opnMe by Boehringer Ingelheim

Dual Vanin Inhibitor

BI-4122

Boehringer Ingelheim

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Summary

BI-4122 is a highly potent and selective dual vanin 1 and vanin 2 inhibitor of the vascular non-inflammatory molecule-1 (vanin 1) and vascular non-inflammatory molecule-2 (vanin 2). In humans, the vanin gene family encodes secreted and membrane-bound ectoenzymes, that convert pantetheine into pantothenic acid and cysteamine. BI-9534 is available as negative control.

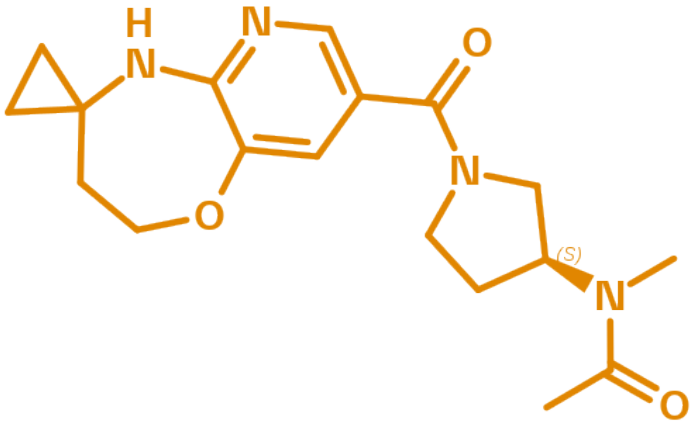

Figure 1: 2D structure of BI-4122

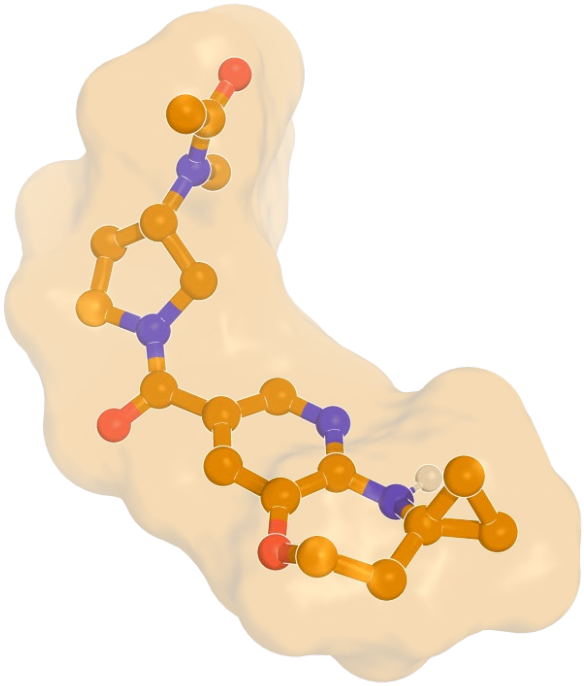

Figure 2: 3D model of BI-4122

Highlights

BI-4122 is an orally available small molecule inhibitor that blocks in a reversible, competitive manner the enzymatic activities of vanin 1 and 2, and therefore potently inhibits the conversion of pantetheine into pantothenic acid and cysteamine.

Target information

Vanin 1 and vanin 2 are single-domain enzymes tethered to the extracellular surface by a glycosylphosphatidylinositol (GPI)-linker but is also shed in from the cell surface into the extracellular milieu. The vanin enzymes function to convert pantetheine into pantothenic acid and cystamine, which is then reduced to cysteamine. Vanin 1 is expressed in immune-competent tissues as well as in intestine, liver, and kidney. Vanin 2 expression is detectable in most tissues with highest expression in spleen, kidney, and blood[1].

The vanin enzymes regulate cellular redox homeostasis, as its reaction products are capable of interfering with glutathione biosynthesis. Studies in vanin 1 knockout mice have shown that the knockout animals were more resistant to oxidative stress and showed a lower inflammatory response compared with their wild-type littermates following challenge with different injury triggers[2]. In addition, vanin 1 knockout mice are resistant to inflammatory bowel disease[3] and the vanins have been postulated to play potential roles in other disorders including malaria susceptibility[4], psoriasis, colitis-associated colon cancer, and post-arterial injury neointimal hyperplasia based on preclinical evidence[5-7].

BI-4122 is a small molecule compound that potently inhibits vanin enzymatic activity in mice and humans with nanomolar potency. It possesses a very favorable selectivity profile and DMPK properties, allowing standard oral application in dose response *in vivo* studies.

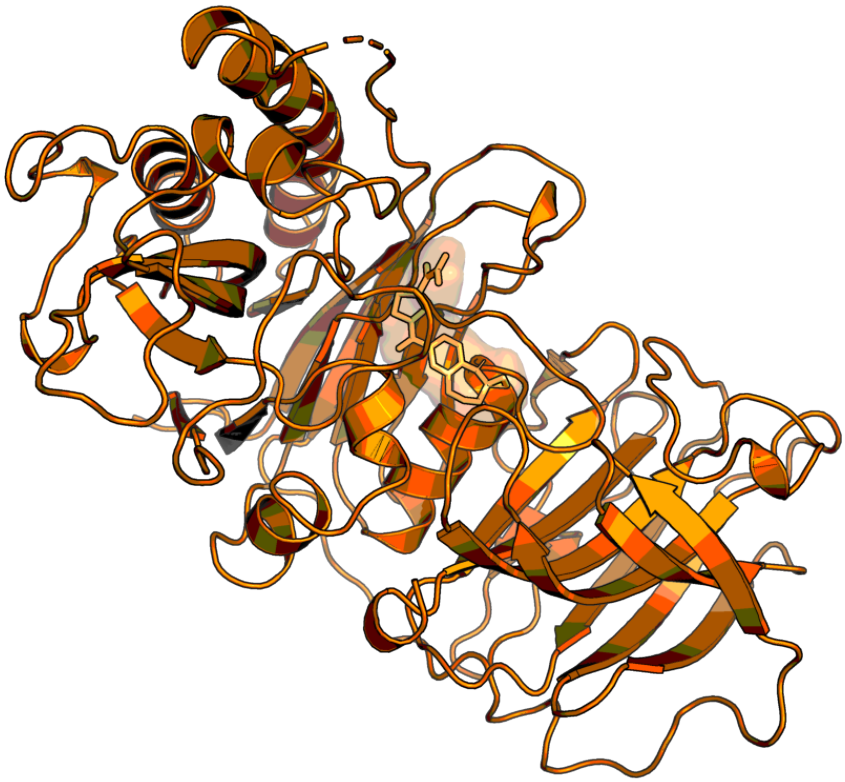

Figure 2: Model of the binding mode of BI-4122 in complex with vanin based on the X-ray structure with a closely related ligand

In vitro activity

The orally bioavailable small molecule BI-4122 inhibits the enzymatic activity of vanin 1 and vanin 2 with IC_{50} of 0.3 and 1.5 nM biochemical potency and 2 nM in a human whole blood assay. The compound is also potently inhibiting vanin enzymatic activity of mouse and rat, making it a suitable tool for model investigations in these species.

Probe name / Negative control	BI-4122	BI-9534
MW [Da][a]	344.42	420.50
Human vanin 1 (IC_{50}) [nM][b]	0.3	129.7
Human vanin 2 (IC_{50}) [nM][b]	11.0	n.a.
Vanin human whole blood (IC_{50}) [nM][c]	6.3	n.a.

[a] The molecule is supplied in salt form; for the molecular weight of the salt, please refer to the vial label.
[b] Enzymatic activity of vanin was evaluated by using D-Pantethine as a substrate and quantifying the production of pantothenic acid via label-free RapidFire mass spectrometry[8].
[c] Enzymatic conversion of D-Pantethine into pantothenic acid in human whole blood was quantified using RapidFire mass spectrometry[8].

In vitro DMPK and CMC parameters

BI-4122 has good solubility in water at all pH values. It shows high permeability in Caco-2 assay but significant efflux in the MDCK permeability assay.

Probe name / Negative control	BI-4122	BI-9534
logD @ pH 7.4, clogD @ pH 7.4	0.0, 0.7	n.a., 2.3
Solubility @ pH 7 [µg/mL]	>1,000	69 (aqueous phase buffered)
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	13.3	14
Caco-2 efflux ratio	1.5	3.6
MDCK permeability P_{appAB} @ 1µM [10^{-6} cm/s]	1.3	2.3
MDCK efflux ratio	13	33.5
Microsomal stability (human/mouse/rat) [% Q_H]	<23 / <26 / <23	76 / 81 / 84
Hepatocyte stability (human/mouse/rat) [% Q_H]	2 / 7 / 18	n.a. / 91 / 90
Plasma protein binding (human/mouse/rat) [%]	13.3 / 26.3. / 16.1	78.7 / 70.3 / 65.4
hERG (IC_{50}) [µM]	>100	>30
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2C8 (IC_{50}) [µM]	>50	n.a.
CYP 2C9 (IC_{50}) [µM]	>50	n.a.
CYP 2C19 (IC_{50}) [µM]	>50	45.8
CYP 2D6 (IC_{50}) [µM]	>50	>50
CYP 1A2 (IC_{50}) [µM]	>50	>50
CYP 2B6 (IC_{50}) [µM]	>50	>50

In vivo DMPK parameters

Pharmacokinetic (PK) properties support an *i.v.* application. In addition, in rodent animal species (rats) administration is suitable for once or twice daily oral dosing in acute or sub-chronic *in vivo* experiments.

BI-4122	Mouse	Rat
Clearance [% Q_H][a]	32.6	35.8
Mean residence time after *i.v.* dose [h][a]	0.6	3.2
t_{max} [h][b]	n.a.	0.3
C_{max} [nM][b]	n.a.	11,025
F [%][b]	n.a.	44
V_{ss} [L/kg][a]	1.1	4.7

[a] *i.v.* dose: mouse: 1 mg/kg, rat: 1mg/kg
[b] *p.o.* dose: mouse: n.a., rat: 3 mg/kg

Negative control

BI-9534, a structurally close analog, can be used as negative control.

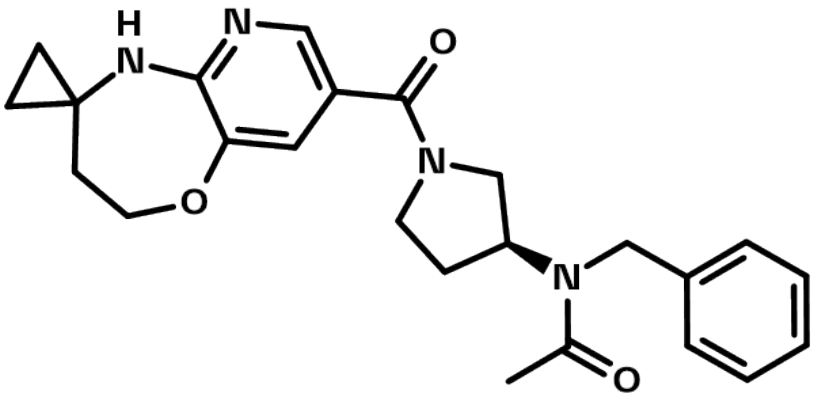

Figure 4: BI-9534 which serves as a negative control

Selectivity

BI-4122 shows no significant activity in a panel of 268 kinases and 68 enzymes/receptors tested at 10 µM. BI-9534 inhibited only one target (COX-1@CE) with > 50% at a 10µM in the SafetyScreen44™.

Selectivity data available	BI-4122	BI-9534
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No

Supplementary data

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

References

1. Kaskow B. J., Proffitt J. M., Blangero J., Moses E. K., Abraham L. J. Diverse biological activities of the vascular non-inflammatory molecules - the Vanin pantetheinases *Biochem Biophys Res Commun* **2012**, 417(2), 653–658. DOI: 10.1016/j.bbrc.2011.11.099, PubMed: 22155241.
2. Berruyer C., Martin F. M., Castellano R., Macone A., Malergue F., Garrido-Urbani S., Millet V., Imbert J., Duprè S., Pitari G., Naquet P., Galland F. Vanin-1-/- mice exhibit a glutathione-mediated tissue resistance to oxidative stress *Mol Cell Biol* **2004**, 24(16), 7214–7224. DOI: 10.1128/MCB.24.16.7214-7224.2004, PubMed: 15282320.
3. Berruyer C., Pouyet L., Millet V., Martin F. M., LeGoffic A., Canonici A., Garcia S., Bagnis C., Naquet P., Galland F. Vanin-1 licenses inflammatory mediator production by gut epithelial cells and controls colitis by antagonizing peroxisome proliferator-activated receptor gamma activity *J Exp Med* **2006**, 203(13), 2817–2827. DOI: 10.1084/jem.20061640, PubMed: 17145956.
4. Rommelaere S., Millet V., Rihet P., Atwell S., Helfer E., Chasson L., Beaumont C., Chimini G., Sambo Mdo R., Viallat A., Penha-Gonçalves C., Galland F., Naquet P. Serum pantetheinase/vanin levels regulate erythrocyte homeostasis and severity of malaria. *Am J Pathol.* **2015**, 185(11):3039-52. DOI: 10.1016/j.ajpath.2015.07.011, PubMed: 26343328.
5. Jansen P. A. M., Kamsteeg M., Rodijk-Olthuis D., van Vlijmen-Willems I. M. J. J., Jongh G. J. de, Bergers M., Tjabringa G. S., Zeeuwen P. L. J. M., Schalkwijk J. Expression of the vanin gene family in normal and inflamed human skin: Induction by proinflammatory cytokines *J Invest Dermatol* **2009**, 129(9), 2167–2174. DOI: 10.1038/jid.2009.67, PubMed: 19322213.
6. Pouyet L., Roisin-Bouffay C., Clément A., Millet V., Garcia S., Chasson L., Issaly N., Rostan A., Hofman P., Naquet P., Galland F. Epithelial vanin-1 controls inflammation-driven carcinogenesis in the colitis-associated colon cancer model *Inflamm Bowel Dis* **2010**, 16(1), 96–104. DOI: 10.1002/ibd.21031, PubMed: 19572375.
7. Dammanahalli K. J., Stevens S., Terkeltaub R. Vanin-1 pantetheinase drives smooth muscle cell activation in post-arterial injury neointimal hyperplasia *PLoS One* **2012**, 7(6), e39106. DOI: 10.1371/journal.pone.0039106, PubMed: 22720042.
8. Doe, J., Smith, J. (2018) *Heteroaromatic compounds as Vanin Inhibtitors* (Patent No. WO2018/228934), WIPO.