opnMe by Boehringer Ingelheim

Cathepsin C inhibitor

BI-9740

Boehringer Ingelheim

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Summary

BI-9740 is very potent, highly selective and orally bioavailable Cathepsin C (CTSC) inhibitor.

Chemical Structure

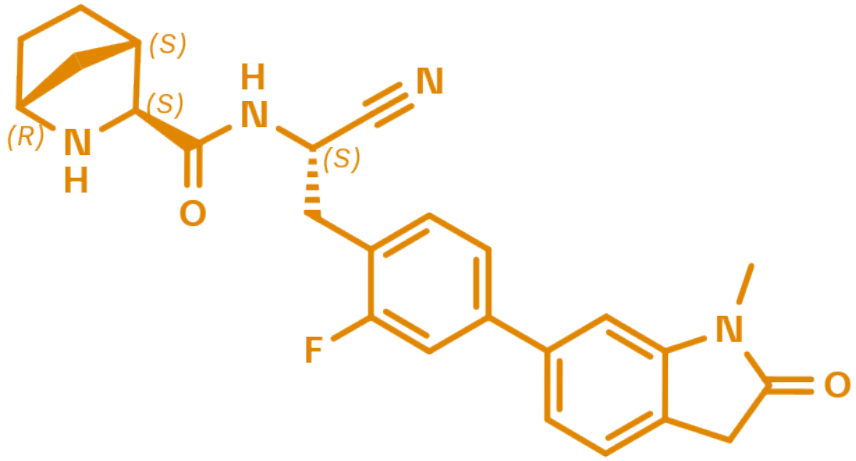


Figure 1: 2D structure of BI-9740

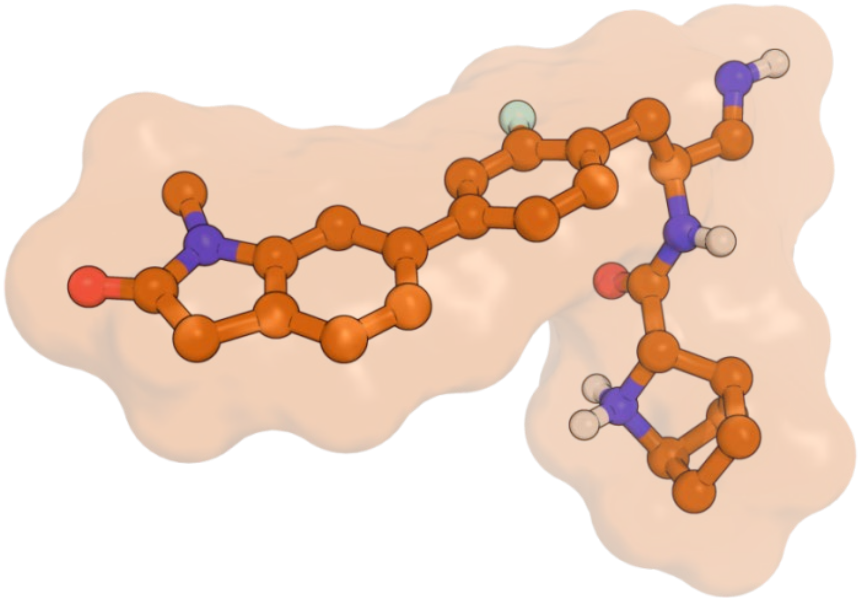


Figure 2: BI-9740, 3D conformation

Highlights

BI-9740 is a very potent and highly selective inhibitor of the enzymatic activity of Cathepsin C. It blocks human CatC *in vitro* with an IC_{50} of 1.8 nM and shows > 1,500x selectivity versus the related proteases Cathepsin B, F, H, K, L and S. BI-9740 displays no activity against 34 unrelated proteases from different classes up to a concentration of 10 µM.
BI-9740 fully inhibits the production of active neutrophil elastase in the human U937 cell line with an IC_{50} of 5.4 nM.
BI-9740 has very good *in vitro* and *in vivo* PK properties in several animal species (mouse, rat, minipig). Treatment of mice with an oral formulation of BI-9740 for 11 consecutive days eliminates active neutrophil elastase in peripheral neutrophils with an ED_{50} of 0.05 mg/kg q.d. The levels of active Cathepsin G and Proteinase 3 are similarly reduced[1].
Its diastereoisomer BI-1821 is available as negative control.

Target information

Cathepsin C (CatC) is a lysosomal cysteine protease. It is expressed at high levels in lung, kidney, and placenta and at moderate to low levels in many other organs. Among immune/inflammatory cells, the mRNA is expressed at high levels in polymorphonuclear leukocytes and alveolar macrophages and their precursor cells[2].
In the bone marrow, CatC activates neutrophil serine proteases (NSPs) during myelopoiesis of neutrophils. Inhibition of CatC leads to a decrease in neutrophil elastase (NE), cathepsin G (CG), proteinase 3 (PR3) and NSP4 activities in circulating neutrophils. Inhibition of Cathepsin C can therefore be used to target pathophysiological processes triggered by enhanced or uncontrolled activity of these proteases[3].
The active sites of CatC from human, rat, mouse, hamster and minipig are mostly conserved. Some non-conserved residues at the outer rim of the active site are not expected to largely influence inhibitor binding between species.

In vitro activity

BI-9740 displays an IC_{50} = 1.8 nM in a biochemical human CatC assay and inhibits NE activity in U937 cell-lysate with an IC_{50} = 5.4 nM. BI-9740 possesses excellent selectivity toward CatK, CatS, CatL, CatB, CatH, CatF.

PROBE NAME / NEGATIVE CONTROL	BI-9740	BI-1821
MW [Da, free base][a]	432.5	432.5
Human CatC + BSA (IC_{50}) [nM][b]	1.8	818
NE activity in U937 cell-lysate (IC_{50}) [nM]	5.4	n.d.
Mouse Cat C + BSA (IC_{50}) [nM][b]	0.6	n.d.
Rat Cat C + BSA (IC_{50}) [nM][b]	2.6	n.d.
Human CatK + BSA (IC_{50}) [µM][b]	3.5	n.d.
Human CatS + BSA (IC_{50}) [µM][b]	32.6	n.d.
Human CatL + BSA (IC_{50}) [µM][b]	>30.0	n.d.
Human CatH + BSA (IC_{50}) [µM][b]	>100	n.d.
Human CatB + BSA (IC_{50}) [µM][b]	>100	n.d.
Human CatF - BSA (IC_{50}) [µM][b]	>100	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] Assay conditions for CatC assay are available in the patent WO2014140075[3]. For CatK, CatS, CatH, CatB and CatF the assay conditions are identical except for the enzyme nature, concentration, buffer and substrates.
For CatC, substrate is Gly-Arg-AMC
For CatK, substrate is Z-Gly-Pro-Arg-AMC
For CatS, substrate is Z-Val-Val-Arg-AMC
For CatL, substrate is Z-Phe-Arg-AMC
For CatH, substrate is H-Arg-AMC
For CatB, substrate is Z-Arg-Arg-AMC
For CatF, substrate is Z-Leu-Arg-AMC

In vitro DMPK and CMC parameters

BI-9740 has high solubility at pH 2.2, 4.5 and 7. BI-9740 has very good *in vitro* PK properties in several animal species (mouse, rat, minipig). The negative control BI-1821 is also highly soluble.

PROBE NAME / NEGATIVE CONTROL	BI-9740	BI-1821
logP @ pH 11	3.1	n.a.

logD @ pH 2 / pH 11	1.0 / 3.0	1.0 / 2.9
Solubility @ pH 6.8 [µg/mL]	25	54
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	38	41
Caco-2 efflux ratio	1	2
MDCK permeability P_{appAB} @ 1µM [10^{-6} cm/s]	6.1	n.a.
MDCK efflux ratio	9.6	n.a.
Microsomal stability (human/mouse/rat) [% Q_H]	<23 / 39 / 80	n.a.
Hepatocyte stability @ 5% plasma (human/mouse/rat) [% Q_H]	<1 / 19 / 13	n.a.
Plasma Protein Binding (human/mouse/rat) [%]	98.5 / 97.7 / 99.9	n.a.
hERG [inh. % @ 10 µM]	39	n.a.
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	n.a.
CYP 2D6 (IC_{50}) [µM]	37	n.a.

In vivo DMPK parameters

BI-9740 has *in vivo* PK properties in several animal species (mouse, rat, minipig) allowing its testing in most of *in vivo* acute and chronic models.

BI-9740	MOUSE	RAT	MINI PIG
Clearance [%Q_H][a]	5	0.6	4
Mean residence time after *i.v.* dose [h]	2	5	3.7
t_{max} [h]	0.3[b] (natrosol)	1.4 [b](suspension)	2.5 [c]
C_{max} [nM]	655[b]	5,590[b]	283[c]

Bone marrow exposure [nM]	15[d]	205[e]	-
F [%]	100[b]	72[b]	30[c]
Vss [L/kg][a]	0.54	0.12	0.38

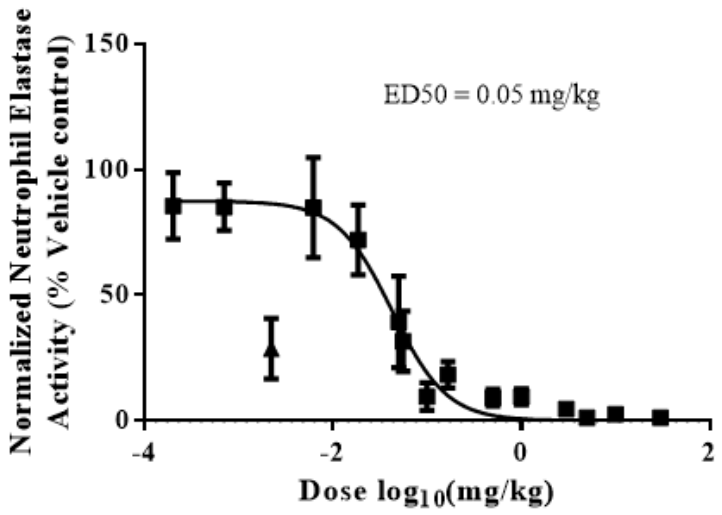
[a] i.v. dose: 0.43 mg/kg
[b] p.o. dose: 2.1 mg/kg
[c] p.o. dose: 4.3 mg/kg, (SUS/ADF)

In vivo pharmacology

A mouse model was used to demonstrate *in vivo* activity. In consideration of neutrophil homeostasis, animals were treated with BI-9740 once daily for 11 consecutive days. On day 12, animals were compound treated, followed by a LPS challenge by inhalation. Four hours later, bronchioalveolar lavage (BAL) was prepared and the activity of Neutrophile Elastase (NE) and of the related proteases Cathepsin G (CatG) and Proteinase 3 (PR3) in the lavage neutrophils was measured.

The production of active Neutrophil Elastase in peripheral neutrophils was completely attenuated by BI-9740 in a dose dependent manner with an ED_{50} of 0.05 mg/kg. The levels of active CatG and PR3 were similarly reduced.

ENZYME	DOSE	% REDUCTION VS LPS CONTROL
NE	0.5 mg/kg	91
PR3	0.5 mg/kg	97
CatG	0.5 mg/kg	100



Data were normalized by setting the mean of the non-LPS (vehicle only) control to 0% and the mean of the LPS control to 100%. A non-linear regression fit to calculate the half-maximal effective dose (ED50) was applied suing GraphPad Prism. Data are shown as mean and SEM.

Negative control

BI-1821 is provided as a negative control for BI-9740.

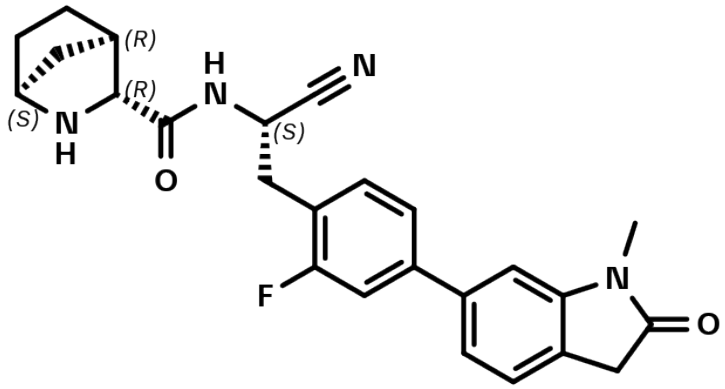


Figure 3: Structure of BI-1821, a negative control for CatC

Selectivity

BI-9740 shows a > 1,000x selectivity versus the related proteases Cathepsin B, F, H, K, L and S and displays no activity against 34 unrelated proteases from different classes up to a concentration of 10 µM.
The testing of BI-9740 against 80 different receptors and transporters identified the following activities:
1) BI-9740 shows agonistic activity on the kappa opioid receptor (KOR) with an EC_{50} of 1.2 µM (protein-free assay).
2) BI-9740 shows inhibitory activity on the 5HT-transporter with an IC_{50} of 0.71 µM (protein-free assay).
The negative control BI-1821 showed more than 50% inhibition @ 10 µM in 3 out of 44 targets (KAPPA(KOP), M2/H, M1H).

SELECTIVITY DATA AVAILABLE PROBE NAME / NEGATIVE CONTROL	BI-9740	BI-1821
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 crystal structure of Cathepsin C in complex with BI-9740 is available via the "Contact us" form.

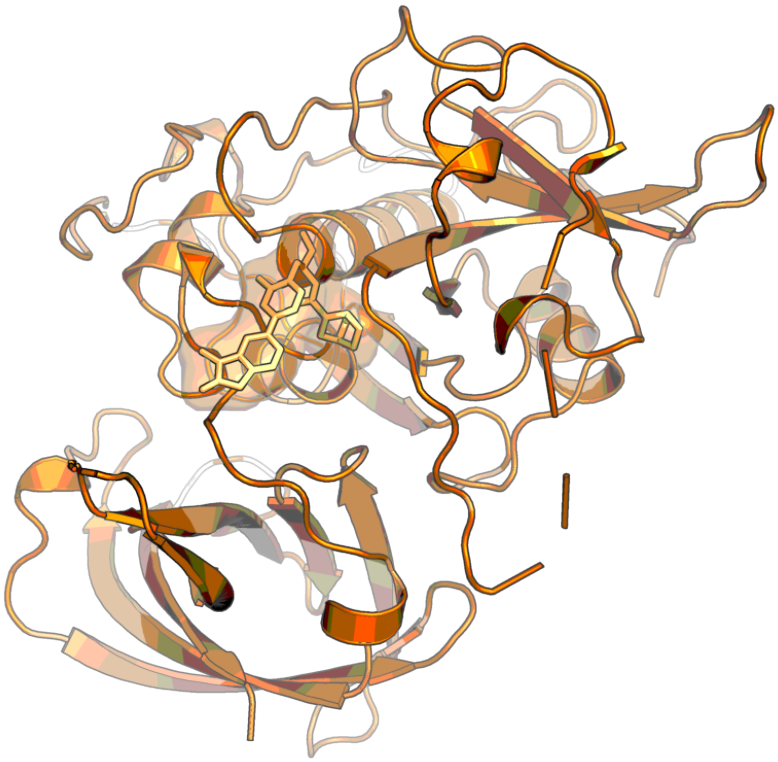


Figure 4: BI-9740, 3D conformation co-crystalized in the CatC protein

Reference molecule(s)

Guay D., Beaulieu C., Percival D. M. Therapeutic Utility and Medicinal Chemistry of Cathepsin C Inhibitors *Current Topics in Medicinal Chemistry* **2010**, *10*, 2010, 708-716. DOI: 10.2174/156802610791113469, PubMed: 20337582.

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

1. Grundl M. Cathepsin C: A challenge for medicinal chemistry ISyCatC II conference in Tour in 2019.

2. Rao N. V., Rao G. V., Hoidal J. R. Human dipeptidyl-peptidase I. Gene characterization, localization, and expression *J Biol Chem* **1997**, 272(15), 10260–10265. DOI: 10.1074/jbc.272.15.10260, PubMed: 9092576.

3. Korkmaz B., Horwitz M. S., Jenne D. E., Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases *Pharmacol Rev* **2010**, 62(4), 726–759. DOI: 10.1124/pr.110.002733, PubMed: 21079042.

4. Anderskewitz R., Grauert M., Grundl M., Haebel P.W., Oost T., Pautsch A., Peters S., Binder, F., Vintonyak V. Substituted 2-Aza-Bicyclo[2.2.1]Heptane-3-Carboxylic acid (Benzyl-cyano-methyl)-amides Inhibitors of Cathepsin C, WO2014140075.