



MLKL inhibitor | BI-8925

Table of contents

Summary	2
Chemical Structure	2
Highlights	3
Target information	3
<i>In vitro</i> activity	4
<i>In vitro</i> DMPK and CMC parameters	4
Negative control	5
Selectivity	5
Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein	5
Reference molecule(s)	6
Summary	6
Supplementary data	6
References	6

Summary

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

Chemical Structure

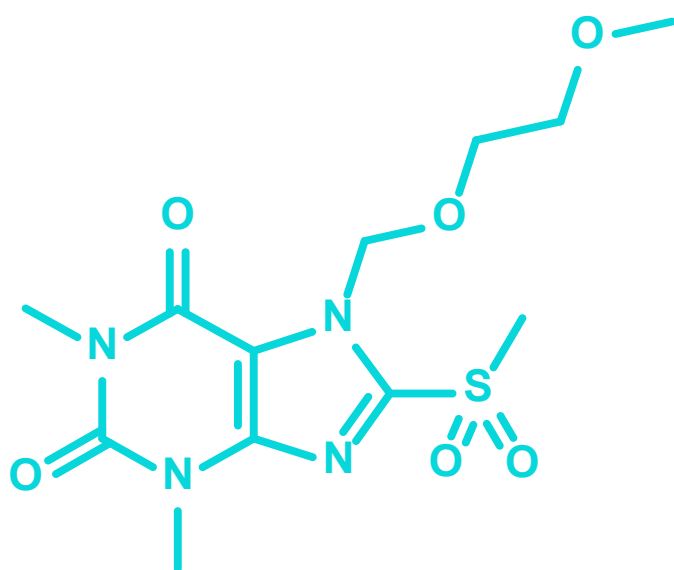


Figure 1: 2-D structure of BI-8925, a MLKL inhibitor

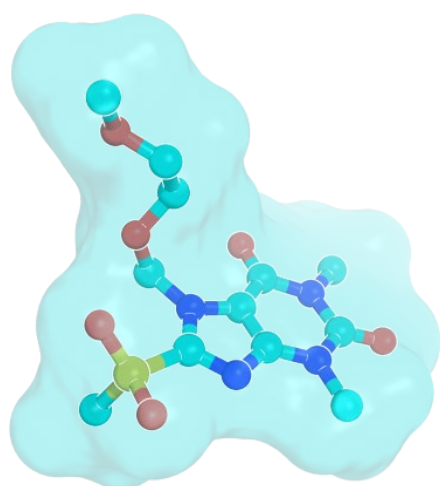


Figure 2: BI-8925, 3D conformation based on the complex with MLKL as solved by X-ray crystallography (PDB code: 6ZZ1)

Highlights

BI-8925 is a covalent inhibitor targeting Cys86 in the N-terminal executioner domain of the necroptosis effector protein mixed lineage kinase domain-like protein (MLKL). MLKL is locked in its auto-inhibited monomeric state by the packing of α -helix 6 against its four-helix bundle in the N-terminal executioner domain. Upon activation through phosphorylation in its C-terminal pseudokinase domain α -helix 6 unfolds and the N-terminal executioner domain can multimerize and integrate into the membrane, which finally leads to Necroptosis. BI-8925 stabilizes the auto-inhibited state by a π - π interaction between its xanthine core and Phe148 located in the auto-inhibitory α -helix 6. Consequently, unfolding of α -helix 6 and multimerization of the MLKL executioner domain is prevented. BI-8925 inhibits 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.

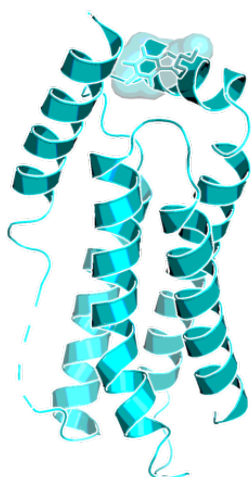


Figure 3: Complex of BI-8925 with MLKL, as solved by X-ray crystallography (PDB code: 6ZZ1). The structure was also solved by NMR spectroscopy (PDB code: 6ZPR). Both structures show a very high level of agreement⁶

In vitro activity

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

PROBE NAME / NEGATIVE CONTROL	BI-8925	BI-8762
MW [Da]	346.36	240.28
Assay A (IC ₅₀) [nM] ^a	541	>100000
Assay B (IC ₅₀) [nM] ^a	271	>100000

^a see reference 6

Assay A: Stimulation of Jurkat FADD^{-/-} cells was achieved with huTNF α 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 huTNF α ; 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 pH2/pH11	1/0.5	-
Solubility @ pH 6.8 [μ g/ml]	75	59
CACO permeability @ pH 7.4 [$*10^{-6}$ cm/s]	17	53
CACO efflux ratio	0.7	0.8

Negative control

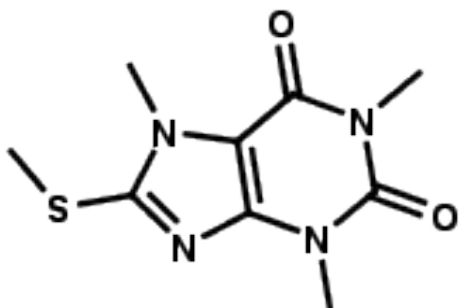



Figure 4: BI-8762 which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	BI-8925	BI-8762
SafetyScreen44™ with kind of support of  eurofins	data in progress - will be added soon	data in progress - will be added soon
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

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

The Xray crystal structure of the target without ligand is available (PDB code: 6ZVO)

The Xray 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

2-D structure files can be downloaded free of charge from [openMe](#).

References

1. Sun L., Wang H., Wang Z., He S., Chen S., Liao D., Wang L., Yan J., Liu W., Lei X., Wang X. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* **2012**, *148*, 213-27. DOI: [10.1016/j.cell.2011.11.031](https://doi.org/10.1016/j.cell.2011.11.031), [PubMed](#).
2. Yan B., Liu L., Huang S., Ren Y., Wang H., Yao Z., Li L., Chen S., Wang X., Zhang Z. Discovery of a new class of highly potent necroptosis inhibitors targeting the mixed lineage kinase domain-like protein. *Chem Commun (Camb)* **2017**, *53*, 3637-3640. DOI: [10.1039/c7cc00667e](https://doi.org/10.1039/c7cc00667e), [PubMed](#).
3. Su L., Quade B., Wang H., Sun L., Wang X., Rizo J. A plug release mechanism for membrane permeation by MLKL. *Structure* **2014**, *22*, 1489-500. DOI: [10.1016/j.str.2014.07.014](https://doi.org/10.1016/j.str.2014.07.014), [PubMed](#).
4. McNamara D. E., Dovey C. M., Hale A. T., Quarato G., Grace C. R., Guibao C. D., Diep J., Nourse A., Cai C. R., Wu H., Kalathur R. C., Green D. R., York J. D., Carette J. E. Moldoveanu T. Direct Activation of Human, MLKL by a Select Repertoire of Inositol Phosphate Metabolites. *Cell Chem Biol* **2019**, *26*, 863-877.e7. DOI: [10.1016/j.chembiol.2019.03.010](https://doi.org/10.1016/j.chembiol.2019.03.010), [PubMed](#).
5. Liao D., Sun L., Liu W., He S., Wang X., Lei X. Necrosulfonamide inhibits necroptosis by selectively targeting the mixed lineage kinase domain-like protein. *MedChemComm* **2014**, *5*. DOI: [10.1039/c3md00278k](https://doi.org/10.1039/c3md00278k).

- Rübelke M., Fiegen D., Bauer M., Binder F., Hamilton J., King J., Thamm S., Nar H., Zeeb M. Locking mixed lineage kinase domain-like protein in its auto-inhibited state prevents necroptosis. *PNAS* (2020) accepted. [DOI: 10.1073/pnas.2017406117](https://doi.org/10.1073/pnas.2017406117)