BET PROTAC | MZ 1

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Summary

MZ 1 is a PROTAC degrader aimed at triggering the intracellular destruction of BET proteins. MZ 1 induces removal of BRD4 over BRD2 and BRD3 in cells.

Chemical Structure

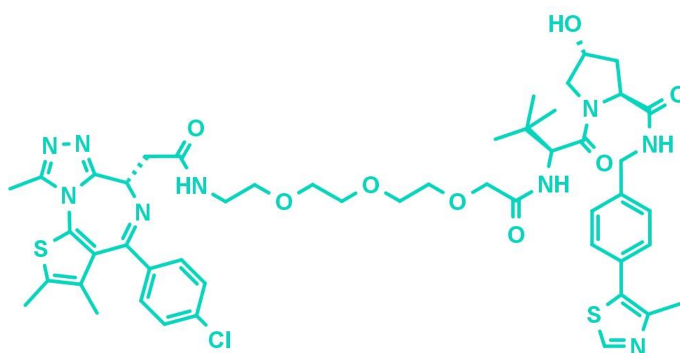


Figure 1: 2-D structure of MZ 1

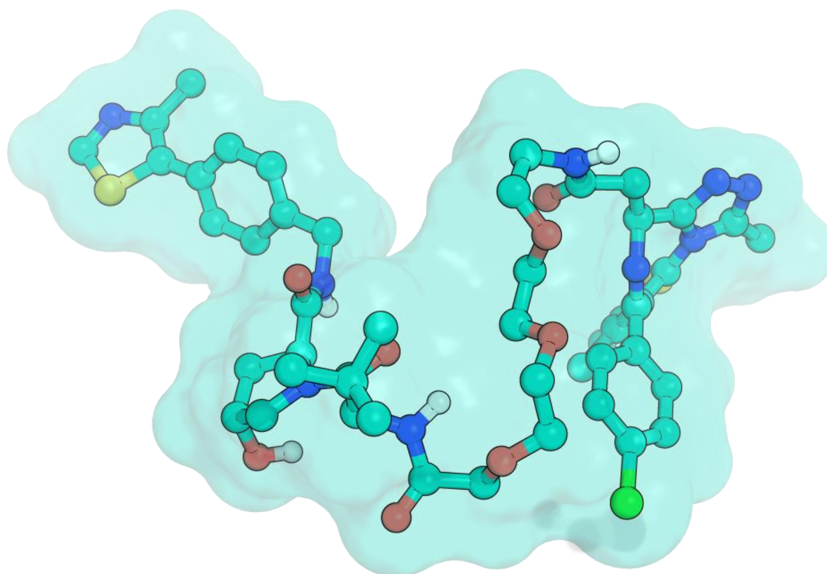


Figure 2: MZ 1, 3D conformation, as observed in complex with BRD4 bromodomain and VHL (PDB 5T35)

Highlights

MZ 1 and the negative control cis MZ 1 have been discovered by Michael Zengerle, Kwok-Ho Chan, and Alessio Ciulli[1] at the Universtity of Dundee. opnMe.com is proud to be able to provide researchers with up to two batches of 5 mg of MZ 1 and one batch of cis MZ 1 for free. Larger quantities of the compounds are available from Tocris.

Inducing macromolecular interactions with small molecules to activate cellular signaling is a challenging goal. PROTACs (proteolysis-targeting chimeras) are bifunctional molecules that recruit a target protein in proximity to an E3 ubiquitin ligase to trigger protein degradation. Structural elucidation of the key ternary ligase-PROTAC-target species and its impact on target degradation selectivity remain elusive. The crystal structure of BRD4 degrader MZ 1 in complex with human VHL and the BRD4 bromodomain ($BRD4^{BD2}$) offers unique insights into the first ternary complex structure of a l.m.w. degrader. The ligand folds into itself to allow formation of specific intermolecular interactions in the ternary complex. Isothermal titration calorimetry studies, supported by surface mutagenesis and proximity assays, are consistent with pronounced cooperative formation of ternary complexes with $BRD4^{BD2}$. Our results elucidate how PROTAC-induced de novo contacts dictate preferential recruitment of a target protein into a stable and cooperative complex with an E3 ligase for selective degradation.[1,2]

Target information

Acetylation of lysine residues is a post-translational modification with broad relevance to cellular signalling and disease biology. Inhibitors that modulate the ‘reading process’ mediated by acetyl lysines are an area of extensive research. The principal readers of ε-N-acetyl lysine (Kac) marks are bromodomains (BRDs), which are a diverse family of evolutionary conserved protein-interaction modules. The conserved BRD fold contains a deep, largely hydrophobic acetyl lysine binding site, which represents an attractive pocket for the development of small, pharmaceutically active molecules. Proteins that contain BRDs have been implicated in the development of a large variety of diseases.

Traditionally small molecules have been used to inhibit the action of a target protein by occupying and blocking a functional region of the protein. An alternative innovative approach is the development of proteolysis targeting chimeras (PROTACs), i.e. heterobifunctional compounds consisting of one moiety that binds a Cullin RING E3 ubiquitin ligase linked to another that binds a desired protein of interest (POI), bringing the ligase and the POI into close spatial proximity This hijacks the intrinsic catalytic activity of the E3 ligase from the natural and directs it toward the POI as a neo-substrate, triggering its poly-ubiquitination and subsequent proteasome-dependent degradation. As a result, a PROTAC acts as a degrader of the target as opposed to just an inhibitor, enabling the effective post-translational elimination of a target gene product in living organisms. This approach presents many advantages compared to conventional target inhibition. One of the most attractive features of the approach is that a PROTAC molecule acts sub-stoichiometrically, i.e. it only needs to bind a molecule of target once to induce its degradation, and

then is released and set free to bind another molecule of target and carry on, as in a catalytic cycle. For this reason, the concentrations required for PROTACs to be active in cells tend to be much lower compared to those needed to be reached and maintained with inhibitors, which can lead to fewer off-target effects and a more selective chemical intervention on the desired target.

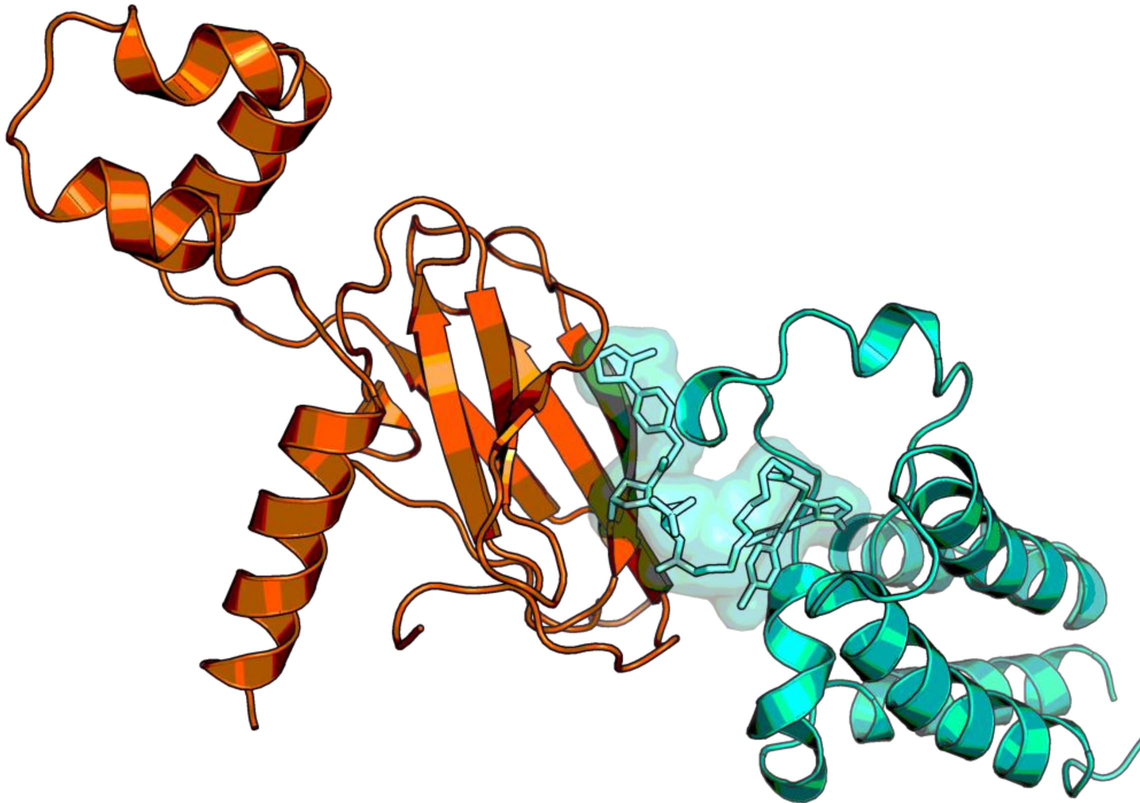


Figure 3: MZ 1, 3D conformation, as observed in complex with BRD4 bromodomain and VHL (PDB 5T35)

In vitro activity

The binary affinity for MZ 1 binding to $BRD4^{BD2}$ and to VCB (VHL-ElonginC-EloginB) is 15 and 66 nM respectively (ITC). The affinity of the negative control “cis MZ 1” to VCB is > 15 μM (ITC). In presence of excess of $BRD4^{BD2}$ MZ 1 the ternary KD for the $BRD4^{BD2::}$MZ 1::VCB is 3.7 nM. MZ 1 degrades the BRD4 protein with a DC_{50} of 2-20 nM depending on the cell line, and with selectivity on DC_{50} of ~10-fold relative to BRD2 and BRD3.

Table: Binding affinities for binary and ternary complexes between MZ 1, VCB and BET bromodomains as measured by isothermal titration calorimetry (for assay conditions/details see Gadd et al, Nat Chem Biol. 2017, 13, 514–521)[1]

	PROBE NAME	MZ 1		
Protein in syringe	**Species in cell**	**K_d (nM)**	**α**	**$\Delta p{<}K_d$**
$BRD2^{BD1}$	MZ 1	62 ± 6	-	-
$BRD2^{BD2}$	MZ 1	60 ± 3	-	-
$BRD3^{BD1}$	MZ 1	21 ± 5	-	-
$BRD3^{BD2}$	MZ 1	13 ± 3	-	-
$BRD4^{BD1}$	MZ 1	39 ± 9	-	-
$BRD4^{BD2}$	MZ 1	15 ± 1	-	-
$BRD2^{BD1}$ KEA	MZ 1	69 ± 9	-	-
$BRD4^{BD1}$ QVK	MZ 1	22 ± 8	-	-
VCB[a]	MZ 1[a]	66 ± 6	-	-
VCB	MZ 1:$BRD2^{BD1}$	24 ± 8	2.9	0.4 ± 0.2
VCB	MZ 1:$BRD2^{BD2}$	28 ± 3	2.3	0.36 ± 0.06
VCB	MZ 1:$BRD3^{BD1}$	19 ± 4	3.5	0.5 ± 0.1
VCB	MZ 1:$BRD3^{BD2}$	7 ± 2	10.7	1.0 ± 0.2

VCB	MZ 1:BRD4^{BD1}	28 ± 6	2.3	0.4 ± 0.1
VCB	MZ 1:BRD4^{BD2}	3.7 ± 0.7	17.6	1.24 ± 0.09
VCB	MZ 1:BRD2^{BD1} KEA	12 ± 7	7.9	0.8 ± 0.3
VCB	MZ 1:BRD4^{BD2} QVK	14.9 ± 0.1	4.2	0.62 ± 0.04

All ITC titrations were performed at 25 °C. Values reported are the mean ± S.E.M. from two independent measurements, except for VCB titration into MZ 1 (line [a]) for which values reported are the mean ± S.E.M. from eight independent measurements.

In vitro DMPK and CMC parameters

MZ 1 is soluble at physiological pH, metabolic stable (hepatocytes) and a PGP substrate (high Caco2 efflux ratio. Therefore, MZ 1 is not suitable for oral administration but suitable for parenteral administration (i.v., i.p. or s.c.). The compound shows moderate inhibition of Cyp3A4 and species differences in plasma protein binding.

PROBE NAME / NEGATIVE CONTROL	MZ 1	CIS MZ 1
MW [Da]	1002.6	1002.6
Solubility @ pH 6.8 [µg/ml]	19	12 (pH 7)
CACO permeability @pH7.4 [$*10^{-6}$ cm/s]	0.034	n.d.
CACO efflux ratio	481	n.d.
Microsomal stability (human/mouse/rat) [% Q_H]	88 / 79 / 73	>95 / >95 / 71
Hepatocyte stability 50% Serum (human/mouse/rat) [% Q_H]	12 / 19 / 58	n.d.
Plasma protein binding (human/mouse/rat/10%FCS) [%f_u]	2.5 / 0.42 / 2.5 / 41	n.d.
CYP 3A4 (IC_{50}) [µM]	5.5	n.d.

CYP 2C8 (IC_{50}) [µM]	24	n.d.
CYP 2C9 (IC_{50}) [µM]	>50	n.d.
CYP 2C19 (IC_{50}) [µM]	>50	n.d
CYP 2D6 (IC_{50}) [µM]	37	n.d.

In vivo DMPK parameters

MZ 1 is suitable for a parenteral administration (i.v., i.p. or s.c.). The compound shows high Clearance in rats and low Clearance in mice. High AUC levels can be obtained, when the compound is administered subcutaneously using a 25% HP-ß-CD formulation. Because of the high Caco2 efflux ratio, the oral exposure is very low.

MZ 1	RAT	MOUSE
Clearance [% Q_H]	156 [a]	20.7 [c]
Mean residence time [h]	0.61 [a]	0.34 [c]
$t_{1/2,term}$ [h]	1.52 [a]	1.04 [c]
V_{ss} [l/kg]	4.05 [a]	0.38 [c]
AUC_{0-inf} [nM·h]	1300 [b]	3760 [d]
c_{max} [nM]	163 [b]	2070 [d]
t_{max} [h]	2 [b]	0.5 [d]
$t_{1/2,term}$ [h]	5.13 [b]	2.95 [d]
c_{max} [nM]	-	<2 [e]

[a] rat *i.v.* 5 mg/kg

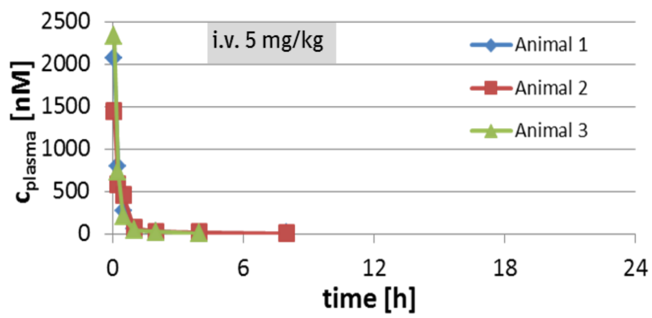
[b] rat *s.c.* 5 mg/kg

[c] mouse *i.v.* mg/kg

[d] mouse *s.c.* mg/kg

[e] mouse *p.o.* mg/kg

A



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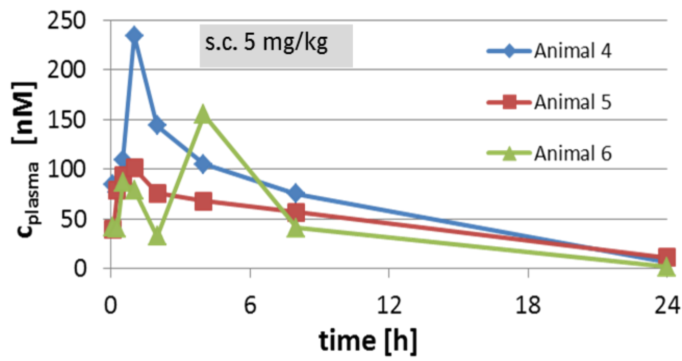

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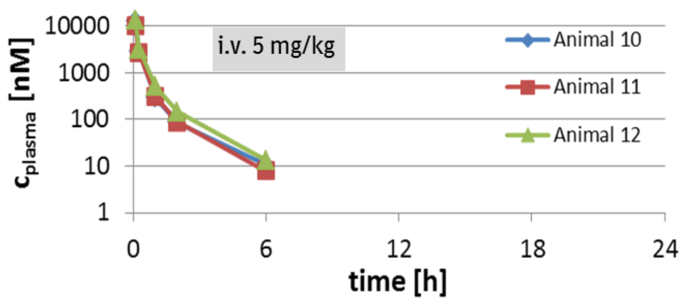

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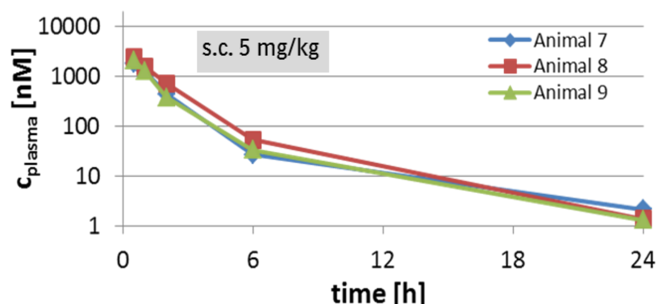

Figure 4: PK curves: **A** Rat *i.v.* 5 mg/kg, **B** Rat *s.c.* 5 mg/kg, **C** mouse *i.v.* 5 mg/kg, **D** mouse *s.c.* 5 mg/kg

In vitro pharmacology

MZ 1 shows antiproliferative and Myc-suppression activity in AML MV4;11 and HL60 cells.

BET Reduction by MZ 1 in HeLa Cells, Antiproliferative Activity, and BRD4/cMyc Reduction in AML Cells[a].[3]

MZ 1											
	pDC_{50}/Dmax (%) in HeLa cells				pIC_{50}		Emax (%)		BRD4/cMyc depletion (%)		(α)
	BRD4 short	BRD4 long	BRD3	BRD2	MV4;11	HL60	MV4;11	HL60	MV4;11	HL60	
MZ 1	8.1/ 98	8.6/ 100	7.0/ 100	7.4/ 98	7.57 ±0.03	6.66 ±0.05	96.1 ±0.3	92.1 ±0.4	96/ 84	82/ 68	7.4

[a]DC_{50}: concentration in molar causing 50% reduction of protein level relative to vehicle control treatment in 24 h. Dmax: maximum reduction of protein level relative to vehicle control treatment. pIC50 was measured after 48 h treatment. Errors on pIC50 values reflect the quality of the curve fitting. Protein depletion % are for 50 nM treatments (4 h) in MV4;11/HL60. For more details please refer to reference 3.

Negative control

"Cis MZ 1" is the (S) hydroxy diastereoisomer of MZ 1. While exhibiting MZ 1 comparable Bromodomain binding affinity it no longer is able to bind and recruit VCB and therefore is not degrading BET proteins in cells. The affinity of the "cis MZ 1" to VCB is > 15 µM (ITC).

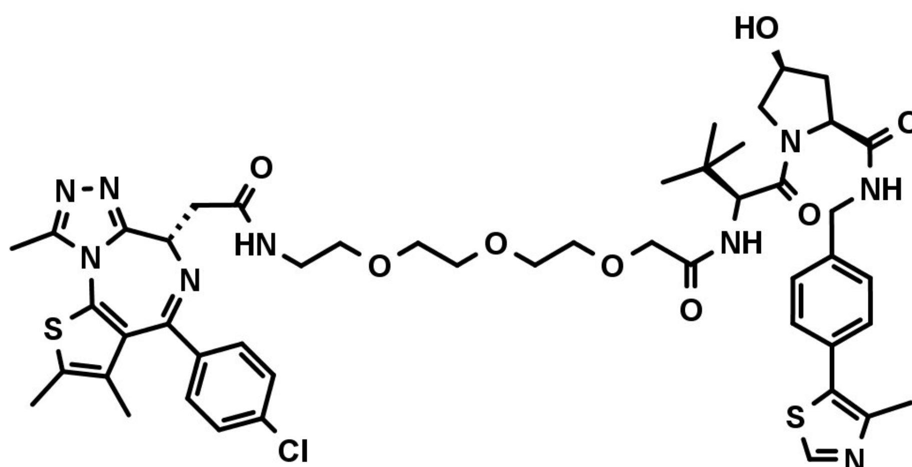

Figure 5: “cis MZ 1” which serves as a negative control

Selectivity

No data available

MZ 1	SELECTIVITY DATA AVAILABLE
Cerep®	No
Panlabs®	No
Invitrogen®	No
DiscoverX®	No
Dundee	No

Co-crystal structure of the MZ 1 in complex with target protein and VCB

The X-ray of the PROTAC MZ 1 in complex with the second bromodomain of BRD4 and pVHL:ElonginC:ElonginB is available in the PDB: 5T35

Reference molecule(s)

dBET (CRBN based PROTAC) is BET bromodomain degrader which is less specific than MZ 1.[4,5]

Summary

MZ 1 is a first in class l.m.w. degrader that tethers JQ1 to a VHL E3 ubiquitin ligase ligand, aimed at triggering the intracellular destruction of BET proteins. MZ 1 potently and rapidly induces reversible, long-lasting, and preferential removal of BRD4 over BRD2 and BRD3 in cells.

MZ 1 and the negative control cis MZ 1 have been discovered by Michael Zengerle, Kwok-Ho Chan, and Alessio Ciulli[1] at the Universtity of Dundee. opnMe.com is proud to be able to provide researchers with up to two batches of 5 mg of MZ 1 and one batch of cisMZ 1 for free. Larger quantities of the compounds are available from Tocris.

Supplementary data

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

References

1. Michael Zengerle, Kwok-Ho Chan, and Alessio Ciulli Selective Small Molecule Induced Degradation of the BET Bromodomain Protein BRD4 *ACS Chem Biol.* **2015**, *10*, 1770–1777. DOI: 10.1021/acschembio.5b00216, PubMed.

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3. Kwok-Ho Chan, Michael Zengerle, Andrea Testa, and Alessio Ciulli Impact of Target Warhead and Linkage Vector on Inducing Protein Degradation: Comparison of Bromodomain and Extra-Terminal (BET) Degraders Derived from Triazolodiazepine (JQ1) and Tetrahydroquinoline (I-BET726) BET Inhibitor Scaffolds *J. Med. Chem.* **2018**, *61*, 504-513. DOI:10.1021/acs.jmedchem.6b01912 , PubMed.

4. Kelly M. DeMars, Changjun Yang, Carolina I. Castro-Rivera and Eduardo Candelario-Jalil Selective degradation of BET proteins with dBET1, a proteolysis-targeting chimera, potently reduces pro-inflammatory responses in lipopolysaccharide-activated microglia *Biochem Biophys Res Commun.* **2018**, *497*, 410-415. DOI: 10.1016/j.bbrc.2018.02.096, PubMed.

5. Georg E. Winter, Dennis L. Buckley, Joshiawa Paulk, Justin M. Roberts, Amanda Souza, Sirano Dhe-Paganon, James E. Bradner DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for in vivo target protein degradation *Science* **2015**, *348*, 1376-1381. DOI: 10.1126/science.aab1433, PubMed.