

BRD7/BRD9 PROTAC | VZ185

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	5
<i>In vivo</i> DMPK parameters	6
Negative control	8
Selectivity	8
Co-crystal structure of the probe compound and the target protein	10
Reference molecule(s)	10
Supplementary data	11
References	11

Summary

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

Chemical Structure

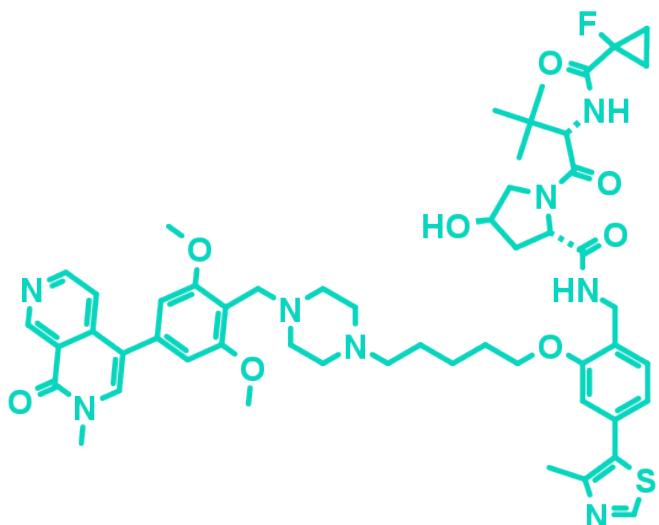


Figure 1: 2-D structure of VZ185

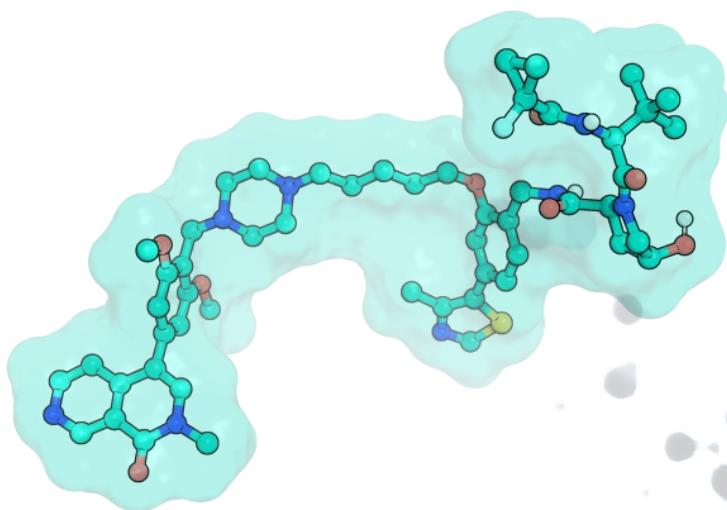


Figure 2: VZ185, 3D conformation

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 identity²) 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](#).

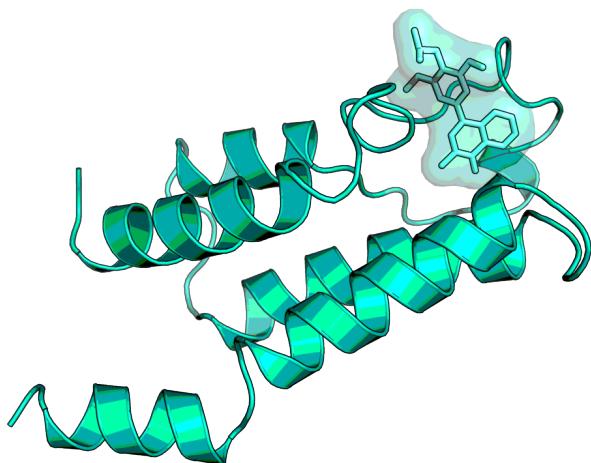


Figure 3: Cocrystal structure of BRD9-BD and compound 5 as shown in reference 1 (PDB code 5EUI)

In vitro activity

VZ185 displays binary and ternary $K_D = 30\text{ nM}$ 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 ΔG of the ternary complex VHL:VZ185:BRD9-BD is -21.7 kcal/mol .¹

PROBE NAME / NEGATIVE CONTROL	VZ185	CIS-VZ185
MW [Da]	995.23	995.23
ITC (VHL binary K_D) [nM] ^a	26 ± 9	n.d.
ITC (VHL ternary K_D i.e. in the presence of BRD9-BD) [nM] ^a	27 ± 3	n.d.

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

^a 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 μ M). 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
logP (calculated)	5.0144	5.0144
CHI LogD @ pH7.4 ^a	2.3	2.4
Aqueous 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 (human mouse) T _{1/2} [min] ^a	>180 >180	>180 >180

^a assay conditions available in reference 1.

In vivo DMPK parameters

VZ185	MOUSE	RAT
Cl (% Q _H) ^a	12	120
Mean residence time (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 5 mg/kg i.v.; ^b 5 mg/kg s.c.

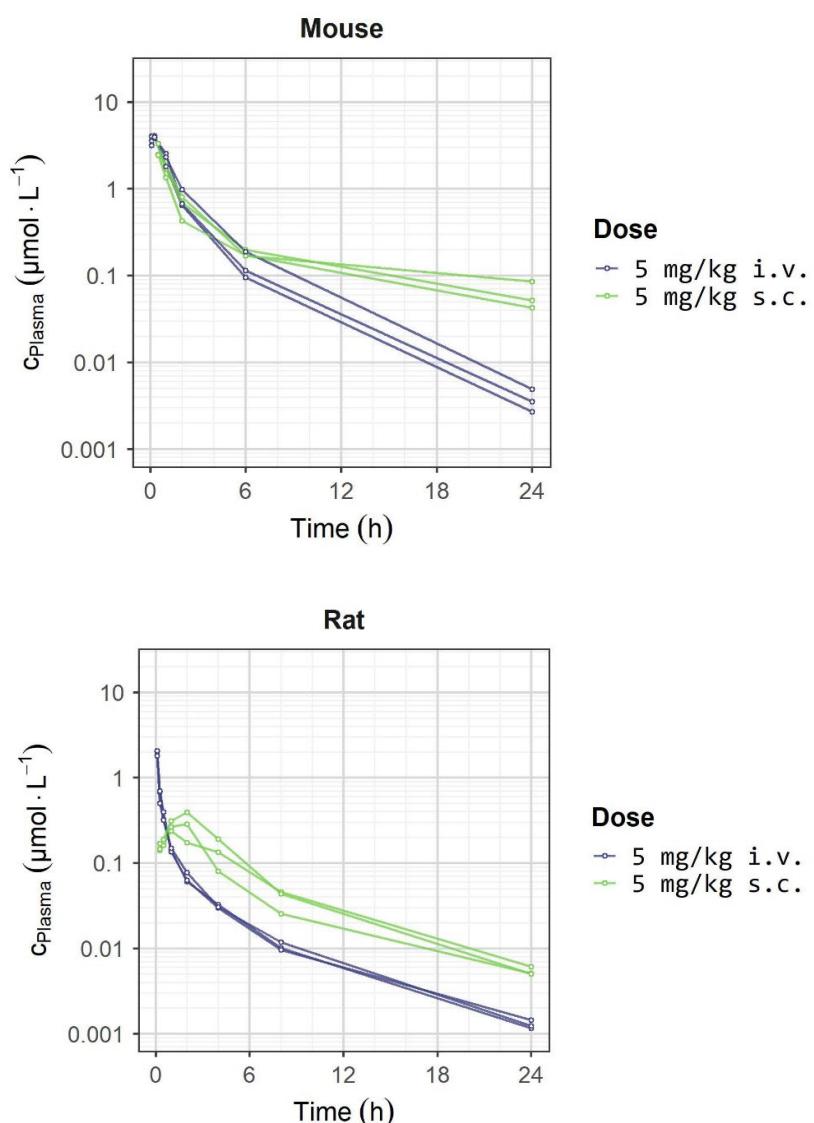


Figure 4: Concentrations of VZ185 in mouse and rat plasma over time

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.

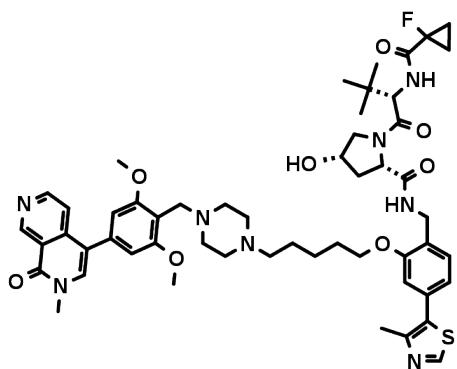


Figure 5: cis VZ185 which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	VZ185	cis VZ185
SafetyScreen™ 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.

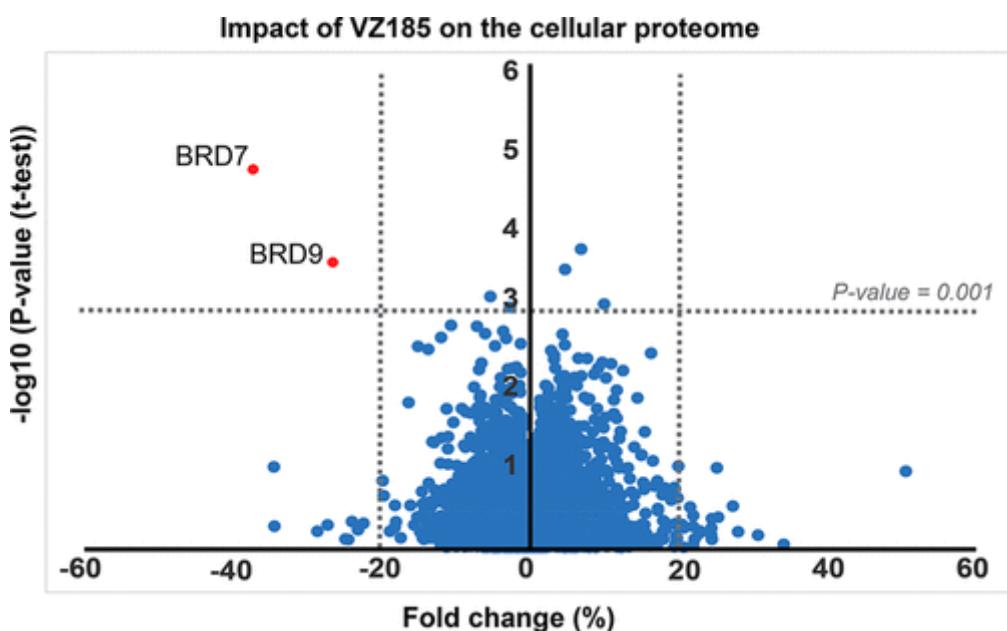


Figure 6: RI-1 cells were treated in triplicate with DMSO, 100 nM VZ185, or 100 nM cis VZ185 for 4 h. Among the 6,273 proteins quantified in this analysis, of those that met the criteria for a statistically significant change in abundance, markedly selective degradation of BRD7 and BRD9 was observed.

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 μ M in the neuroblastoma cell line Kelly Cells.

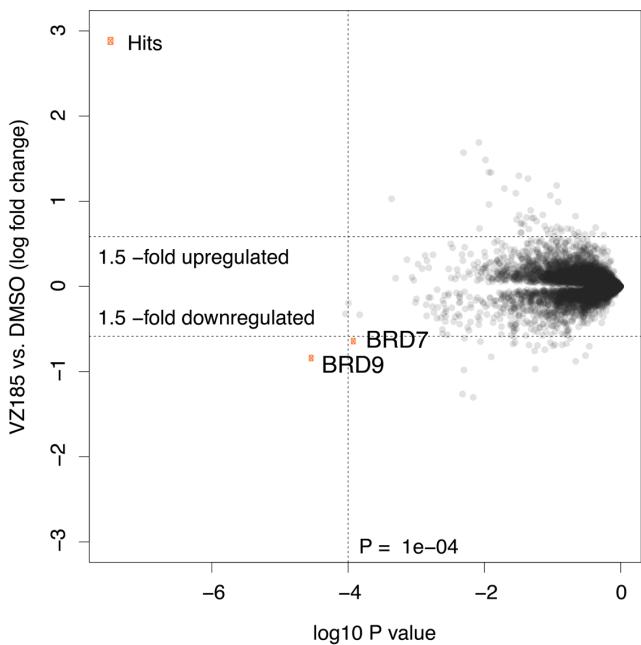


Figure 7: Global protein quantification was used to explore the unbiased proteome-wide selectivity of VZ185 induced degradation. Whole cell protein quantification was performed using label free quantification with the Fischer lab's diaPASEF workflow.

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)

dBRD9²⁵

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

Supplementary data

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

References

1. Zoppi V., Hughes S.J., Maniaci C., Testa A., Gmaschitz T., Wieshofer C., Koegl M., Ricing K., Daniels D.L., Spallarossa A., Ciulli A. Iterative design and optimization of initially inactive Proteolysis Targeting Chimeras (PROTACs) identify VZ185 as a potent, fast and selective von Hippel-Lindau (VHL)-based dual degrader probe of BRD9 and BRD7. *J. Med. Chem.* 2019, **62** (2), 699–726.. [DOI: 10.1021/acs.jmedchem.8b01413](https://doi.org/10.1021/acs.jmedchem.8b01413), [PubMed](#).
2. Theodoulou N. H., Bamborough P., Bannister A. J., Becher I., Bit R. A., Che K.H., Chung C., Dittmann A., Drewes G., Drewry D.H., Gordon L., Grandi P., Leveridge M., Lindon M., Michon A.M., Molnar J., Robson S.C., Tomkinson N.C., Kouzarides T., Prinjha R.K., Humphreys P.G. Discovery of I-BRD9, a Selective Cell Active Chemical Probe for Bromodomain Containing Protein 9 Inhibition. *J. Med. Chem.* 2016, **59** (4), 1425–1439. [DOI: 10.1021/acs.jmedchem.5b00256](https://doi.org/10.1021/acs.jmedchem.5b00256), [PubMed](#).
3. Kadoc C., Hargreaves D.C., Hodges C., Elias L., Ho L., Ranish J., Crabtree G.R. Proteomic and Bioinformatic Analysis of Mammalian SWI/SNF Complexes Identifies Extensive Roles in Human Malignancy. *Nat. Genet.* 2013, **45** (6), 592–601. [DOI: 10.1038/ng.2628](https://doi.org/10.1038/ng.2628), [PubMed](#).
4. Kaeser M.D., Aslanian A., Dong M.-Q., Yates J.R., Emerson B.M. BRD7, a Novel PBAF-Specific SWI/SNF Subunit, is Required for Target Gene Activation and Repression in Embryonic Stem Cells. *J. Biol. Chem.* 2008, **283** (47), 32254–32263. [DOI: 10.1074/jbc.M806061200](https://doi.org/10.1074/jbc.M806061200), [PubMed](#).
5. Narlikar G.J., Sundaramoorthy R., Owen-Hughes T. Mechanisms and Functions of ATP-Dependent Chromatin-Remodeling Enzymes. *Cell* 2013, **154** (3), 490–503. [DOI: 10.1016/j.cell.2013.07.011](https://doi.org/10.1016/j.cell.2013.07.011), [PubMed](#).
6. Hohmann A. F., Vakoc C. R. A Rationale to Target the SWI/SNF Complex for Cancer Therapy. *Trends Genet.* 2014, **30** (8), 356–363. [DOI: 10.1016/j.tig.2014.05.001](https://doi.org/10.1016/j.tig.2014.05.001), [PubMed](#).
7. Martens J.A. Evidence That Swi/Snf Directly Represses Transcription in *S. Cerevisiae*. *Genes Dev.* 2002, **16** (17), 2231–2236. [DOI: 10.1101/gad.1009902](https://doi.org/10.1101/gad.1009902), [PubMed](#).
8. Kang J.U., Koo S.H., Kwon K.C., Park J.W., Kim J.M. Gain at Chromosomal Region 5p15.33, Containing TERT, is the Most Frequent Genetic Event in Early Stages of Non-Small Cell Lung Cancer. *Cancer Genet. Cytogenet.* 2008, **182** (1), 1–11. [DOI: 10.1016/j.cancergencyto.2007.12.004](https://doi.org/10.1016/j.cancergencyto.2007.12.004), [PubMed](#).
9. Scotto L., Narayan G., Nandula S.V., Subramaniyam S., Kaufmann A.M., Wright J.D., Pothuri B., Mansukhani M., Schneider A., Arias-Pulido H., Murty V.V. Integrative Genomics Analysis

- of Chromosome 5p Gain in Cervical Cancer Reveals Target Over-Expressed Genes, Including Drosha. *Mol. Cancer* **2008**, *7*, 58. [DOI: 10.1186/1476-4598-7-58](https://doi.org/10.1186/1476-4598-7-58), [PubMed](#).
10. Yu X., Li Z., Shen J. BRD7: A Novel Tumor Suppressor Gene in Different Cancers. *Am. J. Transl. Res.* **2016**, *8* (2), 742–748. [PMC4846923](#), [PubMed](#).
11. Drost J., Mantovani F., Tocco F., Elkon R., Comel A., Holstege H., Kerkhoven R., Jonkers J., Voorhoeve P.M., Agami R., Del Sal G. BRD7 Is a Candidate Tumour Suppressor Gene Required for P53 Function. *Nat. Cell Biol.* **2010**, *12* (4), 380–389. [DOI: 10.1038/ncb2038](https://doi.org/10.1038/ncb2038), [PubMed](#).
12. Chiu Y.-H., Lee J.Y., Cantley L.C. BRD7, a Tumor Suppressor, Interacts with P85 α and Regulates PI3K Activity. *Mol. Cell* **2014**, *54* (1), 193–202. [DOI: 10.1016/j.molcel.2014.02.016](https://doi.org/10.1016/j.molcel.2014.02.016), [PubMed](#).
13. Harte M.T., O'Brien G.J., Ryan N.M., Gorski J.J., Savage K.I., Crawford N.T., Mullan P.B., Harkin D.P. BRD7, a Subunit of SWI/SNF Complexes, Binds Directly to BRCA1 and Regulates BRCA1-Dependent Transcription. *Cancer Res.* **2010**, *70* (6), 2538–2547. [DOI: 10.1158/0008-5472.CAN-09-2089](https://doi.org/10.1158/0008-5472.CAN-09-2089), [PubMed](#).
14. Niu W., Luo Y., Wang X., Zhou Y., Li H., Wang H., Fu Y., Liu S., Yin S., Li J., Zhao R., Liu Y., Fan S., Li Z., Xiong W., Li X., Li G., Ren C., Tan M., Zhou M. BRD7 Inhibits the Warburg Effect and Tumor Progression Through Inactivation of HIF1 α /LDHA Axis in Breast Cancer. *Cell Death Dis.* **2018**, *9* (5), 519. [DOI: 10.1038/s41419-018-0536-7](https://doi.org/10.1038/s41419-018-0536-7), [PubMed](#).
15. Gatenby R.A., Gillies R.J. Why Do Cancers Have High Aerobic Glycolysis? *Nat. Rev. Cancer* **2004**, *4* (11), 891–899. [DOI: 10.1038/nrc1478](https://doi.org/10.1038/nrc1478), [PubMed](#).
16. Park S.W., Herrema H., Salazar M., Cakir I., Cabi S., Basibuyuk Sahin F., Chiu Y.-H., Cantley L.C., Ozcan U. BRD7 Regulates XBP1s' Activity and Glucose Homeostasis Through its Interaction with the Regulatory Subunits of PI3K. *Cell Metab.* **2014**, *20* (1), 73–84. [DOI: 10.1016/j.cmet.2014.04.006](https://doi.org/10.1016/j.cmet.2014.04.006), [PubMed](#).
17. Pan D., Kobayashi A., Jiang P., Ferrari de Andrade L., Tay R.E., Luoma A.M., Tsoucas D., Qiu X., Lim K., Rao P., Long H.W., Yuan G.C., Doench J., Brown M., Liu X.S., Wucherpfennig K.W. A Major Chromatin Regulator Determines Resistance of Tumor Cells to T Cell-Mediated Killing. *Science* **2018**, *359* (6377), 770–775. [DOI: 10.1126/science.aaq1710](https://doi.org/10.1126/science.aaq1710), [PubMed](#).
18. Clark P.G.K., Vieira L.C.C., Tallant C., Fedorov O., Singleton D.C., Rogers C.M., Monteiro O.P., Bennett J.M., Baronio R., Müller S., Daniels D.L., Méndez J., Knapp S., Brennan P.E., Dixon D.J. LP99: Discovery and Synthesis of the First Selective BRD7/9 Bromodomain Inhibitor. *Angew. Chem. Int. Ed.* **2015**, *54* (21), 6217–6221. [DOI: 10.1002/anie.201501394](https://doi.org/10.1002/anie.201501394), [PubMed](#).
19. Hay D.A., Rogers C.M., Fedorov O., Tallant C., Martin S., Monteiro O.P., Müller S., Knapp S., Schofield C.J., Brennan P.E. Design and Synthesis of Potent and Selective Inhibitors of BRD7 and BRD9 Bromodomains. *MedChemComm* **2015**, *6* (7), 1381–1386. [DOI: 10.1002/anie.201501394](https://doi.org/10.1002/anie.201501394), [PubMed](#).

20. Martin L.J., Koegl M., Bader G., Cockcroft X.-L., Fedorov O., Fiegen D., Gerstberger T., Hofmann M.H., Hohmann A.F., Kessler D., Knapp S., Knesl P., Kornigg S., Müller S., Nar H., Rogers C., Rumpel K., Schaaf O., Steurer S., Tallant C., Vakoc C.R., Zeeb M., Zoephel A., Pearson M., Boehmelt G., McConnell D. Structure-Based Design of an in Vivo Active Selective BRD9 Inhibitor. *J. Med. Chem.* **2016**, 59 (10), 4462–4475. [DOI: 10.1021/acs.jmedchem.5b01865](https://doi.org/10.1021/acs.jmedchem.5b01865), [PubMed](#).
21. Crawford T.D., Vartanian S., Côté A., Bellon S., Duplessis M., Flynn E.M., Hewitt M., Huang H.-R., Kiefer J.R., Murray J., Nasveschuk C.G., Pardo E., Romero F.A., Sandy P., Tang Y., Taylor A.M., Tsui V., Wang J., Wang S., Zawadzke L., Albrecht B.K., Magnuson S.R., Cochran A.G., Stokoe D. Inhibition of Bromodomain-Containing Protein 9 for the Prevention of Epigenetically-Defined Drug Resistance. *Bioorg. Med. Chem. Lett.* **2017**, 27 (15), 3534–3541. [DOI: 10.1016/j.bmcl.2017.05.063](https://doi.org/10.1016/j.bmcl.2017.05.063), [PubMed](#).
22. Hohmann A.F., Martin L.J., Minder J.L., Roe J.-S., Shi J., Steurer S., Bader G., McConnell D., Pearson M., Gerstberger T., Gottschamel T., Thompson D., Suzuki Y., Koegl M., Vakoc C.R. Sensitivity and Engineered Resistance of Myeloid Leukemia Cells to BRD9 Inhibition. *Nat. Chem. Biol.* **2016**, 12 (9), 672–679. [DOI: 10.1038/nchembio.2115](https://doi.org/10.1038/nchembio.2115), [PubMed](#).
23. The data can be accessed via the following link: [Proteomics Dashboard \(fischerlab.org\)](#)
24. Donovan K. A., Ferguson F. M., Bushman J. W., Eleuteri N. A., Bhunia D., Ryu S., Tan L., Shi K., Yue H., Liu X., Dobrovolsky D., Jiang B., Wang J., Hao M., You I., Teng M., Liang Y., Hatcher J., Li Z., Manz T. D., Groendyke B., Hu W., Nam Y., Sengupta S., Cho H., Shin I., Agius M. P., Ghobrial I. M., Ma M. W., Che J., Buhrlage S. J., Sim T., Gray N. S., Fischer E. S. Mapping the Degradable Kinome Provides a Resource for Expedited Degrader Development *Cell*. **2020**, 183(6):1714-1731. [DOI: 10.1016/j.cell.2020.10.038](https://doi.org/10.1016/j.cell.2020.10.038), [PubMed](#).
25. Remillard D., Buckley D.L., Paulk J., Brien G.L., Sonnnett M., Seo H.S., Dastjerdi S., Wühr M., Dhe-Paganon S., Armstrong S.A., Bradner J.E. Degradation of the BAF Complex Factor BRD9 by Heterobifunctional Ligands *Angew. Chem. Int. Ed.* **2017**, 56 (21), 5738–5743. [DOI: 10.1002/anie.201611281](https://doi.org/10.1002/anie.201611281), [PubMed](#).