HCV protease inhibitor | BI-1388

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
Selectivity	6
Co-crystal structure of the BI probe compound and the target protein	6
Reference molecule(s)	6
Summary	6
References	7

Summary

BI-1388 is a highly selective inhibitor of NS3 protease with nanomolar potency across various HCV genotypes and against resistant mutants D168V and R155K.

Chemical Structure

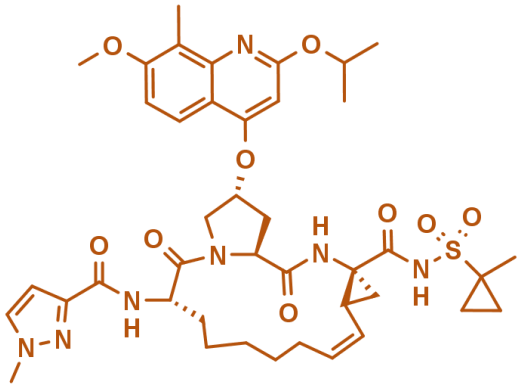

Figure 1: 2-D structure of BI-1388, an inhibitor of HCV NS3 protease

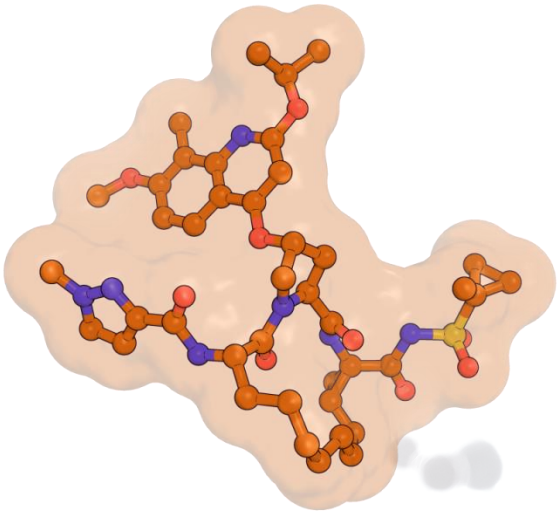

Figure 2: 3-D structure of BI-1388, an inhibitor of HCV NS3 protease

Highlights

BI-1388 binds to the active site of NS3 that is located in the shallow and broad protein-protein interaction surface of the protease- and the helicase domain of the enzyme. It is a nanomolar to picomolar inhibitor of protease activity and of viral replication for various HCV genotypes and for resistant mutants D168V and R155K. BI-1388 was shown to be highly selective against other serine/cysteine proteases, and it is characterised by an exceptional liver partitioning.

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 infection [4].

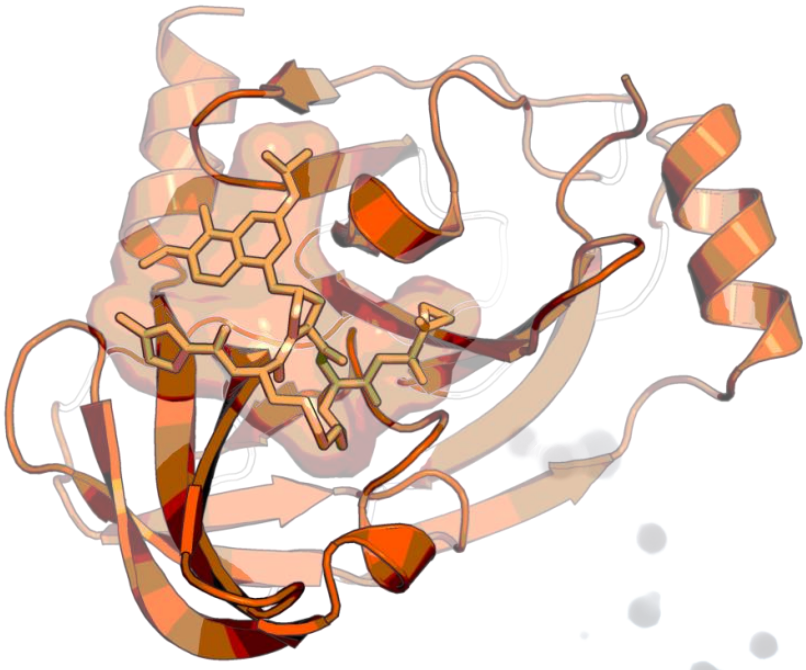

Figure 3: BI-1388 bound to the active site of NS3 (PDB code: 4i31)[3]

In vitro activity

PROBE NAME	BI-1388
MW [Da]	820
IC_{50} [nM][a], WT enzyme, genotype 1a	0.48
IC_{50} [nM][a], WT enzyme, genotype 1b	1.1
IC_{50} [nM][a], WT enzyme, genotype 2a	0.14
IC_{50} [nM][a], WT enzyme, genotype 3a	12
IC_{50} [nM][a], WT enzyme, genotype 4a	0.23
IC_{50} [nM][a], WT enzyme, genotype 5a	0.24
IC_{50} [nM][a], WT enzyme, genotype 6a	0.16
IC_{50} [nM][a], R155K, genotype 1a	4.7
IC_{50} [nM][a], D168V, genotype 1a	58
IC_{50} [nM][a], A156T, genotype 1b	2.7
IC_{50} [nM][a], D156V, genotype 1b	4.3
IC_{50} [nM][a], D168A, genotype 1b	16
IC_{50} [nM][a], D168V, genotype 1b	15
EC_{50} [nM], WT, replicon assay, genotype 1a[b]	0.11
EC_{50} [nM], R155K, replicon assay, genotype 1a[b]	1.0
EC_{50} [nM], WT, replicon assay, genotype 1b[b]	0.11
EC_{50} [nM], D168V, replicon assay, genotype 1b[b]	0.14

[a] Enzymatic assay, see ref. 3 for assay details
[b] Cell-based HCV RNA replication Luciferase reporter assay, see ref. 3 for assay details

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-1388
Aqueous solubility @ pH 7 [µg/ml]	3.4
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	10
CACO efflux ratio	1.2
Microsomal stability: $t_{½}$ [min], RLM	138
Plasma protein binding human [%]	99.800

In vivo DMPK parameters

Pharmacokinetic profile of BI-1388 in rat [a]

ROUTE		
i.v.	CL [ml/min/kg]	19
	T ½ (h)	2.6
	V_{ss} [l/kg][b]	0.97
p.o.	F(%)	14
	Cmax(µM)	1.3
	AUC0-inf(µM*h)	1.4

[a] Dose = i.v., 4 mg/kg (70% PEG-400 and 30% water); p.o., 10 mg/kg (1% MP, 0.3% Tween-80, and Methocel 0.5%)

Selectivity

BI-1388 has been shown to be highly selective against other serine/cysteine proteases.[3]

Activity of BI-1388 against other proteases

PROTEASE	IC_{50}[A]
Caspase I	>10
Cathepsin B	>30
Cathepsin G	>10
Chymotrypsin	>25
Elastase, leucocyte	>30
MMP-1	>10
Trypsin	>10

[a]All values are in µM. Value given is the highest concentration tested, which in all cases gave <50% inhibition.

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

The X-ray structure of BI-1388 in complex with NS3 is available (PDB code: 4i31, 4i32, 4i33 – complexes with WT and resistant mutants D168V and R155K)

Reference molecule(s)

For a recent review of HCV NS3 protease inhibitors see reference 6.

Summary

BI-1388 is a nanomolar to picomolar inhibitor of HCV NS3 protease activity and of viral replication for various HCV genotypes and for resistant mutants D168V and R155K. BI-1388 was shown to be highly selective against other serine/cysteine proteases.

References

1. Youla S. Tsantrizos, Gordon Bolger, Pierre Bonneau, Dale R. Cameron, Nathalie Goudreau, George Kukolj, Steven R. LaPlante, Montse Llinàs-Brunet, Herbert Nar, Daniel Lamarre Macrocyclic Inhibitors of the NS3 Protease as Potential Therapeutic Agents of Hepatitis C Virus Infection, *Angw. Chem. Int. Ed.* **2003**, *42*, 1355-1360, DOI: 10.1002/anie.200390347, PubMed.
2. Xudong Wei, Chutian Shu, Nizar Haddad, Xingzhong Zeng, Nitinchandra D. Patel, Zhulin Tan, Jianxiu Liu, Heewon Lee, Sherry Shen, Scot Campbell, Richard J. Varsolona, Carl A. Busacca, Azad Hossain, Nathan K. Yee, and Chris H. Senanayake A Highly Convergent and Efficient Synthesis of a Macrocyclic Hepatitis C Virus Protease Inhibitor BI 201302, *Org. Lett.* **2013**, *15*, 1016-1019. DOI: 10.1021/ol303498m, PubMed.
3. J. A. O'Meara, C. T. Lemke, C. Godbout, G. Kukolj, L. Lagacé, B. Moreau, D. Thibeault, P. W. White, M. Llinàs-Brunet, Molecular Mechanism by Which a Potent Hepatitis C Virus NS3-NS4A Protease Inhibitor Overcomes Emergence of Resistance, *J. Biol. Chem.* **2013**, *288*, 5673-5681, DOI: 10.1074/jbc.M112.439455, PubMed
4. Daniel Lamarre, Paul C. Anderson, Murray Bailey, Pierre Beaulieu, Gordon Bolger, Pierre Bonneau, Michael Bös, Dale R. Cameron, Mireille Cartier, Michael G. Cordingley, Anne-Marie Faucher, Nathalie Goudreau, Stephen H. Kawai, George Kukolj, Lisette Lagacé, Steven R. LaPlante, Hans Narjes, Marc-André Poupart, Jean Rancourt, Roel E. Sentjens, Roger St George, Bruno Simoneau, Gerhard Steinmann, Diane Thibeault, Youla S. Tsantrizos, Steven M. Weldon, Chan-Loi Yong, Montse Llinàs-Brunet, An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus *Nature* **2003**, *426*, 186-189, DOI: 10.1038/nature02099, PubMed.
5. B. Moreau, J. A. O'Meara, J. Bordeleau, M. Garneau, C. Godbout, V. Gorys, M. Leblanc, E. Villemure, P. W. White, M. Llinàs-Brunet, Discovery of Hepatitis C Virus NS3-4A Protease Inhibitors with Improved Barrier to Resistance and Favorable Liver Distribution, *J. Med. Chem.* **2014**, *57*, 1770-1776, DOI: 10.1021/jm400121t, PubMed
6. J. A. McCauley, T. M. Rudd, Hepatitis C virus NS3/4a protease inhibitors, *Curr. Opin. Pharmacol.* **2016**, *30*, 84-92, DOI: 10.1016/j.coph.2016.07.015, PubMed