Discovery and Initial Optimization of Alkoxyanthranilic Acid Derivatives as Inhibitors of HCV NS5B Polymerase

Kyle Parcella^{a*,†}, Andrew Nickel^{a,†,‡}, Brett R. Beno^a, Steven Sheriff^b, Changhong Wan^{b,#}, Ying-Kai Wang^a, Susan B. Roberts^a, Nicholas A. Meanwell^a, John F. Kadow^{a,§}

^aBristol-Myers Squibb, Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA. ^bBristol-Myers Squibb, Research and Development, PO Box 4000, Princeton, NJ 08540, USA.

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Hepatitis C Virus (HCV)

NS5B Polymerase

Allosteric Inhibitors

Primer Grip

ABSTRACT

Alkoxyanthranilic acid derivatives have been identified to inhibit HCV NS5B polymerase, binding in an allosteric site located at the convergence of the palm and thumb regions. Information from co-crystal structures guided the structural design strategy. Ultimately, two independent structural modifications led to a similar shift in binding mode that when combined led to a synergistic improvement in potency and the identification of inhibitors with sub-micromolar HCV NS5B binding potency.

2016 Elsevier Ltd. All rights reserved.

It is estimated that about 3% of the world's population is infected with hepatitis C virus (HCV).¹ Chronic HCV infection has been one of the leading causes of liver disease accounting for approximately fifteen thousand deaths annually in the US.² HCV is a positive single stranded RNA virus which encodes for a single polypeptide, which is subsequently cleaved into structural and non-structural proteins.³ The non-structural proteins, which have been shown to play critical roles in viral replication, have emerged as popular targets for HCV chemotherapy. In recent years, the standard of care has evolved rapidly from a pegylated interferon- α /ribavirin-based treatment to a combination of direct-acting antivirals.⁴ Of these targets, the RNA-dependent RNA polymerase NS5B has generated particular attention from the medicinal chemistry community.

Several allosteric sites on the enzyme have been investigated in the context of small molecule inhibitors.³ About 10 Å away from the active site in NS5B, a large 15 Å wide and 20 Å deep cleft is known to bind a number of structurally diverse small molecule inhibitors, including HCV-796 (**1**) which was the first non-nucleoside NS5B inhibitor to demonstrate clinical proof of concept (Fig 1).³ Generally speaking, these inhibitors are characterized by varying degrees of resistance to the mutated C316Y NS5B enzyme.

Figure 1. Literature allosteric inhibitors of HCV NS5B polymerase that bind a large cleft 10–20 Å away from the active site.^{3,5}

In an effort to identify a unique inhibitor that binds to the same region of the NS5B enzyme, we initiated a high throughput screening program. Representative compounds from the BMS proprietary compound collection were screened for their ability to inhibit the production of viral RNA by a truncated NS5B genotype 1b enzyme at 50 μ M concentration. The compounds that were active at this concentration were subjected to a counter screen using an NS5B mutant containing the single point

* Corresponding author. Tel.: 203-415-8843;
e-mail address: kyle.parcella@bms.com

[†]These authors contributed equally.

[‡]Current address: Materia, 60 N San Gabriel Blvd. Pasadena, CA 91107.

[#]Current address: Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Rd Ridgefield, CT 06877

[§]Current address: ViiV Healthcare, 5 Research Parkway,
c/o Bristol-Myers Squibb Company, Wallingford CT, 06492

mutation of C316Y. The subset of compounds that were active in the wild-type enzyme and inactive in the mutant were identified as binding to NS5B in the vicinity of Cys316. This screening cascade identified the 3-alkoxyanthranilic acid **5** (Fig 2) as a single-digit micromolar inhibitor of NS5B.

Figure 2. HTS screening hit against wild-type and C316Y mutant HCV NS5B genotype 1b enzyme.⁶

This lead was soaked into crystals of a truncated NS5B enzyme and X-ray quality crystals were obtained showing the compound binding in a pocket often referred to as Palm Site 2. As shown in Fig. 3, **5** binds in the region proximal to Cys316, with the central aromatic ring oriented toward this residue while the chlorobenzyl group projects into a large hydrophobic pocket.⁷ The carboxylate and benzamide carbonyls form hydrogen-bonds with the backbone NHs of Gly449 and Tyr448, respectively.

Figure 3. Co-crystal structure of **5** bound to NS5B (PDB 5TRH). Compound **5** is depicted in ball and stick representation with orange carbon atoms. Protein is shown with a white backbone cartoon and specific residues are displayed in stick representation with green carbon atoms. Hydrogen bonds are denoted as blue prolate ellipsoids. The omit Fo-Fc electron density is contoured at 2 rmsd in magenta mesh. Image generated with The PyMOL Molecular Graphics System (v. 1.8, Schrödinger, LLC).

We focused our initial optimization efforts on the central benzamide portion of the molecule. The X-ray data suggested that the benzamide NH played a role in binding affinity and *N*-methylation resulted in a >6-fold decrease in potency (**6**, Table 1). However, this was attributed to a stabilization of the bio-inactive *cis*-amide rotamer.⁸ To eliminate the issue of rotamers, the cyclic benzamides **7** and **8** were prepared. Of these, the isoindolinone was the preferred ring size and was approximately equipotent to the parent benzamide.

Table 1. Removal of the Benzamide NH.

Cmpd	IC ₅₀ (μM)
6	> 50
7	8.7
8	32

Co-crystallization of the **7**-NS5B complex showed that the ligand binds in a similar orientation as the acyclic amide, with the benzamide carbonyl and hydrophobic chlorophenyl groups residing in the same regions (Fig. 4).⁷ The ligand shifts somewhat, however, pulling the carboxylate far enough away to allow for a water molecule to bridge between Gly449 and the carboxylate oxygen, rather than forming a direct hydrogen-bond as seen in the carboxylate of **5**.

Figure 4. Bound co-crystal structure of **7** and NS5B (PDB 5TRI). Compound **7** is depicted in ball and stick representation with orange carbon atoms. Protein is shown with a white backbone cartoon and specific residues are displayed in stick representation with green carbon atoms while water molecules are shown as red spheres. Hydrogen bonds are denoted as blue prolate ellipsoids. The omit Fo-Fc electron density is contoured at 2.5 rmsd in magenta mesh. Image generated with The PyMOL Molecular Graphics System (v. 1.8, Schrödinger, LLC).

The proximity of the benzamide phenyl ring in both **5** and **7** to Arg386 and its directionality prompted us to consider appending a carboxylate to that region of the molecule with the potential of forming a salt bridge.

Table 2. Dicarboxylate SAR

9		10
Cmpd	R	IC ₅₀ (μM)
9a		4.6
9b		1.2
9c		2.1
10a		6.6
10b		4.4
10c		11
10d		1.7

Co-crystallization studies of **9b**, the most potent of the dicarboxylates, with the NS5B enzyme afforded X-ray quality crystals⁷. The co-crystal structure shows that the appended acetate indeed forms a salt bridge with Arg386, as anticipated, and additionally establishes a hydrogen-bond with the OH of Tyr415 (Fig. 5). It was anticipated that dicarboxylic acids of this type would be poorly absorbed, so we turned our attention to the possibility of replacing the benzoic acid with a more readily absorbed surrogate that would be able to maintain the ability to accept a hydrogen bond from Gly449.

Figure 5. Bound co-crystal structure of **9b** and NS5B (PDB 5TRJ). Compound **9b** is depicted in ball and stick representation with orange carbon atoms. Protein is shown with a white backbone cartoon and specific residues are displayed in stick representation with green carbon atoms. Hydrogen bonds are denoted as blue prolate ellipsoids. The omit Fo-Fc electron density is contoured at 2 rmsd in magenta mesh. Image generated with The PyMOL Molecular Graphics System (v. 1.8, Schrödinger, LLC).

A number of H-bond accepting isosteres were investigated as benzoate alternatives (Table 3). Neutral species (i.e. amides **11b** and **12b**) were not tolerated. Diffuse anions, such as tetrazole

11a or the acyl sulfonamides **11c** and **12c** maintained measurable activity but were generally weaker than the parent acids. Transposition of the carboxylic acid was also investigated and was found to have relatively minor effects on potency. This was surprising considering the fact that extended carboxylates such as *meta*-glycinamide **12d** were not anticipated to be able to form the same H-bond to Gly449 as seen in **5**. Subsequent co-crystallization of the NS5B-**12d** complex (Fig. 6) revealed that **12d** binds in the same region of the enzyme proximal to Cys316, with the ligand shifting slightly relative to **5** to allow for the extended carboxylate to accept a hydrogen-bond from the OH of Ser288⁷. This new interaction compensates for the lost hydrogen-bond to Gly449.

Table 3. Benzoate Alternatives

11

12

13

Cmpd	R	IC ₅₀ (μM)
11a		25
11b		> 50
11c		24
11d		10
12a		8.3
12b		> 50
12c		15
12d		6.2
13		0.88

Figure 6. Bound co-crystal structure of **12d** and NS5B (PDB 5TRK). Compound **12d** is depicted in ball and stick representation with orange carbon atoms. Protein is shown with a white backbone cartoon and specific residues are displayed in stick representation with green carbon atoms while water molecules are shown as red spheres. Hydrogen bonds are denoted as blue prolate ellipsoids. The omit Fo-Fc electron density is contoured at 2 rmsd in magenta mesh. Image generated with The PyMOL Molecular Graphics System (v. 1.8, Schrödinger, LLC).

The process of relocating the carboxylic acid of **5** led to a shifted binding mode in **12d** that is similar to what was seen in the co-crystal structure of the NS5B-**7** complex (Fig. 4). Combining the two structural modifications into a hybrid molecule **13** resulted in a synergistic effect on potency with the IC₅₀ value = 0.88 μM. Despite the improvement in NS5B enzyme potency compared to the initial lead **5**, **13** lacked whole cell activity in the HCV replicon assay (EC₅₀ > 100 μM)⁹ presumably due to the presence of the carboxylic acid moiety which may limit cell permeability. Further work is clearly needed to gain whole cell activity in this series but this was not pursued in deference to an alternative chemotype (*vide infra*).

In summary, functionalized anthranilic acid derivatives have been identified to bind in an allosteric site located at the junction of the thumb and palm regions of NS5B polymerase. This initial SAR work guided by co-crystallographic studies resulted in compound **13** with a 10-fold improvement in potency compared to the initial HTS hit **5**. Unfortunately, **13** was inactive in a replicon assay, indicating that considerably additional optimization may be required. However, the X-ray crystallographic observations were exploited after an overlay of the bound forms of anthranilic acid **5** with the benzofuran **1** suggested an interesting avenue for drug design (Fig. 7). The central benzene ring of **5** projects in a direction that would correspond to the C5 position of the benzofuran, a relationship that suggested the potential for merging elements of both chemotypes by exploring substitution at the C5 position of **1**. An exploration based on this hypothesis as a means of improving activity ultimately led to an inhibitor which was advanced into phase 1 clinical studies¹⁰.

Figure 7. Overlay of crystal structures NS5B bound to **5** and **1** which are depicted in ball and stick representation with orange and green carbon atoms, respectively.

Acknowledgments

I'd like to thank all those who helped make this paper possible, including Mark Witmer and especially Kyle Eastman for his time editing and mentorship. Use of the IMCA-CAT beamline 17-ID and 17-BM at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Hauptman-Woodward Medical Research Institute. This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

References and notes

1. Hanafiah M, Flansman G, Wiersma S. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology*. **2013** 57, 4, 1333-42.
2. Centers for Disease Control and Prevention: Viral Hepatitis- Hepatitis C. www.cdc.gov/hepatitis/hcv. Accessed 10/26/15.
3. Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T. Ross, B. S. Nucleoside, Nucleotide, and Non-Nucleoside Inhibitors of Hepatitis C Virus NS5B RNA-Dependent RNA-Polymerase *J. Med. Chem.*, **2012**, 55, 2481–2531.
4. Kwong, A. The HCV Revolution Did Not Happen Overnight. *ACS Med. Chem. Lett.*, **2014**, 5, 214–220.
5. Pfefferkorn, J.; Greene, M.; Nugent, R.; Gross, R.; Mitchell, M.; Finzel, B.; Harris, M.; Wells, P.; Shelly, J.; Anstadt, R.; Kilkuskie, R.; Kopta, L.; Schwende, F. Inhibitors of HCV NS5B polymerase. Part I: Evaluation of the southern region of (2Z)-2-(benzoylamino)-3-(5-phenyl-2-furyl)acrylic acid. *Bioorg. Med. Chem. Lett.* **2005**, 15, 2481-2486.
6. IC₅₀ values were determined in a HCV NS5B RdRP enzyme assay described in Wang Y-K.; Rigat K.; Roberts S.; Gao M. *Anal. Biochem.* **2006**, 359, 106-111.
7. Coordinates and data for structures of the following compounds in complex with NS5B 1b WT have been deposited in the wwPDB with the following IDs: **5** (5TRH), **7** (5TRI), **9b** (5TRJ), **12d** (5TRK).
8. Itai, A; Toriumi, Y; Tonrioka, N; Kagechika, H; Isao Azumaya, I; Shudo, K. *Tet. Lett.* **1989**, 30, 45, 6177-6180.
9. EC₅₀ values were determined in a HCV replicon luciferase whole cell assay, as described in Eastman, K.; Parcella, K. Kadow, J. US Patent 20140275154.
10. Yeung, K-S; Parcella, K.; Beno, B.; Bender, J; Grant-Young, K.; Rigat, K.; Wang, Y-K; Liu, M.; Lemm, J.; Mosure, K.; Hanumegowda, U.; Zhuo, X.; Parker, D.; Sinz, M.; Santone, K.; Smith, D.; Li, J.; Fraunhofer, K.; Delmonte, A.; Colston, E.; Pasquinelli, C.; Gao, M.; Meanwell, N.; Roberts, S.; Knipe, J.; Kadow, J. Discovery of BMS-929075 an HCV NS5B replicase allosteric inhibitor advanced to phase 1 clinical studies. *250th ACS National Meeting & Exposition*, Boston, MA, United States, August 16-20, **2015**, MEDI-32. Manuscript in preparation.

**Discovery and Initial Optimization of
Alkoxyanthranilic Acid Derivatives as Inhibitors
of HCV NS5B Polymerase**

Leave this area blank for abstract info.

Kyle Parcella, Andrew Nickel, Brett R. Beno, Steven Sheriff, Changhong Wan, Ying-Kai Wang,
Susan B. Roberts, Nicholas A. Meanwell, John F. Kadow