. *J. Med. Chem.* **2014**, *57*, 1753–1769. (c). Neelamkavil, S. F.; Agrawal, S.; Bara, T.; Bennett, C.; Bhat, S.; Biswas, D.; Brockunier, L.; Buist, N.; Burnette, D.; Cartwright, M.; Chackalamannil, S.; Chase, R.; Chelliah, M.; Chen, A.; Clasby, M.; Colandrea, V. J.; Davies, I. W.; Eagen, K.; Guo, Z.; Han, Y.; Howe, J.; Jayne, C.; Josien, H.; Kargman, S.; Marcantonio, K.; Miao, S.; Miller, R.; Nolting, A.; Pinto, P.; Rajagopalan, M.; Ruck, R. T.; Shah, U.; Soriano, A.; Sperbeck, D.; Velazquez, F.; Wu, J.; Xia, Y.; Venkatraman, S. *ACS Med. Chem. Lett.*, **2016**, *7*, 111–116

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20. At the early stage of our HCV NS5B program, we encountered chemical contamination issues of low double-digit nM IC_{50} HCV NS5B polymerase non-nucleoside inhibitors with low single-digit pM IC_{50} HCV NS5A inhibitors when synthesizing these two classes of molecules in the same chemistry lab without much precautions. Therefore, the gt-1a HCV replicon bearing double mutants (e.g. L31V+Y93H) in the region of HCV NS5A protein was utilized as a surrogate, in which HCV NS5A inhibitors show significantly diminished potency as compared to that observed in the corresponding wide-type gt-1a relpicon. For potent HCV NS5B inhibitors, they demonstrate good potency in the gt-1b (wide-type) replicon, while exhibiting comparable potency against both the gt-1a (wide-type) and gt-1a (L31V+Y93H) replicons.

21. The stereochemistry of the chial center was elucidated on the basis of the method reported in Harada, N.; Watanabe, M.; Kuwahara, S.; Sugio, A.; Kasai. Y.; Ichikawa, A. *Tetrahedron: Asymmetry* **2000**, *11*, 1249 and Taji, H.; Kasai, Y.; Sugio, A.; Kuwahara, S.; Watanabe, M.; Harada, N.; Ichikawa, A. *Chirality* **2002**, *14*, 81. The detailed experimental procedure was described in reference 19b.

22. See the supplemental material for detailed experimental procedure and stereochemistry determination on the basis of the known stereochemistry of the chiral starting material and the spectroscopic data, including ^{1}H NMR, COSY, NOSEY, and chiral HPLC data.

Graphical abstract

Discovery of novel potent HCV NS5B polymerase non-nucleoside inhibitors bearing a fused benzofuran scaffold

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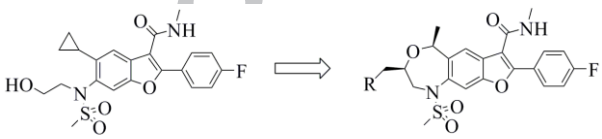
This letter describes the discovery of a fused benzofuran scaffold viable for preparing a series of novel potent HCV NS5B non-nucleoside inhibitors. Designed on the basis of the functionalized benzofuran derivative *Nesbuvir* (**HCV-796**), these compounds presumably bind similarly to the allosteric binding site in the "palm" domain of HCV NS5B protein. SAR of each potential hydrogen-bonding interaction site of this novel scaffold is discussed along with some preliminary genotypic profile and PK data of several advanced compounds.



Nesbuvir (**HCV-796**) (**1**)