opnMe by Boehringer Ingelheim

Bacterial PROTAC

BI-8255

Boehringer Ingelheim

Table of contents

Summary 2
Chemical Structure 2
Highlights 3
Target information 3
In vitro activity 4
In vitro DMPK and CMC parameters 5
In vivo DMPK parameters 5
In vivo pharmacology 5
Negative control 5
Selectivity 6
Reference molecule(s) 6
Supplementary data 6
References 7

Summary

Gram-positive bacteria and mycobacteria utilize an essential proteolytic complex consisting of the ClpC unfoldases and ClpP proteases. With BI-8255 that represents a proof-of-concept compound targeting the eukaryotic protein BRDT to the mycobacterial ClpC1 unfoldase, we have established a versatile research tool enabling the inducible degradation of bacterial proteins fused to BRDT.

Chemical Structure

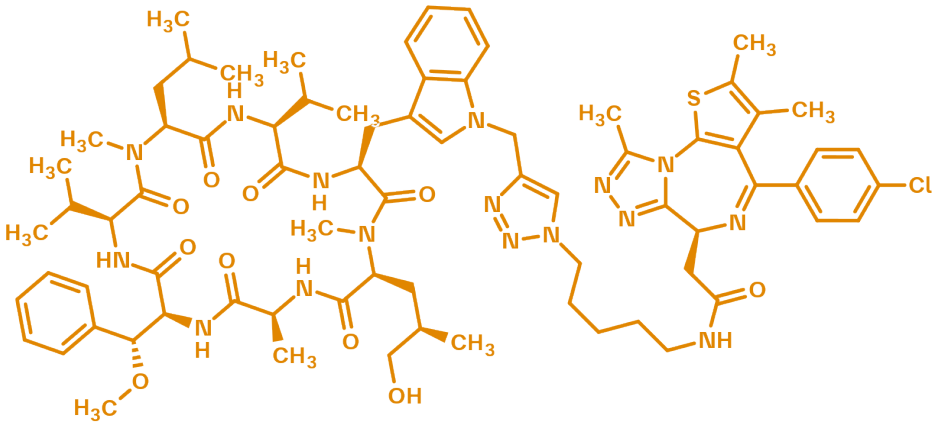

Figure 1: 2D structure of BI-8255, a bacterial PROTAC targeting the mycobacterial ClpC1 unfoldase on one end and the eukaryotic protein BRDT on the other end.

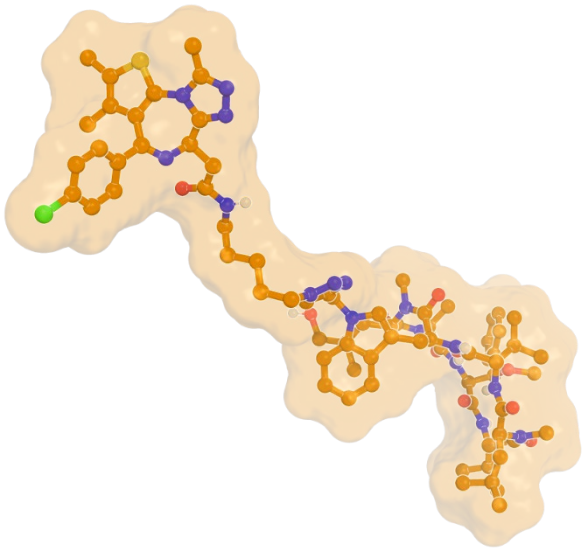

Figure 2: BI-8255, low energy 3D conformation, derived from an X-ray structure of a complex of ClpC1 with a related compound

Highlights

BI-8255 is a first PROTAC molecule reprogramming the bacterial degradation machinery. Tethering the epigenetic regulator BRDT via BI-8255 to the mycobacterial unfoldase ClpC1 resulted in the selective cellular degradation of BRDT alone as well as BRDT fusion proteins in mycobacteria. Thus, we established a versatile research tool enabling the inducible degradation of bacterial proteins. We encourage researchers to use this concept to target new bacterial proteins. We refer to this system as BID (BacPROTAC Inducible Degradation), which is the bacterial counterpart to the AID (Auxin induced degradation) system prominently used in eukaryotic cells. The BID approach can be used for the temporarily controlled knockdown of mycobacterial proteins.

Target information

ClpC1 is part of the ClpC1P1P2 degradation complex, which mycobacteria use for protein quality control, in analogy to the ubiquitination-dependent proteasome in eucaryotic cells. Situated at the top of this complex, ClpC1 acts as an ATP-driven unfoldase, translocating the unfolded peptide chains into the protease compartment formed by the ClpP subunits. By inducing proximity of ClpC1 to BRDT, BI-8255 leads to efficient degradation of the BRDT protein, a mechanism which was successfully adapted to target fusion proteins in mycobacteria[1,2]. Derived from dCymM of the natural product cyclomarin, BI-8255 has nanomolar binding affinity to the unfoldase ClpC1, as well as a similarly strong affinity to BRDT via the corresponding JQ-1 ligand. The induced proximity towards the ClpC1P1P2 protease leads to cellular degradation of the protein of interest (POI). BI-8255 has been previously described and characterized as BacPROTAC-5[1].

In summary, using cell permeable BacPROTACs, we have demonstrated that recruitment of BRDT as a model protein to ClpCP enables the selective degradation of fused POIs in mycobacteria.

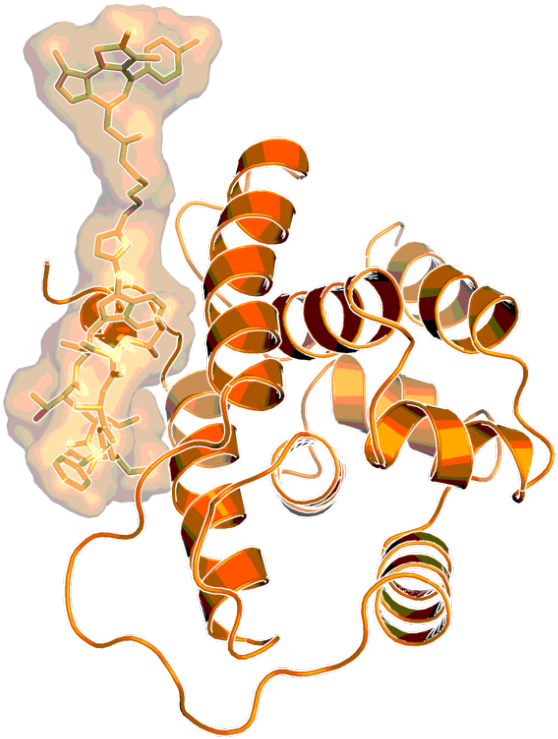

Figure 3: Model of a complex of ClpC1 with BI-8225, based on an X-ray structure of a complex with a related compound

In vitro activity

BI-8255 displays binding affinity to $ClpC1_{NTD}$ of 0.2 µM in ITC titration assays[1].

PROBE NAME / NEGATIVE CONTROL	BI-8255	BI-8256
MW [Da, free base][a]	1452.2	1452.2
ITC (KD) [µM][b]	0.2	0.2

[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]Binding affinity and BRDT degradation was determined in the literature[2].

ITC experiments were performed in a buffer containing 50 mM Tris pH 7.5, 300 mM NaCl, 0.5 mM TCEP. Each titration consisted of 19 injections with intervals of 150 s (the first injection of

0.4 mL was followed by 18 injections of 2 mL) at constant stirring at 750 rpm. DMSO concentration was matched between cell and syringe to be 4% (v/v).

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-8255	BI-8256
logD @ pH 11	5.05	5.05
Solubility @ pH 6.8 [µg/mL]	< 1	< 1
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	< 2.1	< 2.1
Caco-2 efflux ratio	-	-
MDCK permeability P_{appAB} @ 1 µM [10^{-6} cm/s]	0	0.1
MDCK efflux ratio	1.1	0.2
Microsomal stability (human/mouse/rat) [% Q_H]	84 / 74 / 45	87 / 76 / <23
CYP 3A4 (IC_{50}) [µM]	0.3	0.4
CYP 2C8 (IC_{50}) [µM]	>50	>50
CYP 2C9 (IC_{50}) [µM]	>50	>50
CYP 2C19 (IC_{50}) [µM]	>50	>50

In vivo DMPK parameters

No *in vivo* profiling was performed for the compounds.

In vivo pharmacology

In vivo experiments were performed in mycobacteria expressing BRDT or fusion proteins containing BRDT according to the experiments described in literature reference 1.

Negative control

The negative control BI-8256 differs in its configuration at the JQ-1 binding motif, leading to equally potent binding of the compound to $ClpC1_{NTD}$ but lacking the ability to induce degradation of BRDT.

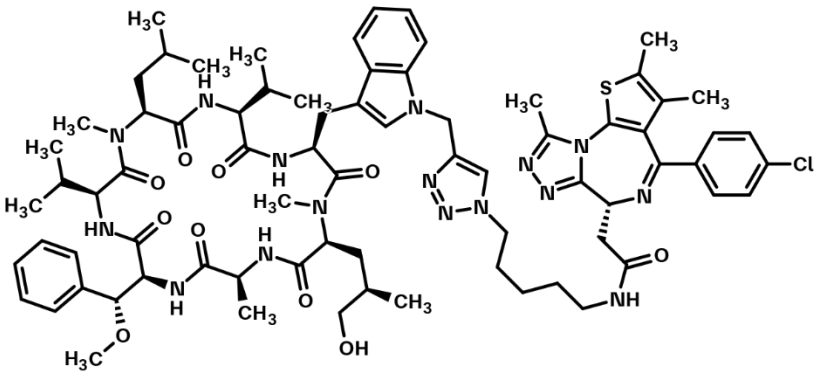

Figure 4: BI-8256 serves as a negative control with respect to BRDT binding.

Selectivity

BI-8255 inhibits COX1@CE and COX2@CE with 68 and 59% respectively. All other targets measured in the SafetyScreen44 are below 50% inhibition. BI-8256 inhibits no targets from the SafetyScreen44™above 50%. (Compounds are considered to be selective, if they do not hit any of the measured targets in the SafetyScreen44™ > 50%; panel measured at 10 µM).

SELECTIVITY DATA AVAILABLE	BI-8255	BI-8256
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

There are no other tool compounds available.

Supplementary data

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

References

1. Morreale F. E., Kleine S., Leodolter J., Junker S., Hoi D. M., Ovchinnikov S., Okun A., Kley J., Kurzbauer R., Junk L., Guha S., Podlesainksi D., Kazmaier U., Boehmelt G., Weinstabl H., Rumpel K., Schmiedel V. M., Hartl M., Haselbach D., Meinhart A., Kaiser M., Clausen T. BacPROTACs mediate targeted protein degradation in bacteria *Cell* **2022**, *185*, 2338-2353. DOI: org/10.1016/j.cell.2022.05.009, PubMed.

2. Junk. L., Schmiedel V. M., Guha S., Fischel K., Greb P., Vill K., Krisilia V., van Geelen L., Rumpel K., Kaur P., Krishnamurthy R. V., Narayanan S., Shandil R. K., Singh M., Kofink C., Mantoulidis A., Biber P., Gmaschitz G., Kazmaier U., Meinhart A., Leodolter J., Hoi D., Junker S., Morreale F. E., Clausen T., Kalscheuer R., Weinstabl H., Boehmelt G. Homo-BacPROTAC-induced degradation of ClpC1 as a strategy against drug-resistant mycobacteria *Nat Commun*. **2024**, 15(1),2005. DOI:10.1038/s41467-024-46218-7, PubMed.