opnMe by Boehringer Ingelheim

BRD7/BRD9 PROTAC

VZ185

Boehringer Ingelheim

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Summary

VZ185 is a VHL-based, potent and selective PROTAC (proteolysis-targeting chimera) degrader of the BAF/PBAF complexes subunits BRD7 & BRD9.

Chemical Structure

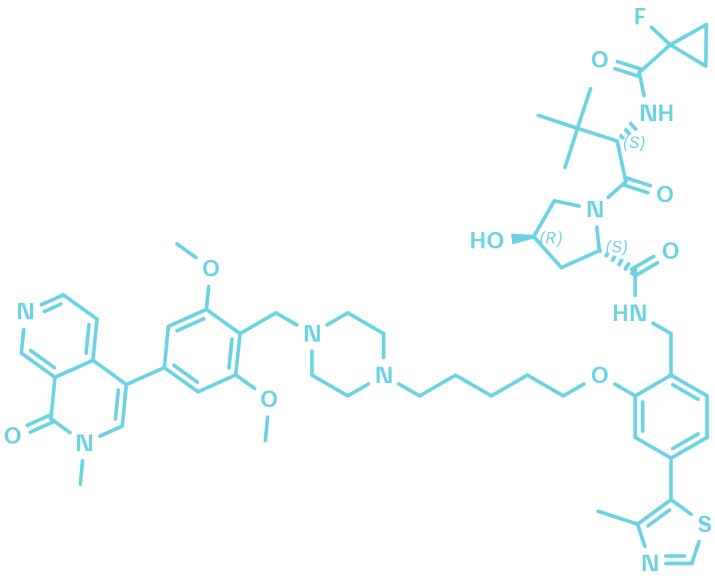

Figure 1: 2D structure of VZ185

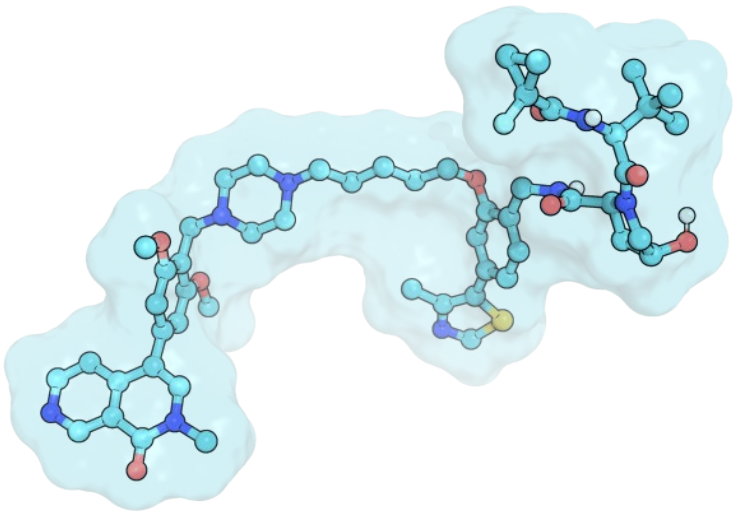

Figure 2: VZ185, 3D conformation

Highlights

VZ185 is a potent, fast, and selective degrader of BRD9 and its close homolog BRD7. It has shown a good *in vivo* PK profile, with high stability in plasma and microsomes as well as high aqueous kinetic solubility (up to ~100 µM). This compound is suitable for both *in vitro* and *in vivo* studies. The molecule and its negative control cis VZ185 have been discovered by Vittoria Zoppi, Scott Hughes, Chiara Maniaci, Andrea Testa, and Alessio Ciulli at the University of Dundee, School of Life Sciences, in collaboration with researchers from Boehringer Ingelheim and Promega.

Target information

Developing PROTACs (proteolysis-targeting chimera) to redirect the ubiquitination activity of E3 ligases and potently degrade a target protein within cells can be a lengthy and unpredictable process, and it remains unclear whether any combination of E3 and target might be productive for degradation. We describe a probe-quality degrader for a ligase–target pair deemed unsuitable: the von Hippel–Lindau (VHL) and BRD9, a bromodomain-containing subunit of the SWI/SNF chromatin remodeling complex BAF. VHL-based degraders could be optimized from suboptimal compounds in two rounds by systematically varying conjugation patterns and linkers and monitoring cellular degradation activities, kinetic profiles, and ubiquitination, as well as ternary complex formation thermodynamics. The emerged structure–activity relationships guided the discovery of VZ185, a potent, fast, and selective degrader of BRD9 and of its close homolog BRD7. Our findings qualify a new chemical tool for BRD7/9 knockdown and provide a roadmap for PROTAC development against seemingly incompatible target–ligase combinations[1].

BRD9 and its close homolog BRD7 (85% sequence identity[2]) are bromodomain-containing subunits of the BAF (BRG-/BRM-associated factor) and PBAF (Polybromo-associated BAF) complexes, respectively[3,4]. BAF and PBAF represent two variants of the SWI/SNF complex, one of the four mammalian ATP-dependent chromatin remodeling complexes. The SWI/SNF complexes control gene expression, DNA replication and DNA repair by modulating access to promoters and coding regions of DNA through modification of the degree of compactness of chromatin[5-7]. Mounting evidence from genetics and sequencing of cancer-associated mutations have spurred efforts to unravel yet largely elusive physiological roles of BAF/PBAF subunits and to develop targeted therapeutics in cancer and other human diseases[3]. In particular, BRD9 is overexpressed in several malignancies, such as cervical cancer and in non-small cell lung cancer (NSCLC)[8,9]. In contrast, BRD7 gene has been proposed as candidate tumor suppressor gene,[10–13] as it regulates breast cancer cell metabolism,[14] and acts as negative regulator of aerobic glycolysis essential for tumor progression[15]. BRD7 also promotes X-box binding protein 1 (XBP1) nuclear translocation, which prevents the development of insulin-resistance disorders[16]. In contrast to these roles, it has been recently shown that inactivation of the BRD7 gene sensitizes tumor cells to T cell-mediated killing,

suggesting that knockdown of BRD7 could be an attractive target for cancer immunotherapy[17]. Potent and selective inhibitors that bind to the BRD7/9 bromodomains have recently emerged from structure-guided medicinal chemistry campaigns, including compounds I-BRD9,[2] LP99,[18] ketone "compound 28",[19] BI-7273 and BI-9564,[1,20] and GNE-375[21]. These BRD7/9 inhibitors have been used in cells to help clarify the roles of the BRD7/9 bromodomain in oncogenesis and other disease states. For example, pharmacological studies of inhibitors BI-7273 and BI-9564 in combination with domain-swap protein engineering revealed that an active bromodomain of BRD9 is required to sustain MYC transcription and proliferation of leukemic cells[20,22]. These findings and availability of bromodomain ligands prompted us to initiate a PROTAC medicinal chemistry campaign to target BRD7 and BRD9 proteins for degradation[1]. Larger quantities of the compounds are available from Tocris.

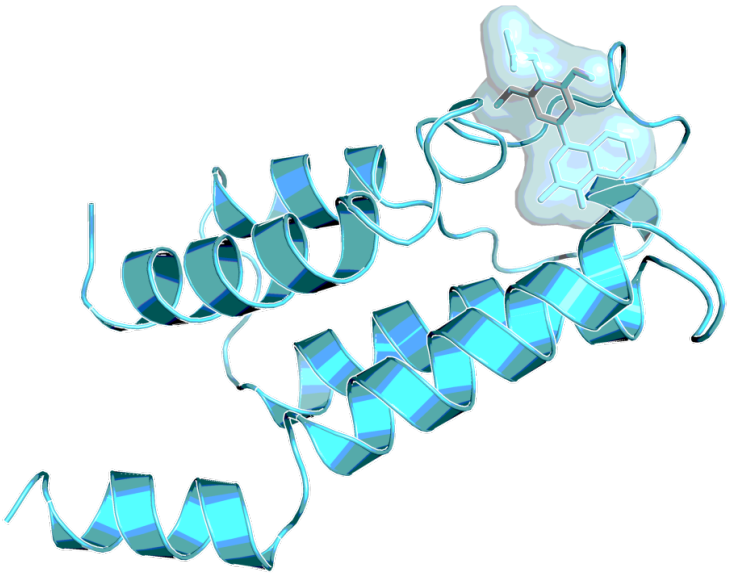

Figure 3: Cocrystal structure of BRD9-BD and compound 5 as shown in reference 1 (PDB code 5EUI)

In vitro activity

VZ185 displays binary and ternary K_D = 30 nM to VHL in both ITC and FP binding assays and degrades BRD9 and BRD7 proteins with DC_{50} values of 1.8 nM and 4.5 nM and D_{max} of 95% (in RI-1 cell lines)[1]. The total stability rG of the ternary complex VHL:VZ185:BRD9-BD is –21.7 kcal/mol[1].

PROBE NAME / NEATIVE CONTROL	VZ185	cis-VZ185
MW [Da, free base][a]	995.2	995.2

ITC (VHL binary K_D) [nM][b]	26 ± 9	n.d.
ITC (VHL ternary K_D *i.e.* in the presence of BRD9-BD) [nM][b]	27 ± 3	n.d.
ITC (BRD9-BD binary K_D) [nM][b]	5.1 ± 0.6	n.d.
ITC total rG (kcal mol^{-1})[b]	–21.7	n.d.
FP (VHL binary K_D) [nM][b]	35 ± 5	n.d.
FP (VHL ternary K_D *i.e.* in the presence of BRD9-BD) [nM][b]	35 ± 6	n.d.
ITC/FP (Cooperativity, α)[b]	1.0	n.d.
Western Blot degradation assay (DC_{50}, 8 h in RI-1 cells, BRD9 / BRD7) [nM][b]	1.8 / 4.5	n.d.
Live-cell degradation (DC_{50}, in HEK293 cells, HiBiT-Brd9 / HiBiT-Brd7) [nM][b]	4.0 / 34.5	n.d.
WES degradation assay (DC_{50}, 18 h BRD9, EOL-1 / A204 cells) [nM][b]	2.3 / 8.3	n.d.
CellTiterGlo (Cell viability EC_{50}, EOL-1 / A204 cells) [nM][b]	3.4 / 39.8	n.d.

[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] Assay conditions available in reference 1

In vitro DMPK and CMC parameters

In vitro PK data further showed high stabilities of VZ185 in both plasma and microsomes from both human and mouse species, as well as high aqueous kinetic solubility (up to ~100 µM). Together, the data qualify VZ185 as a novel high-quality degrader probe for cellular and potentially *in vivo* investigations.

PROBE NAME / NEGATIVE CONTROL	VZ185	CIS-VZ185
logD @ pH 11	4.1	4.2
CHI logD @ pH 7.4[a]	2.3	2.4
Solubility (nephelometry) [µM][a]	85	79

PAMPA permeability @ pH7.4 P_e [nm/s][a]	0.01	0.36
PAMPA permeability @ pH7.4 recovery [%][a]	70	87
Microsomal stability (human / mouse) [mL/min/g liver][a]	3.8 / 1.2	8.1 / 2.4
Plasma stability $T_{1/2}$ (human / mouse) [min][a]	>180 / >180	>180 / >180

[a] Assay conditions available in reference 1

In vivo DMPK parameters

VZ185	MOUSE	RAT
Clearance [% Q_H][a]	12	120
Mean residence time after *i.v.* dose [h][a]	2.1	2.1
t_{max} [h][b]	0.5	1.7
F [%][b]	Quantitatively bioavailable	Quantitatively bioavailable
V_{ss} [L/kg]	1.3	10
AUCinf[a] [h*nmol/L]	7,800	1,000
AUCinf[b] [h*nmol/L]	7,400	1,600
AUD[a] [h*nmol/L]	7,800	1,000
AUD[b] [h*nmol/L]	6,700	1,600

[a] *i.v.* dose: 5 mg/kg
[b] *s.c.* dose: 5 mg/kg

Negative control

cis-VZ185 is the (S) hydroxy diastereoisomer of VZ185. While exhibiting comparable bromodomain binding affinity it no longer is able to bind and recruit the E3 ligase VHL and therefore does not induce the degradation of BRD7 and BRD9 proteins in cells.

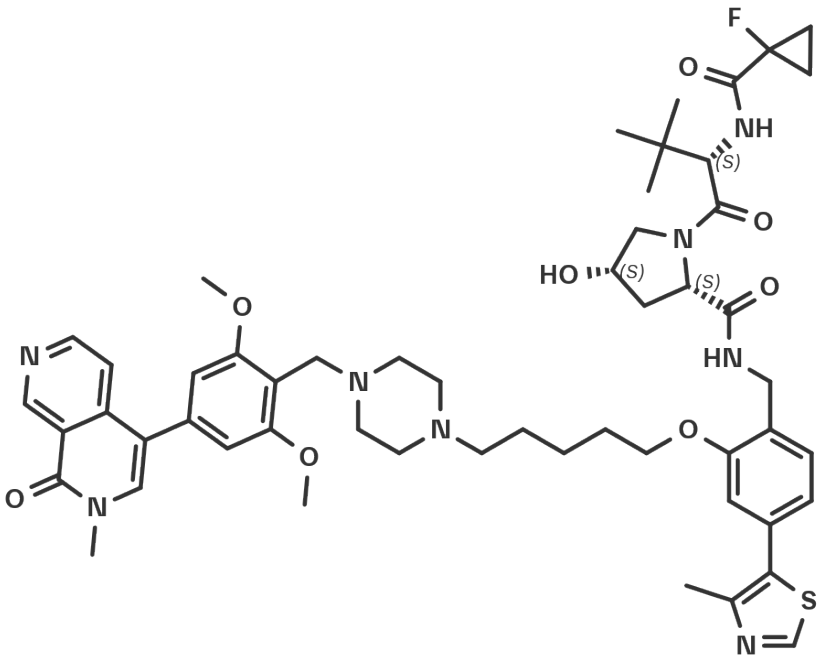

Figure 4: cis VZ185 which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	VZ185	cis-VZ185
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

To assess the cellular selectivity of VZ185 for BRD7/9 degradation and identify potential degradation off-targets, multiplexed isobaric tagging mass spectrometry proteomic experiments were performed to monitor protein levels in a quantitative and unbiased manner.

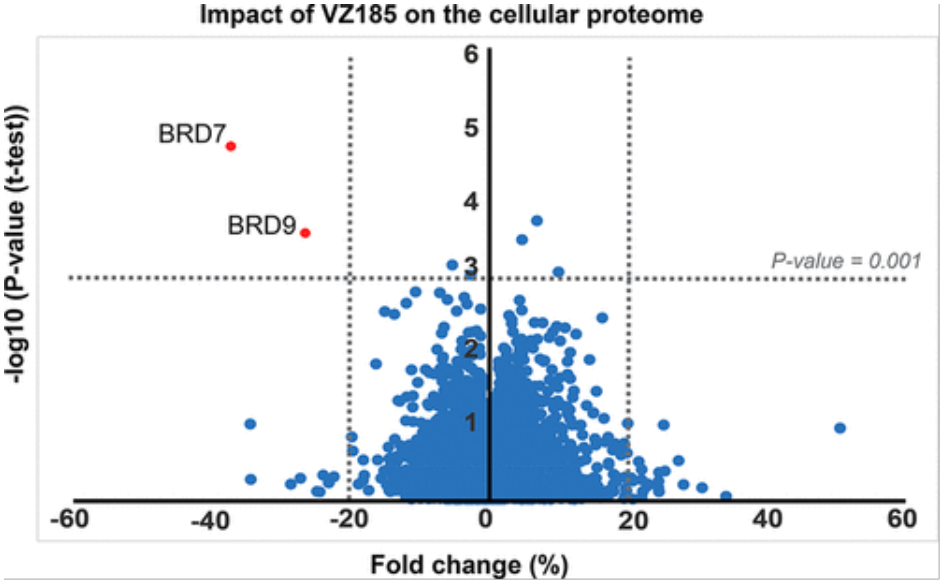

Figure 5: RI-1 cells were treated in triplicate with DMSO, 100 nM VZ185, or 100 nM cis VZ185 for 4 h. Among the 6,273 proteins quantified in this analysis, of those that met the criteria for a statistically significant change in abundance, markedly selective deg

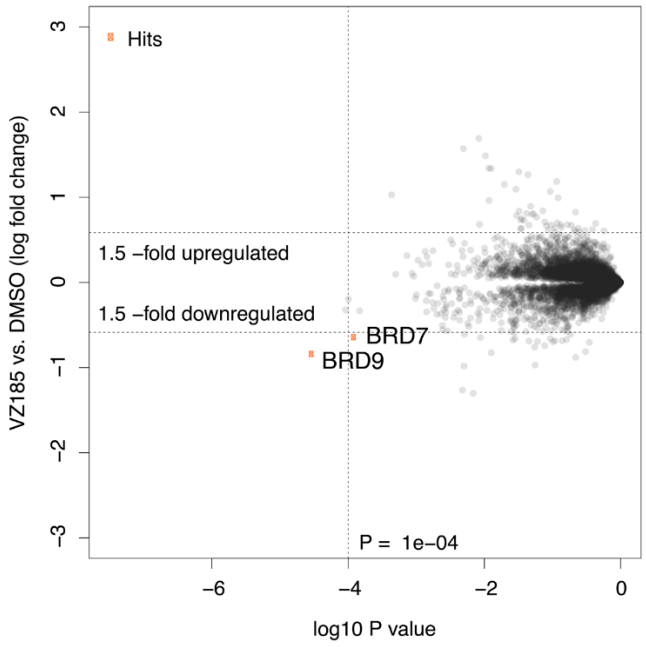

Figure 6: Global protein quantification was used to explore the unbiased proteome-wide selectivity of VZ185 induced degradation. Whole cell protein quantification was performed using label free quantification with the Fischer lab's diaPASEF workflow.

As expected, BRD7/9 proteins were not depleted by treatment with negative control cis VZ185. Protein levels of other bromodomain-containing proteins or other BAF/PBAF subunits remained unaffected. To confirm selectivity over key potential off-target proteins within the bromodomain protein family, live cell kinetic analyses of endogenously tagged BRD2/3/4 and SMARCA4 proteins expressing LgBiT were performed, which showed no degradation of these proteins in the presence of VZ185. Together these results confirmed VZ185 as an effective and highly selective degrader of BRD7/9 proteins in cells[1].

The PROTAC VZ185 was tested by the Eric Fisher Laboratory - Dana-Farber Cancer Institute as part of their Degradation Proteomics Initiative[23,24]. It induces selective degradation of BRD9 and BRD7 after 5 h of treatment at 0.1 µM in the neuroblastoma cell line Kelly Cells.

Of the 7,742 proteins quantified in this experiment, only BRD9 and BRD7 were found to be significantly downregulated in response to VZ185 treatment. Statistical analysis was performed using a moderated t-test in Bioconductor's limma package to generate hit lists containing log2 Fold Change and P-values for each protein. The data are also displayed in the scatterplot above.

Co-crystal structure of the probe compound and the target protein

No ternary structure of VZ185 is available.

Reference molecule(s)

dBRD9[25]

We also offer the BRD7 and BRD9 inhibitors BI-7273 and BI-9564 for free on opnme.com[20].

Supplementary data

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

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