opnMe by Boehringer Ingelheim

LOX-1 inhibitor

BI-0115

Boehringer Ingelheim

Table of contents

Summary

BI-0115 is a small molecule inhibitor of Lectin-like ox-LDL receptor 1 (LOX-1) with a unique mode of action[1]. It displays a clean Eurofins Safety Panel 44™ profile. This tool compound has an acceptable *in vitro* profile that showed clear inhibition of oxLDL internalization. It is supplemented by the negative control BI-1580.

Chemical Structure

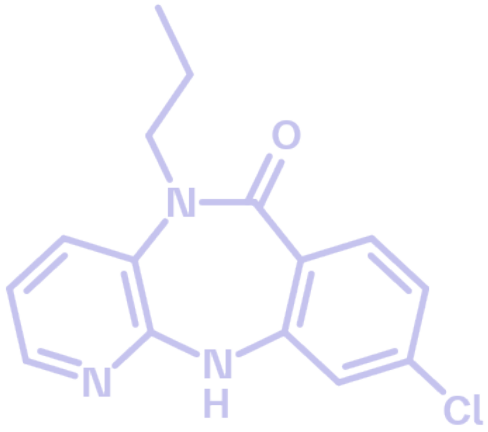

Figure 1: 2D structure of BI-0115, a small molecule inhibitor of LOX-1.

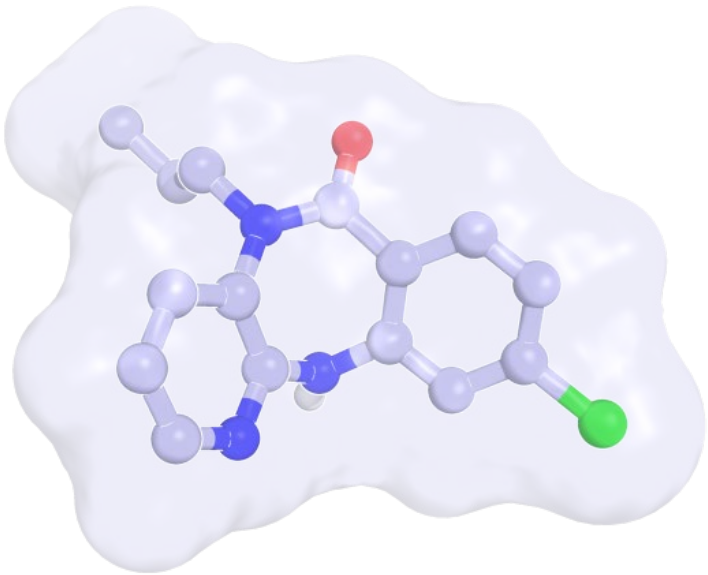

Figure 2: 3D conformation of BI-0115, a small molecule inhibitor of LOX-1.

Highlights

BI-0115 is a highly selective inhibitor of Lectin-like ox-LDL receptor 1 (LOX-1). This mechanism is mediated by stabilizing a protein-protein interaction. It has shown an acceptable *in vitro* profile with a clear inhibition of oxLDL internalization. This chemical probe may be a valuable tool to study LOX-1-mediated signal transduction pathways and cellular effects in different pathogenic pathways.

Target information

Elevated plasma levels of oxidized low-density lipoproteins (oxLDL) play a role in proatherogenic processes like plaque formation and destabilization that are postulated to affect cardiovascular health negatively[2]. Lectin-like ox-LDL receptor 1 (LOX-1) is the major cell surface receptor for oxLDL in a wide variety of different cell types and binds to and internalizes oxLDL, which leads to plaque formation in arteries[3,4]. It is a 273 amino acid long Type II membrane protein with a short N-terminal intracellular region followed by the transmembrane domain. On the extracellular side, the long neck domain is predicted to be a long alpha helix ending in the C-terminal C-type lectin-like domain (CTLD). LOX-1 is a constitutively dimeric protein mediated via an inter chain disulphide bridge at Cys140[5,6,7]. Oligomerization of LOX-1 receptors is an important step and regulated via the neck domain. It has been proposed that a complex of three LOX-1 dimers is needed to ligand ox-LDL and then internalize it via vascular endothelial cells[8,9].

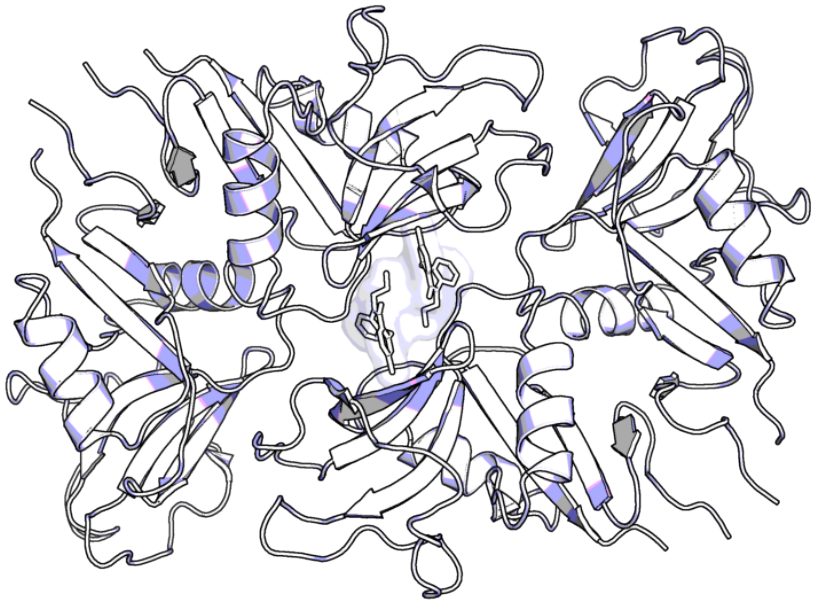

Figure 3: Stabilization of a LOX-1 receptor tetramer by two molecules of BI-0115 (PDB code 6TL9)[1].

In vitro activity

BI-0115 shows an IC_{50} potency of 5.4 µM in the LOX-1 cellular uptake assay. This compares well with a K_d value of 4.3 µM in surface plasmon resonance (SPR) and a K_d of 6.99 µM in isothermal titration calorimetry (ITC)[1]. To exclude unspecific and non-target related mechanisms, scavenger receptor class B type I (SR-BI), an alternative scavenger receptor with low sequence and structural homology to human LOX-1, has been used as counter target. BI-0115 shows no activity up to 100 µM in this assay[1].

PROBE NAME / NEGATIVE CONTROL	BI-0115	BI-1580
MW [Da, free base][a]	287.8	267.3
LOX-1 (IC_{50}) [µM][b]	5.4	>100
SR-B1 (IC_{50}) [µM][b]	>172	>100
SPR (K_d) [µM][b]	4.3	n.d.
ITC (K_d) [µM][b]	6.99	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] For a detailed description of the LOX-1, SR-B1, SPR, and ITC assays, please refer to reference 1. For any additional questions, please reach out to the opnMe team using the Contact form available on the opnMe portal.

In vitro DMPK and CMC parameters

BI-0115 displays a moderate solubility at pH 7 and a moderate permeability. Its stability in human, rat and mouse liver microsomes is not optimal, qualifying the compound primarily as an *in vitro* tool[1]. BI-0115 has a good selectivity against the hERG channel (IC_{50} > 10 µM).

PROBE NAME / NEGATIVE CONTROL	BI-0115	BI-1580
logD @ pH 11	3.4	3.3
Solubility @ pH 7 [µg/mL]	0.001	n.d.
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	30	n.d.
Caco-2 efflux ratio	0.7	n.d.
Microsomal stability (human/rat/mouse) [% Q_H]	65 / >88 / <88	n.d.
hERG (IC_{50}) [µM]	>10	n.d.

Negative control

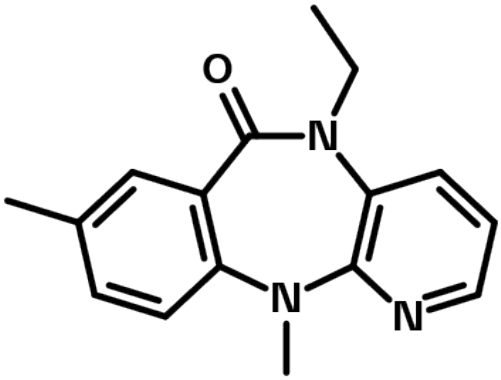

Figure 4: BI-1580 serves as a negative control.

Selectivity

BI-0115 has a clean Eurofins Safety Panel 44™ profile and it shows no hERG channel inhibition. The selectivity of BI-0115 versus other paralogues of the C-type lectin-like family has not been tested.

SELECTIVITY DATA AVAILABLE	BI-0115	BI-1580
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 X-ray structure of LOX-1 in complex with BI-0115 is available via PDB code 6TL9[1].

Reference molecules

Please see references 10 – 12.

Supplementary data

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

References

1. Schnapp G., Neubauer H., Büttner F. H., Handschuh S., Lingard I., Heilker R., Klinder K., Prestle J., Walter R., Wolff M., Zeeb M., Debaene F., Nar H., Fiegen D. A small-molecule inhibitor of lectin-like oxidized LDL receptor-1 acts by stabilizing an inactive receptor tetramer state *Commun Chem* **2020**, 3(1), 75. DOI: 10.1038/s42004-020-0321-2, PubMed: 36703453.

2. Suzuki T., Kohno H., Hasegawa A., Toshima S., Amaki T., Kurabayashi M [Masahiko], Nagai R., Kurabayashi M [Masa-hiko], Shimada K., Nakamura H., Teramoto T., Yamaguchi H., Nishiyama S., Takahashi H., Michishita I., Sugano Z., Konoshi K. Diagnostic implications of circulating oxidized low density lipoprotein levels as a biochemical risk marker of coronary artery disease *Clin Biochem* **2002**, 35(5), 347–353. DOI: 10.1016/S0009-9120(02)00326-0, PubMed: 12270763.

3. Dunn S., Vohra R. S., Murphy J. E., Homer-Vanniasinkam S., Walker J. H., Ponnambalam S. The lectin-like oxidized low-density-lipoprotein receptor: A pro-inflammatory factor in vascular disease *Biochem J* **2008**, 409(2), 349–355. DOI: 10.1042/bj20071196, PubMed: 18092947.

4. Sawamura T., Kume N., Aoyama T., Moriwaki H., Hoshikawa H., Aiba Y., Tanaka T., Miwa S., Katsura Y., Kita T., Masaki T. An endothelial receptor for oxidized low-density lipoprotein *Nature* **1997**, 386(6620), 73–77. DOI: 10.1038/386073a0, PubMed: 9052782.

5. Park H., Adsit F. G., Boyington J. C. The 1.4 angstrom crystal structure of the human oxidized low density lipoprotein receptor lox-1 *J Biol Chem* **2005**, 280(14), 13593–13599. DOI: 10.1074/jbc.m500768200, PubMed: 15695803.

6. Ohki I., Ishigaki T., Oyama T., Matsunaga S., Xie Q., Ohnishi-Kameyama M., Murata T., Tsuchiya D., Machida S., Morikawa K., Tate S. Crystal structure of human lectin-like, oxidized low-density lipoprotein receptor 1 ligand binding domain and its ligand recognition mode to OxLDL *Structure* **2005**, 13(6), 905–917. DOI: 10.1016/j.str.2005.03.016, PubMed: 15939022.

7. Nakano S., Sugihara M., Yamada R., Katayanagi K., Tate S. Structural implication for the impaired binding of W150A mutant LOX-1 to oxidized low density lipoprotein, OxLDL *Biochim Biophys Acta* **2012**, 1824(5), 739–749. DOI: 10.1016/j.bbapap.2012.02.003, PubMed: 22369967.

8. Xie Q., Matsunaga S., Niimi S., Ogawa S., Tokuyasu K., Sakakibara Y., Machida S. Human lectin-like oxidized low-density lipoprotein receptor-1 functions as a dimer in living cells *DNA Cell Biol* **2004**, 23(2), 111–117. DOI: 10.1089/104454904322759920, PubMed: 15000751.

9. Cao W., Calabro V., Root A., Yan G., Lam K., Olland S., Sanford J., Robak A., Zollner R., Lu Z., Ait-Zahra M., Agostinelli R., Tchistiakova L., Gill D., Harnish D., Paulsen J., Shih H. H. Oligomerization is required for the activity of recombinant soluble LOX-1 *The FEBS Journal* **2009**, 276(17), 4909–4920. DOI: 10.1111/j.1742-4658.2009.07190.x., PubMed: 19664054.

10. Thakkar S., Wang X., Khaidakov M., Dai Y., Gokulan K., Mehta J. L., Varughese K. I. Structure-based Design Targeted at LOX-1, a Receptor for Oxidized Low-Density Lipoprotein *Sci Rep* **2015**, 5, 16740. DOI: 10.1038/srep16740, PubMed: 26578342.

11. Falconi M., Ciccone S., D'Arrigo P., Viani F., Sorge R., Novelli G., Patrizi P., Desideri A., Biocca S. Design of a novel LOX-1 receptor antagonist mimicking the natural substrate *Biochem Biophys Res Commun* **2013**, 438(2), 340–345. DOI: 10.1016/j.bbrc.2013.07.073, PubMed: 23892036.

12. Biocca S., Iacovelli F., Matarazzo S., Vindigni G., Oteri F., Desideri A., Falconi M. Molecular mechanism of statin-mediated LOX-1 inhibition *Cell Cycle* **2015**, 14(10), 1583–1595. DOI: 10.1080/15384101.2015.1026486, PubMed: 25950192.