opnMe by Boehringer Ingelheim

MLKL inhibitor

BI-8925

Boehringer Ingelheim

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Summary

BI-8925 is the first covalent tool compound for the necroptosis effector protein MLKL with a structurally understood mode of action.

Chemical Structure

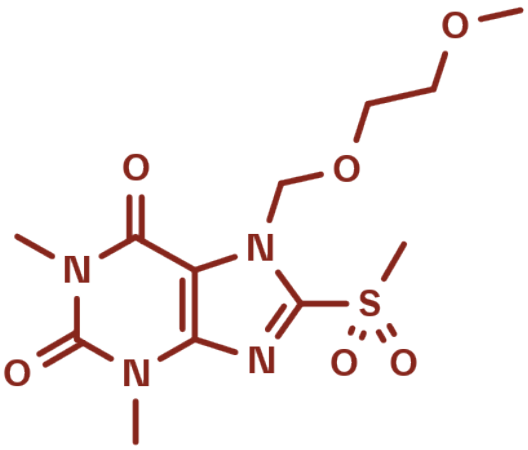

Figure 1: 2D structure of BI-8925, a MLKL inhibitor

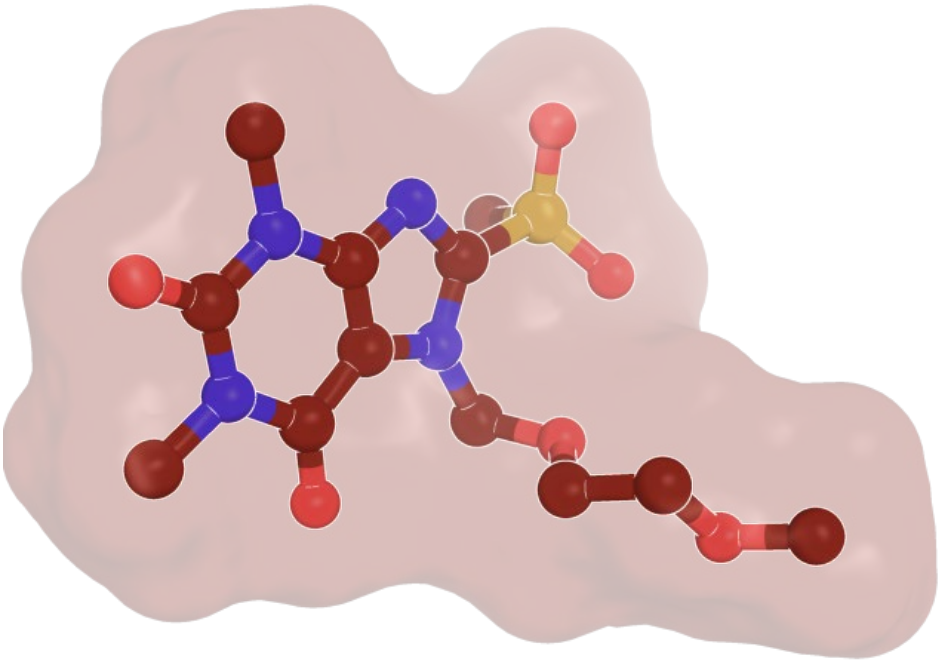

Figure 2: BI-8925, 3D conformation based on the complex with MLKL as solved by X-ray crystallography (PDB code: 6ZZ1)

Highlights

BI-8925 is an inhibitor of the MLKL protein, belonging to the xanthine class. It is the first covalent tool compound for MLKL, with a structurally understood mode of action. It works by stabilizing the inactive state of MLKL by an essential π–π stacking interaction. The molecule is found to inhibit necroptosis in Jurkat and U937 cells with an IC_{50} of 541 and 271 nM, respectively.

Target information

Mixed lineage kinase domain-like protein (MLKL) is the effector protein in the signal pathway leading to Necroptosis. MLKL is comprised of two domains. The C-terminal pseudokinase domain is connected via two brace helices to the N-terminal four helix bundle domain. The N-terminal executioner domain is locked in its inactive state by the auto-inhibitory first brace helix (α-helix 6). Upon TNF signalling in the absence of caspase activity MLKL becomes activated via the kinases RIPK1 and RIPK3 through phosphorylation in its pseudokinase domain. Upon activation the auto-inhibitory brace helix unfolds and the N-terminal executioner domain of MLKL multimerizes and integrates into the membrane, which then leads to membrane rupture and necroptosis.

Figure 3: Complex of BI-8925 with MLKL, as solved by X-ray crystallography (PDB code: 6ZZ1). The structure was also solved by NMR spectroscopy (PDB code: 6ZPR). Both structures show a very high level of agreement[6]

In vitro activity

BI-8925 displays nM activity in two different cellular necroptosis assays.

PROBE NAME / NEGATIVE CONTROL	BI-8925	BI-8762
MW [Da, free base][a]	346.4	240.3
Assay A (IC_{50}) [nM][b]	541	>100000

Assay B (IC_{50}) [nM][b]	271	>100000

[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] See reference 6

Assay A: Stimulation of Jurkat FADD-/- cells was achieved with huTNFα and subsequent cell viability analysis was carried out with Cell Titer Glo® Luminescent Cell Viability Assay

Assay B: U937 cells were treated with the caspase inhibitor zVAD-fmk and stimulated with huTNFα; cell viability analysis was performed with Cell Titer Glo® Luminescent Cell Viability Assay.

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-8925	BI-8762
logD @ pH 2 / 11	1 / 0.5	- / 1.0
Solubility @ pH 6.8 [µg/mL]	75	59
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	17	53
Caco-2 efflux ratio	0.7	0.8

Negative control

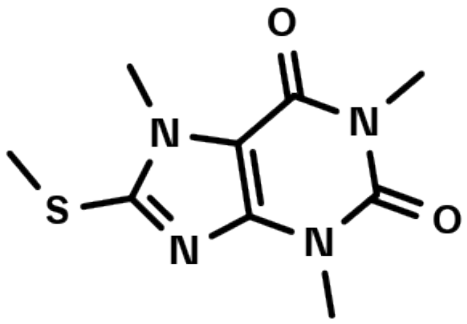

Figure 4: BI-8762 which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	BI-8925	BI-8762
SafetyScreen44™ with kind of 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 the target without ligand is available (PDB code: 6ZVO)

The X-ray crystal structure of the target in complex with BI-8925 is available (PDB code: 6ZZ1)

See figure 3

The NMR structure of the target without ligand is available (PDB code: 6ZLE)

The NMR structure of the target in complex with BI-8925 is available (PDB code: 6ZPR)

Reference molecule(s)

Necrosulfonamide (NSA)

Summary

BI-8925 is a covalent inhibitor of MLKL with a structurally and functionally characterized mode of action and good activity in cells (IC_{50} = 541 nM).

Supplementary data

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

References

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