

HCV protease inhibitor

BI-1230

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
Negative control.....	5
Selectivity.....	6
Co-crystal structure of the BI probe compound and the target protein.....	6
Reference molecule(s).....	6
Supplementary data	6
References.....	6

Summary

BI-1230 is a nanomolar inhibitor of HCV protease and of viral replication with good *in vivo* PK characteristics.

Chemical Structure

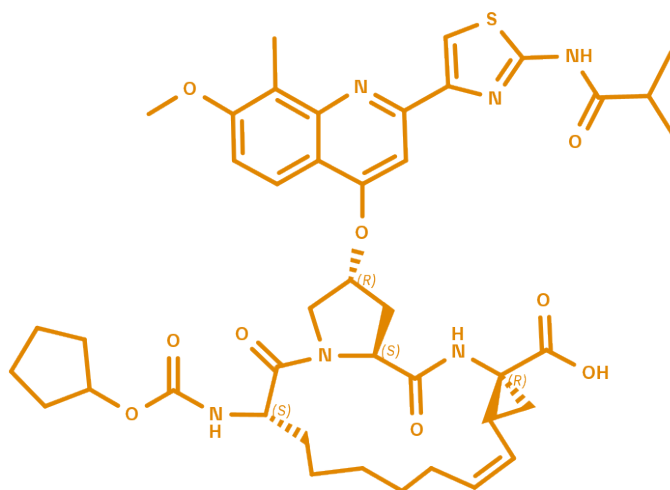


Figure 1: 2D structure of BI-1230, an inhibitor of HCV NS3 protease

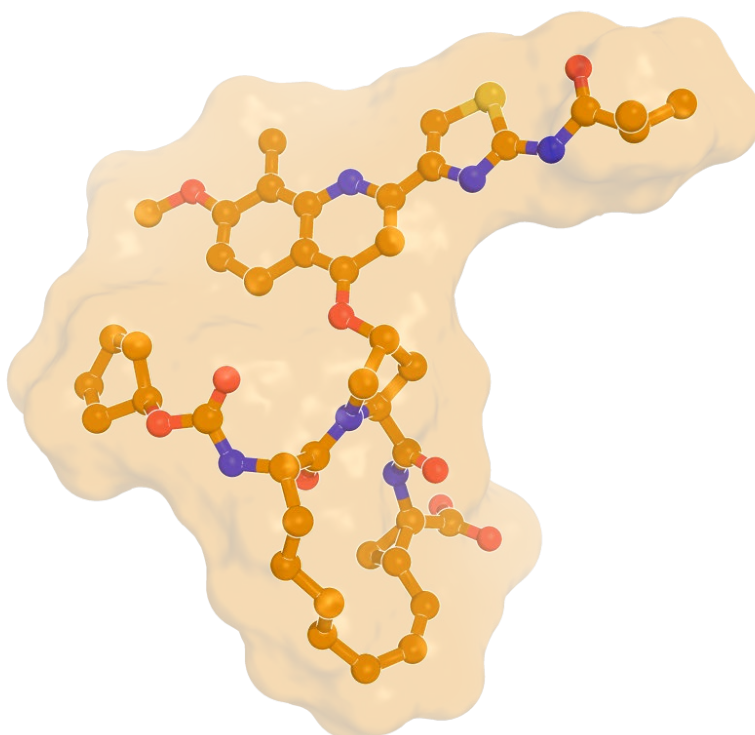


Figure 2: 3D structure of BI-1230, an inhibitor of HCV NS3 protease

Highlights

BI-1230 is a single-digit nanomolar inhibitor of HCV NS3 protease activity and of viral replication. It binds to the active site of NS3 and was shown to be highly selective against other serine/cysteine proteases. BI-1230 shows good *in vivo* PK properties, including half-life and bioavailability. As such, this compound is a valuable tool for both *in vitro* and *in vivo* studies. We also offer [faldaprevir](#) from our infectious diseases pipeline for pre-clinical research purposes.

Target information

HCV NS3 protease is a 180-amino acid chymotrypsin-like serine protease. Its function is the auto-proteolytic cleavage of HCV viral polyprotein (~3000 aa) into individual, non-structural (NS) proteins with various functions. Thus, it is an essential component of HCV replication and infectivity. The NS3 protein contains two functional domains: a serine protease- and a helicase domain. The active site of NS3 is located in the shallow and wide protein-protein interaction surface of these domains. BI-1230 and other known NS3 inhibitors cover significant parts of this interaction surface in addition to the active site. Boehringer Ingelheim was the first company to establish proof-of-concept in humans for an HCV NS3 protease inhibitor as a treatment of HCV infection⁴.

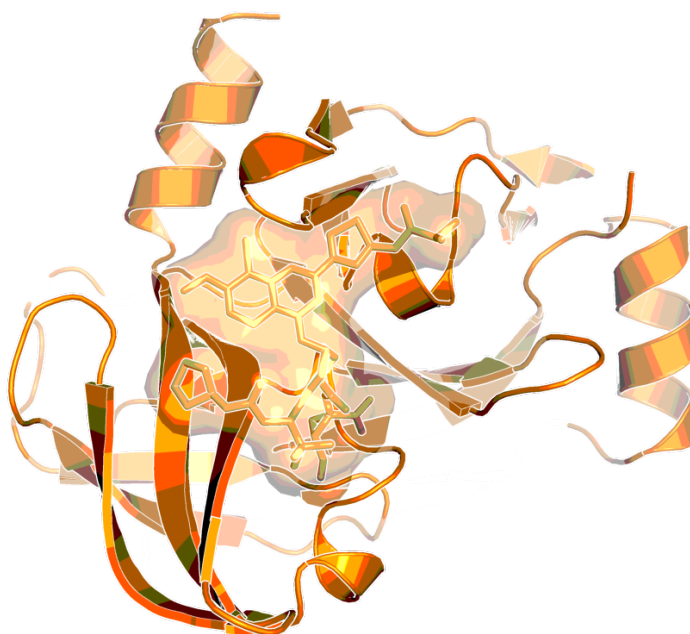


Figure 3: 3: X-ray structure of HCV NS3 protease with faldaprevir, a close analog of BI-1230 (PDB code: 3p8n)

In vitro activity

PROBE NAME / NEGATIVE CONTROL	BI-1230	BI-1675
MW [Da, free base] ^a	817.0	554.6
IC ₅₀ [nM] ^b	6.7	4870
EC ₅₀ [nM], replicon assay, genotype 1a ^c	4.6	n.d.
EC ₅₀ [nM], replicon assay, genotype 1b ^c	<1.8	n.d.

^a For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

^b Enzymatic assay, NS3-NS4A heterodimer, fluorogenic substrate, 60 min incubation

^c Cell-based HCV PV RNA replication Luciferase reporter assay, genotype background 1a and 1b, Huh7 cells, 72 h incubation

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-1230	BI-1675
logD @ pH 11	2.7	0.4
Solubility @ pH 7 [µg/mL]	<39	n.d.
Caco-2 permeability AB @ pH 7.4 [$\times 10^{-6}$ cm/s]	8.7	0.1
Caco-2 efflux ratio	0.3	2.5
Microsomal stability [% Q _H]	<24	n.d.
Plasma Protein Binding human [%]	99.9	n.d.
CYP 1A2 (IC ₅₀) [µM]	13.9	n.d.
CYP 2C9 (IC ₅₀) [µM]	4	n.d.
CYP 2C19 (IC ₅₀) [µM]	2	n.d.
CYP 2D6 (IC ₅₀) [µM]	>30	n.d.

In vivo DMPK parameters

In vivo DMPK parameters of BI-1230 in rat and dog.^{a,b}

ROUTE		RAT ^A	DOG ^B
<i>i.v.</i>	Clearance [mL/min/kg]	15	1.9
	Mean residence time after <i>i.v.</i> dose [h]	2.3	3.4
	V _{ss} [L/kg]	2.1	0.4
<i>p.o.</i>	T _{1/2} [h]	2.1	5.1
	t _{max} [h]	1.8	1.7
	C _{max} [nM]	405	7370
	AUC _{0-inf} [nM*h]	2550	49700
	F [%]	42	92

^a *i.v.* dose: 1.6 mg/kg, *p.o.* dose: 4 mg/kg

^b *i.v.* dose: 1.1 mg/kg, *p.o.* dose: 2.2 mg/kg

Negative control

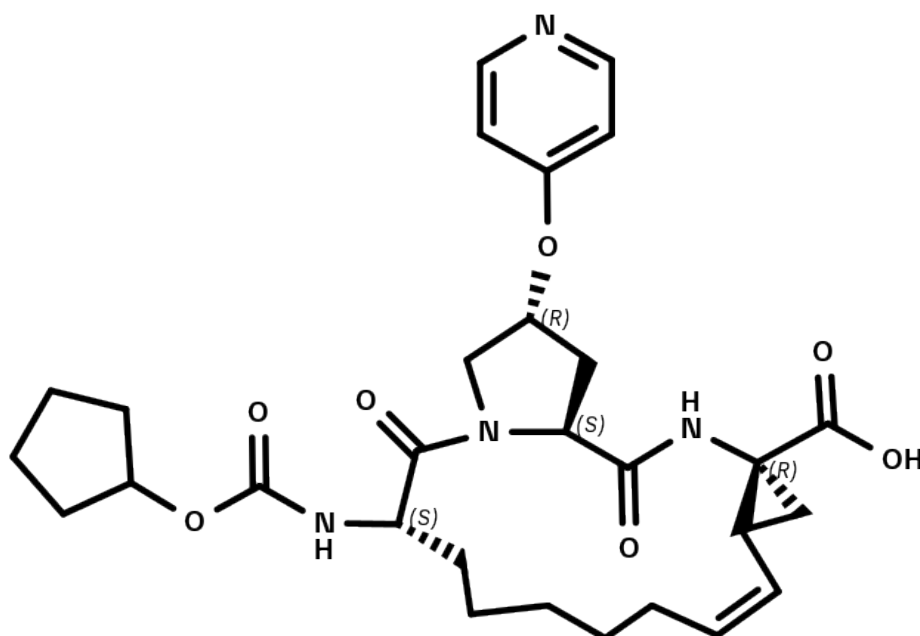


Figure 4: Structure of BI-1675 offered as negative control

Selectivity

BI-1230 is highly selective against other serine/cysteine proteases.

SELECTIVITY DATA AVAILABLE	BI-1230	BI-1675
SafetyScreen44™ with kind support of  eurofins	Yes	Yes
PDSP ⁶	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

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

An X-ray structure of BI-1230 in complex with NS3 is not available. However, the structure with the highly related [faldaprevir](#) (BI 201335) was solved.

Reference molecule(s)

For a recent review of HCV NS3 protease inhibitors see reference 5.

Supplementary data

2D structures can be downloaded free of charge from [openMe](#).

References

1. Tsantrizos Y. S., Bolger G., Bonneau P., Cameron D. R., Goudreau N., Kukolj G., LaPlante S. R., Llinàs-Brunet M., Nar H., Lamarre D. Macrocyclic inhibitors of the NS3

- protease as potential therapeutic agents of hepatitis C virus infection *Angew Chem Int Ed Engl* **2003**, 42(12), 1356–1360. DOI: [10.1002/anie.200390347](https://doi.org/10.1002/anie.200390347), PubMed: [12671967](https://pubmed.ncbi.nlm.nih.gov/12671967/).
2. Wei X., Shu C., Haddad N., Zeng X., Patel N. D., Tan Z., Liu J., Lee H., Shen S., Campbell S., Varsolona R. J., Busacca C. A., Hossain A., Yee N. K., Senanayake C. H. A highly convergent and efficient synthesis of a macrocyclic hepatitis C virus protease inhibitor BI 201302 *Org Lett* **2013**, 15(5), 1016–1019. DOI: [10.1021/ol303498m](https://doi.org/10.1021/ol303498m), PubMed: [23406520](https://pubmed.ncbi.nlm.nih.gov/23406520/).
 3. Llinas-Brunet M., Bailes M., Bhardwaj P., Forgione P., Ghironi E., Goudreau N., Halmos T., Rancourt J. Macrocyclic peptides active against the hepatitis c virus **2005**, WO 2005/028501A1, US 8552205. Patent: [WO2005028501A1](https://patents.google.com/patent/WO2005028501A1).
 4. Lamarre D., Anderson P. C., Bailey M., Beaulieu P., Bolger G., Bonneau P., Bös M., Cameron D. R., Cartier M., Cordingley M. G., Faucher A.-M., Goudreau N., Kawai S. H., Kukolj G., Lagacé L., LaPlante S. R., Narjes H., Poupart M.-A., Rancourt J., Sentjens R. E., St George R., Simoneau B., Steinmann G., Thibeault D., Tsantrizos Y. S., Weldon S. M., Yong C.-L., Llinàs-Brunet M. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus *Nature* **2003**, 426(6963), 186–189. DOI: [10.1038/nature02099](https://doi.org/10.1038/nature02099), PubMed: [14578911](https://pubmed.ncbi.nlm.nih.gov/14578911/).
 5. McCauley J. A., Rudd M. T. Hepatitis C virus NS3/4a protease inhibitors *Curr Opin Pharmacol* **2016**, 30, 84–92. DOI: [10.1016/j.coph.2016.07.015](https://doi.org/10.1016/j.coph.2016.07.015), PubMed: [27544488](https://pubmed.ncbi.nlm.nih.gov/27544488/).
 6. Kroeze W. K., Sassano M. F., Huang X.P., Lansu K., McCorvy J. D., Giguère P. M., Sciaky N., Roth B. L. PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome *Nat Struct Mol Biol*. **2015**, 22(5):362-9. DOI: [10.1038/nsmb.3014](https://doi.org/10.1038/nsmb.3014), PubMed: [PubMed](https://pubmed.ncbi.nlm.nih.gov/).