Bioorganic & Medicinal Chemistry Letters 28 (2018) 963–968

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

Min Zhong *, Eric Peng, Ningwu Huang, Qi Huang, Anja Huq, Meiyen Lau, Richard Colonno, Leping Li *

Presidio Pharmaceuticals, Inc., 1700 Owens Street, Suite 184, San Francisco, CA 94158, USA

ARTICLE INFO

Article history:
Received 27 September 2017
Revised 12 January 2018
Accepted 17 January 2018
Available online 31 January 2018

Keywords:
HCV
NS5B polymerase
Non-nucleoside inhibitor
Fused benzofuran

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.

It was estimated that >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*(**PPI-668**))[14] and an HCV NS3/4A protease inhibitor as a new all-oral combination regimen.

Nesbuvir (**HCV-796**) (**1**, Fig. 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 α-2b) 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] (Fig. 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 hydrogen-bonding interaction region of this new scaffold and some preliminary genotypic profile and rat PK data of several advanced compounds.

* Corresponding author at: MyoKardia, Inc., 333 Allerton Avenue, South San Francisco, CA 94080, USA (M. Zhong). Assembly Biosciences, Inc., 409 Illinois Street, San Francisco, CA 94158, USA (L. Li).

E-mail addresses: mzhong@myokardia.com (M. Zhong), lli@assemblybio.com (L. Li).

https://doi.org/10.1016/j.bmcl.2018.01.029
0960-894X/

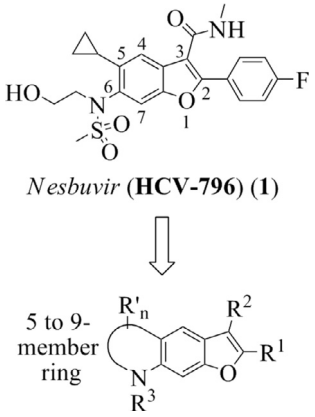
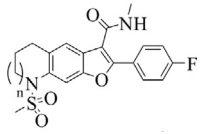


Fig. 1. Chemical structures of *nesbuvir* (**HCV-796**) (**1**) and a fused benzofuran scaffold.

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 is completely diminished, demonstrating the complementary effect of these two classes of HCV inhibitors.

In view that the cyclopropyl moiety in *nesbuvir* (**1**) (Fig. 1) mainly has hydrophobic interaction with the protein and the $-CH_2-CH_2OH$ 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.

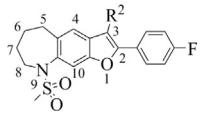
Table 1
SAR of the ring size in the fused benzofuran analogs (**2a**–**e**).



Entry	Compd.	n	CLogP[a]	HCV gt-1b EC_{50} (μM)[b]
1	**1**		2.16	0.012 0.009[c]
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] The data was generated in gt-1b (wide-type)_LucNeo Luciferase HCV replicon.
[c] Reported value, see Ref. 15a.

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 $-C(O)NHS(O)_2Me$ (**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 **2 k** (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 ($-S(O)_2Me$ 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 $-S(O)_2Me$ group with –C(O)Me (**2s**, entry 5) or –C(O)OMe (**2t**, entry 6) was not tolerated.