Discovery and characterization of novel small molecule degraders of BRD4 that act through the recruitment of DCAF11

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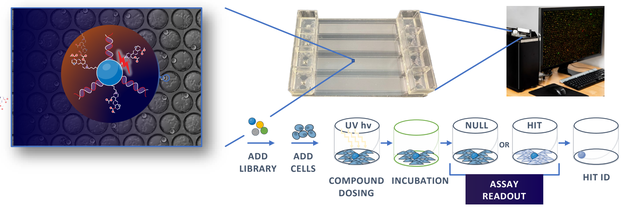
Abstract

Targeted protein degradation (TPD) through the ubiquitin proteasome system (UPS) is a rapidly growing drug discovery approach to eliminate pathogenic proteins. Strategies for TPD have focused on heterobifunctional degraders in which an E3 ligase and a target protein are brought into proximity. Optimization of drug-like properties has often been challenging. Monovalent degraders represent an alternative approach, in which small molecules are designed to bind to the target protein and induce its degradation through the recruitment of an E3 ligase complex. However, until now, the discovery of monovalent degraders has relied on serendipity. Using our ultra-high throughput cell-based screening platform, which measures degradation of target proteins upon exposure to diverse E3 ligase agnostic chemical libraries, we identified several monovalent degraders of the bromodomain extra-terminal (BET) protein BRD4. Optimization of hits produced a lead compound, PLX-3618, which elicited full, rapid, and selective degradation of BRD4, strong down-regulation of the MYC oncogene, and potent anti-proliferative activity in AML. Further characterization confirmed BRD4 degradation was mediated via the UPS, and a ubiquitin ligase-focused CRISPR screen identified CUL4B^{DCAF11} as the E3 complex responsible for PLX-3618-mediated degradation of BRD4. Protein-protein interaction studies verified a BRD4/PLX-3618/DCAF11 ternary complex, and mutational studies provided further insights into the DCAF11-mediated degradation mechanism. *In vivo* activity of PLX-3618 versus a pan-BET inhibitor was assessed using the AML MV-4-11 xenograft model. Treatment with PLX-3618 resulted in complete tumor regression, whereas the inhibitor only resulted in tumor growth inhibition. Collectively, these results demonstrate the efficient discovery of novel small molecule degraders using Plexium's proprietary platform, and subsequent characterization of the degradation mechanism highlights the discovery of DCAF11 as an E3 ligase substrate receptor amenable to redirection for neo-substrate degradation.

Plexium's screening platform and initial hit identification, validation, and optimization



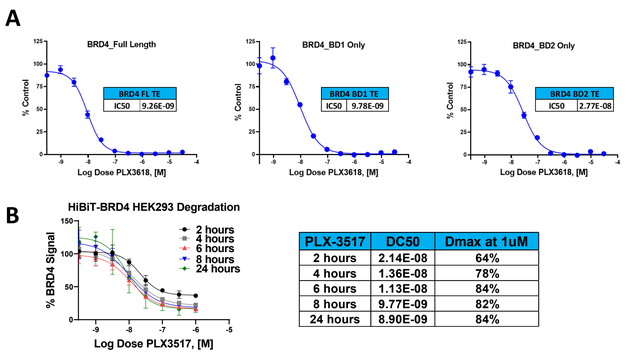
Molecule ID	IC50	DC50
PLX-3517	1.03E-08	<1.0E-07

Optimization

PLX-3618	
TE IC_{50}	DC_{50}
10.9 nM	12.2 nM

Figure 1: A. Overview of the Plexium ultra-high throughput screening workflow. B. Lane view of Plexium Picowell device with increased magnification showing example of a hit well (BRD4 = green; Tubulin = red) C. NanoLuc-BRD4 target engagement binding curve of screening hit compound, PLX-3517. Bars represent standard deviation (SD) D. Western blot analysis of BRD4 degradation using PLX-3517 in HEK-293T cells. Table shows DC_{50} calculated from western blot and IC_{50} from target engagement assay shown in B. Optimization yielded lead tool compound, PLX-3618, demonstrating improved degradation potency.

Plexium monovalent degraders bind both bromodomains and have rapid degradation kinetics



PLX-3517	DC50	Dmax at 1uM
2 hours	2.14E-08	64%
4 hours	1.36E-08	78%
6 hours	1.13E-08	84%
8 hours	9.77E-09	82%
24 hours	8.90E-09	84%

Figure 2: A. NanoLuc-BRD4 full length, NanoLuc-BRD4 BD1, and NanoLuc-BRD4 BD2 target engagement binding curves of optimized lead tool compound, PLX-3618. Bars represent standard deviation (SD) B. BRD4 degradation using PLX-3517 in HiBiT-BRD4-HEK293 cells. Bars represent SD. Table shows DC_{50} values calculated from degradation curves and maximum degradation (Dmax) at 1μM dose.

Plexium monovalent degraders require the proteasome to selectively degrade BRD4

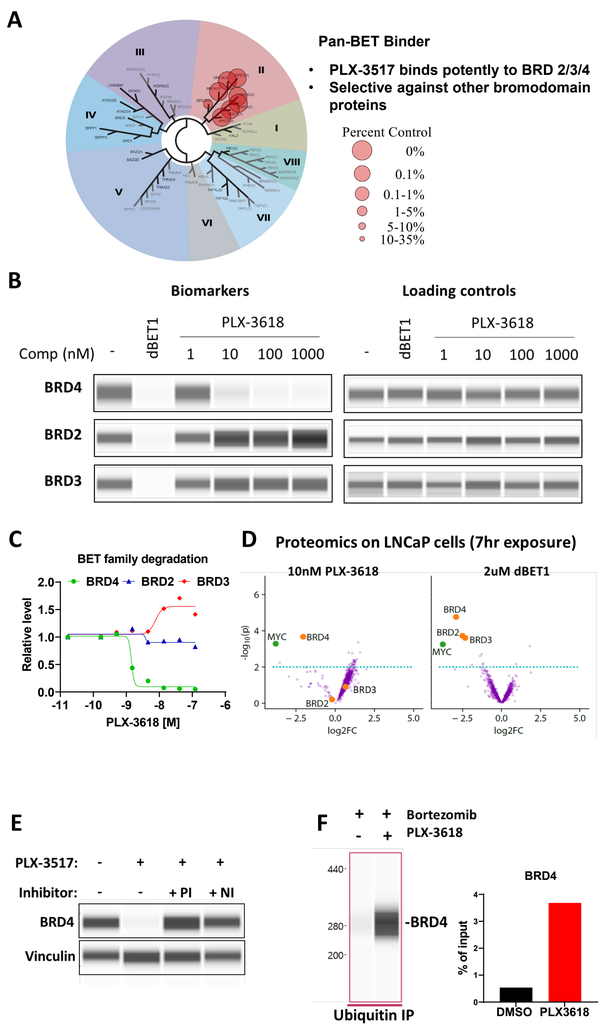
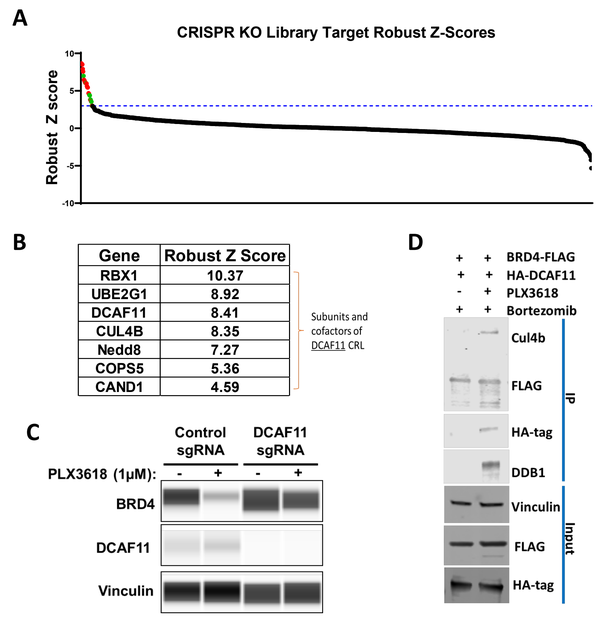


Figure 3: A. BROMO*scan* binding data (Eurofins) using 1μM PLX-3618. Data demonstrates binding is selective to BET-family proteins (Class II). B. BET protein degradation selectivity comparing PLX-3618 to 10μM dBET1 (pan-BET degrader PROTAC) in MV-4-11 cells. PLX-3618 selectively degrades BRD4, whereas dBET1 degrades all BET proteins. C. Degradation dose response curves, comparing BRD2, BRD3, and BRD4 in MV-4-11 cells treated with PLX-3618 for 24h. Data quantified from western blot analyses. D. Proteomic changes in LNCaP cells in response to 7h exposure to 10nM PLX-3618 or 2uM dBET1. E. Degradation is mediated by the proteasome. HEK-293T cells were incubated +/- proteasome inhibitor (PI; 100nM bortezomib) or neddylation inhibitor (NI; 1μM Pevonedistat) for 2h, followed by a 6h incubation with 100nM PLX-3517. F. PLX-3618 induces BRD4 ubiquitination. Ubiquitinated proteins were immunoprecipitated following PLX-3618 treatment in HEK-293T cells, and subsequently subjected to western blot analysis, probing for BRD4.

DCAF11 is required for PLX-3618 mediated degradation of BRD4



Gene	Robust Z Score
RBX1	10.37
UBE2G1	8.92
DCAF11	8.41
CUL4B	8.35
Nedd8	7.27
COPS5	5.36
CAND1	4.59

Subunits and cofactors of DCAF11 CRL

Figure 4: A. CRISPR knockout (KO) screening data utilizing a ubiquitin proteasome system focused sgRNA library (Synthego). BRD4 degradation was assessed via immunofluorescence after CRISPR KO and subsequent treatment with PLX-3618. Hits were identified using robust Z-score calculations (0.675*(Xi-median)/MAD). Red dots = proteasome subunits; green dots = CUL4B-DCAF11 CRL subunits. B. Table showing CUL4B-DCAF11 CRL subunits identified in CRISPR screen. C. CRISPR-mediated knockout of DCAF11 verified its involvement in degrading BRD4 upon exposure to PLX-3618. D. FLAG-BRD4 immunoprecipitation experiments demonstrated PLX-3618-induced interactions with HA-tagged DCAF11 and co-purification of selected endogenous components of CRL4B^{DCAF11}.

PLX-3618-mediated degradation of BRD4 requires binding to both bromodomains

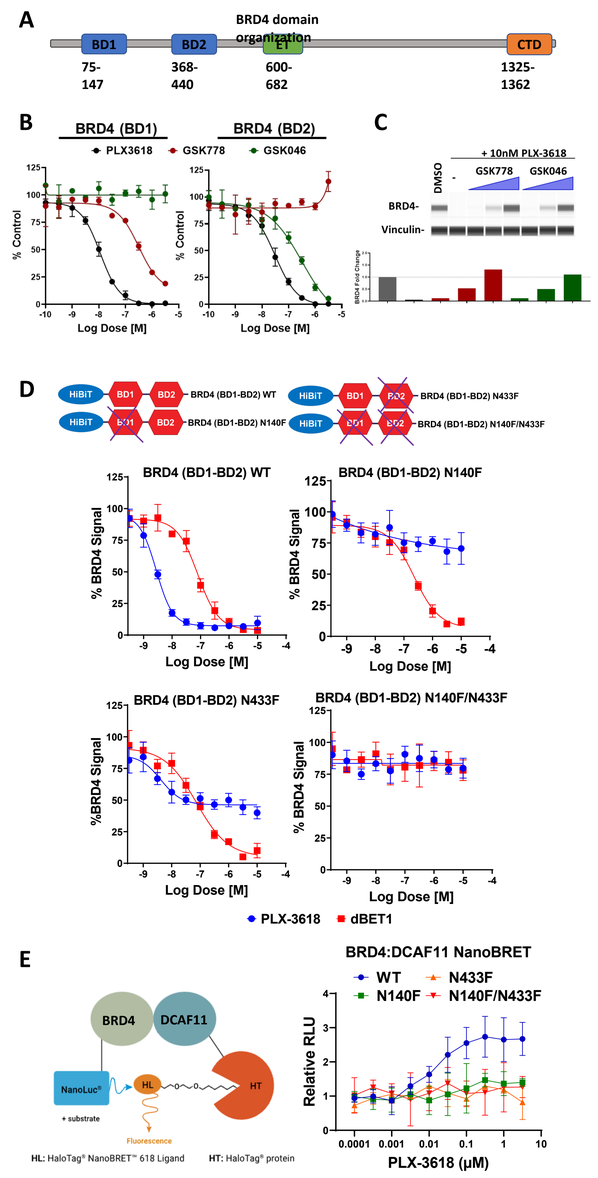
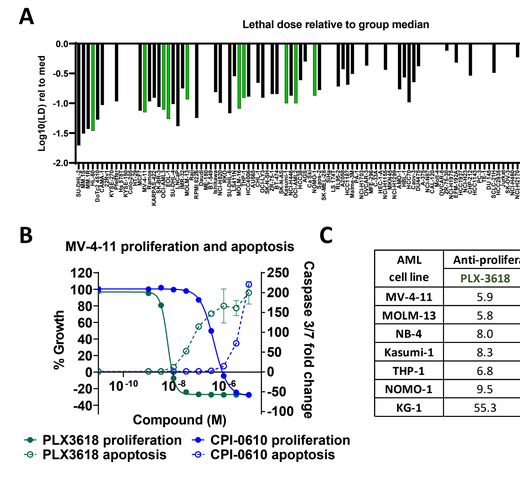


Figure 5: A. Domain organization of BRD4 with corresponding amino acid ranges. BRD4 contains two N-terminal bromodomains (BD1 and BD2), an extra-terminal domain (ET), and C-terminal domain (CTD). B. Bromodomain selective tool inhibitors GSK778 (BD1-selective) and GSK046 (BD2-selective) bind selectively in the BRD4 isolated bromodomain target engagement assays. C. Competition experiment using BD-selective tool inhibitors. Both BD1 and BD2-selective inhibitors compete for PLX-3618-mediated degradation of BRD4. D. Bromodomain-specific point mutations known to abrogate acetylated histone binding were introduced into HiBiT protein constructs and used to monitor PLX-3618-mediated degradation (schematic at top shows fusion constructs and point mutations). Mutational analysis confirmed the requirement of both bromodomains for full degradation of BRD4 by PLX-3618. E. PPI NanoBRET assay, with constructs harboring bromodomain point mutations, show that binding to both bromodomains are required for efficient BRD4:DCAF11 ternary complex formation.

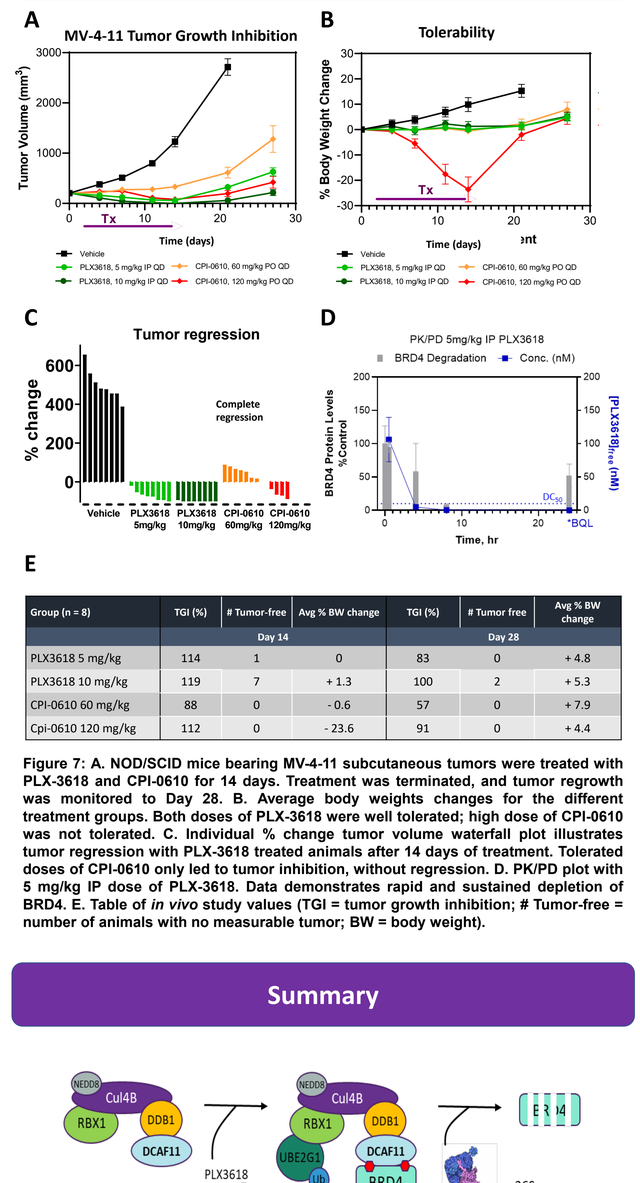
Potent anti-tumor activity identified in AML



AML cell line	Anti-proliferative EC_{50} (nM)	
	PLX-3618	CPI-0610
MV-4-11	5.9	412.0
MOLM-13	5.8	473.6
NB-4	8.0	760.4
Kasumi-1	8.3	262.9
THP-1	6.8	377.3
NOMO-1	9.5	386.6
KG-1	55.3	520.4

Figure 6: A. AML tumor lines are sensitive to BRD4 degradation. Cell killing activity of PLX-3618 in a panel of cancer cell lines (Crown Biosciences). Cells were treated with PLX-3618 for 72h and cell viability was monitored using CellTiter Glo. Lethal doses (minimum concentration of PLX-3618 that lead to cell number decrease below Day0 value) were plotted relative to the group. 10 out of 10 AML lines tested (highlighted in green) experienced reduction in cell number upon treatment with PLX-3618. B. MV-4-11 growth inhibition and apoptosis induction curves, comparing PLX-3618 vs. the pan-BET inhibitor, CPI-0610, after treatment for 72h. Cell viability was monitored using CellTiter Glo. Apoptosis was evaluated using Caspase Glo 3/7. C. Table comparing proliferative EC_{50} values for PLX-3618 vs. CPI-0610 in panel of AML tumor lines

PLX-3618 elicits potent anti-tumor activity *in vivo*



E

Group (n = 8)	TGI (%)	# Tumor-free	Avg % BW change	TGI (%)	# Tumor free	Avg % BW change
	Day 14			Day 28		
PLX3618 5 mg/kg	114	1	0	83	0	+ 4.8
PLX3618 10 mg/kg	119	7	+ 1.3	100	2	+ 5.3
CPI-0610 60 mg/kg	88	0	- 0.6	57	0	+ 7.9
Cpi-0610 120 mg/kg	112	0	- 23.6	91	0	+ 4.4

Figure 7: A. NOD/SCID mice bearing MV-4-11 subcutaneous tumors were treated with PLX-3618 and CPI-0610 for 14 days. Treatment was terminated, and tumor regrowth was monitored to Day 28. B. Average body weights changes for the different treatment groups. Both doses of PLX-3618 were well tolerated; high dose of CPI-0610 was not tolerated. C. Individual % change tumor volume waterfall plot illustrates tumor regression with PLX-3618 treated animals after 14 days of treatment. Tolerated doses of CPI-0610 only led to tumor inhibition, without regression. D. PK/PD plot with 5 mg/kg IP dose of PLX-3618. Data demonstrates rapid and sustained depletion of BRD4. E. Table of *in vivo* study values (TGI = tumor growth inhibition; # Tumor-free = number of animals with no measurable tumor; BW = body weight).

Summary

- Plexium's ultra high-throughput screening platform was used to identify potent and selective BRD4 monovalent degraders
- A cullin-RING ligase complex containing the E3 substrate receptor, DCAF11, was identified as the required CRL for PLX-3618-mediated degradation of BRD4
- Mechanistic studies demonstrate BRD4:PLX-3618:DCAF11 ternary complexes, as well as unique bromodomain requirements for efficient target degradation
- BRD4 degradation led to potent anti-proliferative activity in AML tumor models, both *in vitro* and *in vivo;* complete tumor regression observed with well-tolerated doses
- Insights obtained from the BRD4 monovalent degrader discovery program are being applied to enable Plexium's drug discovery pipeline