Hepatitis C virus (HCV) NS5B polymerase inhibitor | BI-0588

Table of contents

Summary	**2**
Chemical Structure	**2**
Highlights	**3**
Target information	**3**
***In vitro* activity**	**4**
***In vitro* DMPK and CMC parameters**	**4**
***In vivo* DMPK parameters**	**5**
***In vivo* pharmacology**	**6**
Negative control	**6**
Selectivity	**7**
Co-crystal structure of the BI probe compound and the target protein.	**7**
Reference molecule(s)	**7**
Supplementary data	**8**
References	**8**

Summary

BI-0588 is one of the most potent and specific allosteric inhibitors of HCV polymerase known, as demonstrated by its cell-based antiviral replicon activity in the single-digit nM range for genotype(GT)-1. Furthermore, BI-0588 was optimized to combine good *in vitro* metabolic stability, acceptable cell-permeability and good solubility (pH 7). Following *in vivo* cross-species PK profiling, this compound was further progressed into preclinical drug development. Hence, the molecule is suitable for *in vitro* as well as *in vivo* experiments.

Chemical Structure

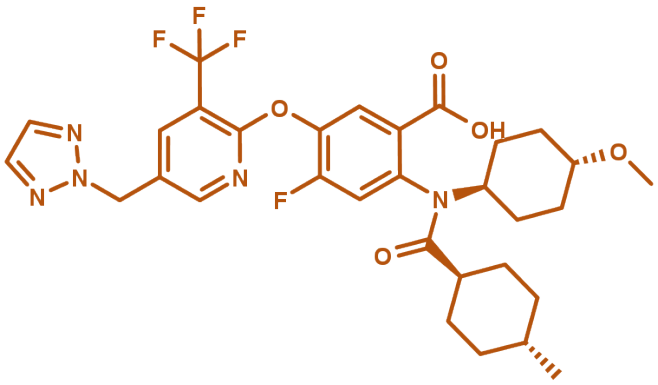

Figure 1: 2-D structure of BI-0588

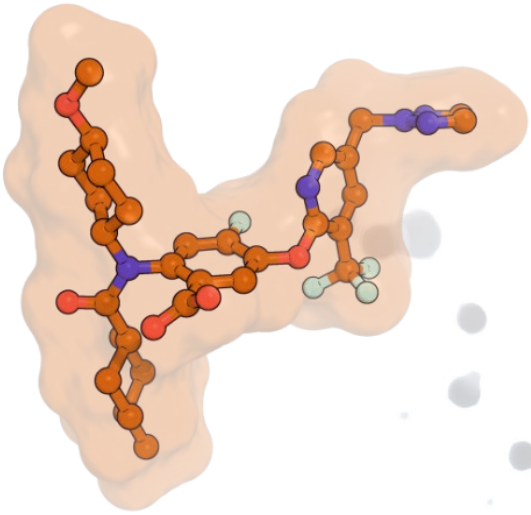

Figure 2: BI-0588, 3D conformation

Highlights

BI-0588 is a highly potent and selective allosteric thumb pocket-2 inhibitor of Hepatitis C virus (HCV) NS5B polymerase and complements our allosteric thumb pocket-1 inhibitor, deleobuvir. It has demonstrated optimized antiviral activity in a cell-based replicon system, with activity in the low nanomolar range for genotype-1 HCV replication. Its overall CMC-ADME-PK profile is good. BI-0588 is also suitable for both *in vitro* and *in vivo* experiments, as it has shown sufficient plasma exposure in rodents and was found to predominantly localize to the liver.

Target information

HCV NS5B is an RNA-dependent RNA polymerase that is essential for the replication of the genome of the hepatitis C virus. BI-0588 inhibits the polymerase activity by binding to the allosteric pocket-2 located in the thumb domain of NS5B.[5]

Figure 3: Binding model of BI-0588 on basis of an X-ray structure with a related inhibitor (PDB code: 4JJS)

In vitro activity

BI-0588 is a potent and specific allosteric pocket-2 inhibitor of the virally-encoded NS5B polymerase, and inhibited genotype (GT)-1 HCV replication in the cell-based replicon system in the single-digit nM range with EC_{50}= 2.3 nM and EC_{50} = 3.0 nM for GT1a and GT1b, respectively.

PROBE NAME / NEGATIVE CONTROL	BI-0588	BI-0900
MW [Da]	633.6	551.5
HCV-POLd21 (IC_{50}) [nM][a]	60 nM	6,400 nM
HCVLUC1 (EC_{50}) [nM][b]	3.3 nM	>10,000 nM
Rep GT 1a/1b (EC_{50}) [nM][c]	2.3 / 3.0 nM	

[a] Scintillation proximity HCV polymerase assay with recombinant protein His-NS5Bd21. This assays measures the incorporation of 3H-UTP during the elongation of RNA primer.

[b] Cell-based luciferase reporter HCV RNA replication assay, MP-1 cells with 10% FBS, 28 h incubation.

[c] Cell-based HCV replicon assay using RT-PCR for RNA quantification, genotype background 1a and 1b, Huh7 cells, 72 h incubation.

In vitro DMPK and CMC parameters

BI-0588 is highly soluble at neutral pH while being cell-permeable. A hallmark is its good metabolic stability in hepatocyte across several species.

PROBE NAME / NEGATIVE CONTROL	BI-0588	BI-0900
$logD_{7.4}$	1.8	n.a.
Solubility @ pH 6.8 [µg/ml]	193	n.a.
CACO permeability @ pH 7.4 [$*10^{-6}$ cm/s]	3.0	n.a.

CACO efflux ratio	2.7	n.a.
Microsomal stability (human) [% Q_H]	<24%	n.a.
Hepatocyte stability (human / dog / rat) [% Q_H]	<12 / <19 / 10	n.a.
Plasma protein binding (human / dog / rat) [%]	99.6 / 99.5 / 99.8	n.a.
hERG [inh. % @ 10 µM]	1.9%	n.a.
CYP 3A4 (IC_{50}) [µM]	8.1	n.a.
CYP 2C9 (IC_{50}) [µM]	6.5	n.a.
CYP 2C19 (IC_{50}) [µM]	9.4	n.a.
CYP 2D6 (IC_{50}) [µM]	>30	n.a.
CYP 1A2 (IC_{50}) [µM]	>30	n.a.

In vivo DMPK parameters

BI-0588 demonstrates sufficient plasma exposure in rat and dog species. As often observed for this anthranilic acid chemical series, BI-0588 illustrates that *in vitro* hepatic clearance was not predictive of the *in vivo* clearance, and biliary excretion of the parent compound significantly contributes to clearance mechanism.

BI-0588	DOG	RAT
Clearance [% Q_H]	49	44
Mean residence time after iv dose [l/kg]	1.0	3.3
t_{max} [h]	0.7	1.0

C_{max} [nM]	1,100	650
F [%]	44	41
V_{ss} [l/kg]	1.0	6.4

Oral dosing: 5 mg/kg (1% MP, 0.3% Tween-80, 0.5% methocel); *i.v.* dosing: 2 mg/kg (70% PEG-400)

In vivo pharmacology

During the course of this drug development project, the lack of a small animal model to study HCV biology and virus-host interaction imposed an additional challenge.

Boehringer Ingelheim's scientists developed a novel *in vitro-in vivo* proportionality method to assess a human **Liver Corrected Inhibitory Quotient** (LCIQ)[3] predictive of clinical efficacy. Given that Hepatitis-C virus replicates predominantly in the human liver, the main objective was to predict the local drug concentration – i.e. drug's concentration within the human liver at C_{min} – and the required dose to achieve such local exposure.

Plasma exposure and liver concentration were measured in rodents, and then coupled with allometry scaling to guide candidate selection.

Research Target Profile: LCIQ >500 [(plasma C_{min})/ EC_{50}]*$Kp_{(liver)}$ >500]

Human dose prediction to achieve LCIQ500: 357 mg (bid)

[Rep Gt1b EC_{50}: 0.003 µM; liver Kp: 78; estimated C_{min}: 0.02 µM; predicted C_{max}: 0.80 µM]

We found that BI-0588 localizes predominantly into the liver where HCV mainly replicates, given its high liver partitioning coefficient ([liver]/[plasma]= Kp_{liver}: 78 in rodents). Thus, BI-0588 reached candidate selection milestone based on its propensity to reach LCIQ>500 (liver-corrected inhibitory quotient greater than 500) with a human-predicted dosing of 357 mg (bid).

Negative control

Despite close structural similarity, BI-0900 bears a tetrahydropyrane (THP) ring instead of a 4-methylcyclohexane ring, thus imparting beneficial interaction in the deep lipophilic subpocket. This translate into a 107-fold and >3000-fold potency shift in HCV-POLd21 and HCV Luc1 assays, respectively.

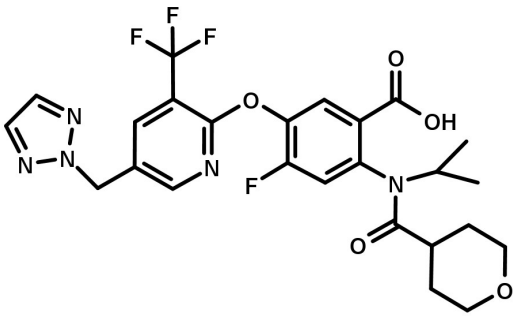

Figure 4: BI-0900 which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	BI-0588	BI-0900
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

The Xray crystal structure of the target in complex with a related inhibitor is available (PDB code: 4JJS)[2].

Reference molecule(s)

See reference 6.

Supplementary data

2-D structure files can be downloaded free of charge from opnMe.

References

1. Stammers T.A., Coulombe R., Duplessis M., Fazal G., Gagnon A., Garneau M., Goulet S., Jakalian A., LaPlante S., Rancourt J., Thavonekham B., Wernic D., Kukolj G., Beaulieu PL. Anthranilic acid-based Thumb Pocket 2 HCV NS5B polymerase inhibitors with sub-micromolar potency in the cell-based replicon assay. *Bioorg. Med. Chem. Lett.* **2013**, 23, 6879-6885. DOI: https://doi.org/10.1016/j.bmcl.2013.09.102, PubMed.
2. Hucke O., Coulombe R., Bonneau P., Bertrand-Laperle M., Brochu C., Gillard J., Joly MA., Landry S., Lepage O., Llinàs-Brunet M., Pesant M., Poirier M., Poirier M., McKercher G., Marquis M., Kukolj G., Beaulieu PL., Stammers TA. Molecular Dynamics Simulations and Structure-Based Rational Design Lead to Allosteric HCV NS5B Polymerase Thumb Pocket 2 Inhibitor with Picomolar Cellular Replicon Potency. *J. Med. Chem.* **2014**, 57, 1932-1943. DOI: https://doi.org/10.1021/jm4004522, PubMed.
3. Duan J., Bolger G., Garneau M., Amad M., Batonga J., Montpetit H., Otis F., Jutras M., Lapeyre N., Rhéaume M., Kukolj G., White PW., Bethell RC., Cordingley MG. The Liver Partition Coefficient-Corrected Inhibitory Quotient and the Pharmacokinetic-Pharmacodynamic Relationship of Directly Acting Anti-Hepatitis C Virus Agents in Humans. Antimicrob Agents Chemother. **2012**, 56(10), 5381-5386. DOI: https://aac.asm.org/content/56/10/5381, PubMed.
4. Beaulieu PL., Coulombe R., Duan J., Fazal G., Godbout C., Hucke O., Jakalian A., Joly MA., Lepage O., Llinàs-Brunet M., Naud J., Poirier M., Rioux N., Thavonekham B., Kukolj G., Stammers TA. Structure-based design of novel HCV NS5B thumb pocket 2 allosteric inhibitors with submicromolar gt1 replicon potency: Discovery of a quinazolinone chemotype. *Bioorg. Med. Chem. Lett.* **2013**, 23, 4132-4140. DOI:10.1016/j.bmcl.2013.05.037, PubMed.
5. Beaulieu PL., Bös M., Cordingley M.G., Chabot C., Fazal G., Garneau M., Gillard J.R., Jolicoeur E., LaPlante S., McKercher G., Poirier M., Poupart M.A., Tsantrizos Y.S., Duan J., Kukolj G. Discovery of the First Thumb Pocket 1 NS5B Polymerase Inhibitor (BILB 1941) with Demonstrated Antiviral Activity in Patients Chronically Infected with Genotype 1 Hepatitis C Virus (HCV). *J. Med. Chem.* **2012** 55, 7650-7666. DOI:https://pubs.acs.org/doi/10.1021/jm3006788, PubMed.
6. Court JJ., Poisson C., Ardzinski A., Bilimoria D., Chan L., Chandupatla K., Chauret N., Collier P.N., Das S.K., Denis F., Dorsch W., Iyer G., Lauffer D., L'Heureux L., Li P., Luisi B.S., Mani N., Nanthakumar S., Nicolas O., Rao BG., Ronkin S., Selliah S., Shawgo R.S., Tang Q., Waal N.D., Yannopoulos C.G., Green J. Discovery of Novel Thiophene-Based, Thumb Pocket 2 Allosteric Inhibitors of the Hepatitis C NS5B Polymerase with Improved Potency and Physicochemical Profiles. *J. Med. Chem.* **2016**, 59, 6293-6302 DOI: 10.1021/acs.jmedchem.6b00541, PubMed.