Cathepsin C inhibitor | BI-9740

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Summary

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

Chemical Structure

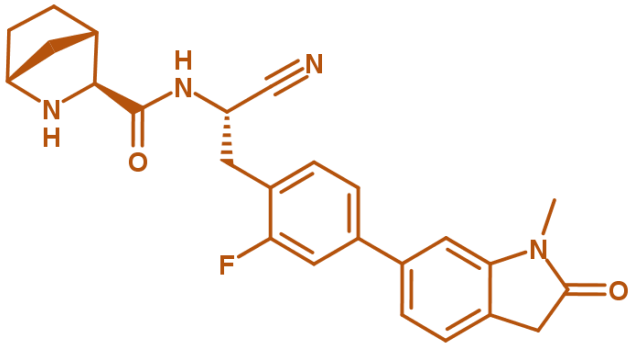


Figure 1: 2-D structure of BI-9740

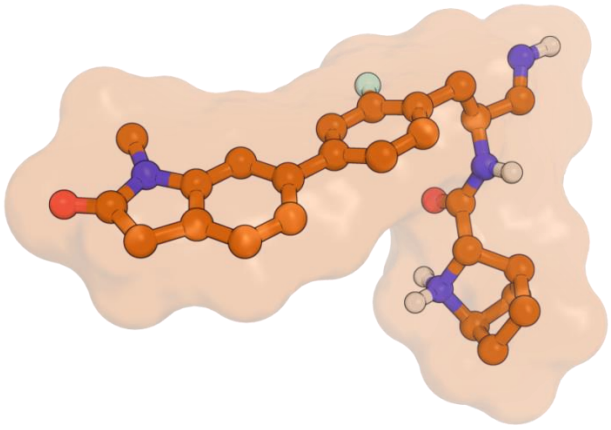


Figure 2: BI-9740, 3-D conformation

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 > 1500x 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, mini pig). 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. More information about the compounds can be obtained via the "Contact us" formular (including slides of the oral presentation given by Marc Grundl at the ISyCatC II conference in Tour in 2019).

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[1].
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[2].
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 humCatC 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	BI-9740
MW [Da]	432
Human CatC + BSA (IC_{50}) [nM][a]	1.8

NE activity in U937 cell-lysate (IC_{50}) [nM]	5.4
Mouse Cat C + BSA (IC_{50}) [nM] [a]	0.6
Rat Cat C + BSA (IC_{50}) [nM] [a]	2.6
Human CatK + BSA (IC_{50}) [µM] [a]	3.5
Human CatS + BSA (IC_{50}) [µM] [a]	32.6
Human CatL + BSA (IC_{50}) [µM] [a]	>30.0
Human CatH + BSA (IC_{50}) [µM] [a]	> 100
Human CatB + BSA (IC_{50}) [µM] [a]	> 100
Human CatF - BSA (IC_{50}) [µM] [a]	> 100

[a]Assay conditions for CatC assay are available in the patent WO2014140075. For CatK, CatS, CatH, CatB and CatF the assay conditions are identical except for the enzyme nature, concentration, buffer and substrates. More detailed experimental conditions can always be obtained via the "Contact us" formular.

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, mini pig).

PROBE NAME	BI-9740
logP	3.1
Solubility @ pH 6.8 [µg/ml]	25
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	79
CACO efflux ratio	1
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	6.1
MDCK efflux ratio	9.6
Microsomal stability (human/mouse/rat) [% Q_H]	<23 | 39 | 80
Hepatocyte stability @ 5% plasma (human/mouse/rat) [% Q_H]	<1 | 19 | 13
Plasma protein binding (human/mouse/rat) [%]	98.5 | 97.7 | 99.9
hERG [inh. % @ 10 µM]	39
CYP 3A4 (IC_{50}) [µM]	>50
CYP 2C8 (IC_{50}) [µM]	>50
CYP 2C9 (IC_{50}) [µM]	>50
CYP 2C19 (IC_{50}) [µM]	>50
CYP 2D6 (IC_{50}) [µM]	37

In vivo DMPK parameters

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

BI-9740	MOUSE	RAT	MINI PIG
Clearance [% Q_H][b]	5	0.6	4
Mean residence time after iv dose [h]	2	5	3.7
t_{max} [h]	0.3 (after oral dose 5 µmol/kg, natrosol)	1.4 (after oral dose 5 µmol/kg, suspension)	2.5 (after oral dose 10 µmol/kg, SUS/ ADJ)
C_{max} [nM]	655	5590	283
F [%]	100	72	30
V_{ss} [l/kg]	0.54	0.12	0.38

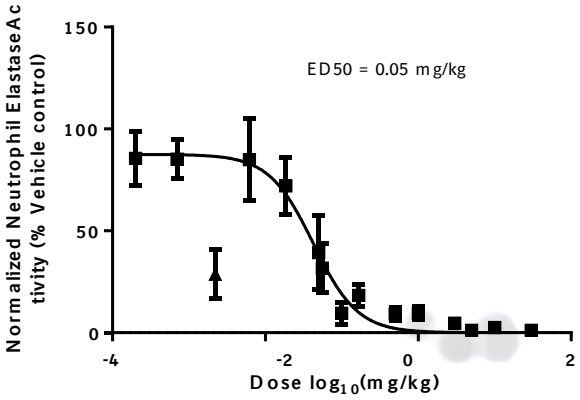
[b] 0.43 [mg/kg]

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 re shown as mean and SEM.

Selectivity

BI-9740 shows a > 1000x 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).

BI-9740	SELECTIVITY DATA AVAILABLE
Cerep®	Yes
Panlabs®	No
Invitrogen®	No
DiscoverX®	No
Dundee	No

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

The Xray crystal structure of Cathepsin C in complex with BI-9740 is available via the "Contact us" form.

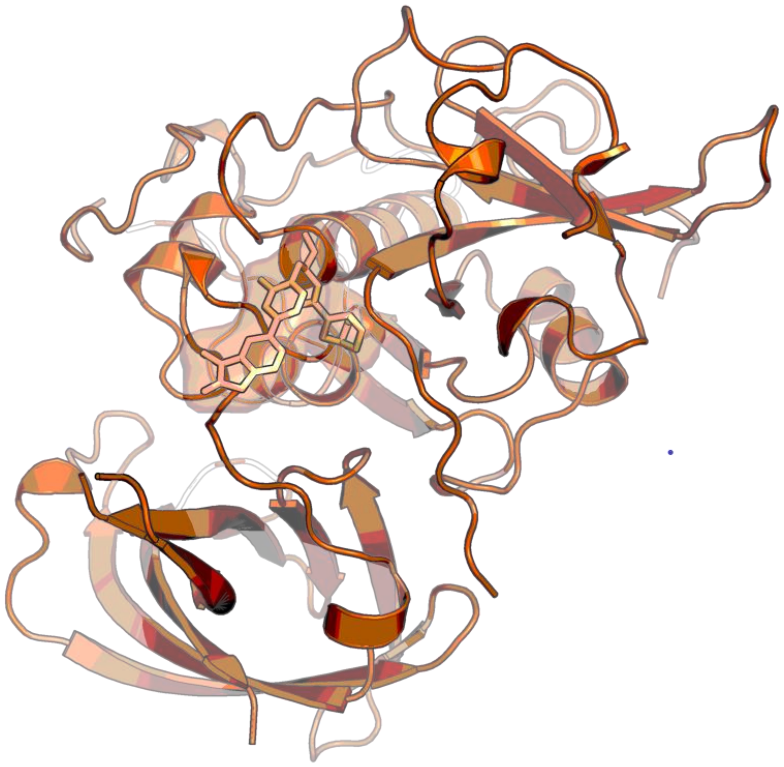


Figure 3: BI-9740, 3-D conformation co-crystalized in the CatC protein

Reference molecule(s)

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

Summary

BI-9740 is very potent, highly selective and orally bioavailable CatC inhibitor. BI-9740 shows no species selectivity and displays low nM IC_{50} in human, mouse and rat CatC assays. BI-9740 has high solubility at pH 2.2, 4.5 and 7. BI-9740 has very good *in vitro* and *in vivo* PK properties in several animal species (mouse, rat, mini pig).

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

1. Narayanam V. Rao, Gopna V. Rao and John R. Hoidal Human Dipeptidylpeptidase I Gene characterization, localization and expression *Journal of Biological Chemistry* **1997,** *272*, 10260-10265 DOI: 10.1074/jbc.272.15.10260, PubMed.

2. Brice Korkmaz, Marshall S. Horwitz, Dieter E. Jenne and Francis Gauthier Neutrophil Elastase, Proteinase 3, and Cathepsin G as Therapeutic Targets in Human Diseases *Pharmcological Review* **2010**, *62*, 726-759 DOI: 10.1124/pr.110.002733, PubMed