Bifunctional degraders of BRPF1 that retain the antiproliferative phenotype

Matilda Bingham[1], Rosie Crampton[1], Thomas Pesnot[1], Anne-Chloe Nassoy[1], Ralph Kirk[1], Daniele Narducci[1], Andrew Scott[2], Gary Nelson[3], Habiba Begum[2], Lynette Ongeri[3], Darryl Turner[2], Rhoanne McPherson[2], Vincent Rao[2], Niall Martin[2], Daniel Glynn[1]

[1] Medicinal Chemistry Department, Concept Life Sciences, Frith Knoll Road, Chapel-en-le-Frith, High Peak, SK23 0PG (UK)

[2] Biology Department, Concept Life Sciences, Nine Edinburgh BioQuarter, 9 Little France Road, Edinburgh, EH16 4UX (UK)

[3] ADMET Department Concept Life Sciences, Frith Knoll Road, Chapel-en-le-Frith, High Peak, SK23 0PG (UK).

BRPF1 as an Actionable Target

Utilising our Integrated Drug Discovery Platform consisting of in-house PROTAC synthesis, PROTAC ADME characterisation and biological screening capabilities in haematological malignancies, we disclose our initial investigations into the role of BRPF1 in Acute Myeloid Leukemia (AML). We have designed a series of BRPF1 PROTACs to test the hypothesis that small-molecule degraders of BRPF1 will have profound antiproliferative effects in AML cell lines that harbour MLL translocations. In addition to their potential as new medicines, PROTACs are a powerful tool in the arsenal of techniques available for target validation.

Introduction

The BRPF (Bromodomain and PHD Finger-containing) family of proteins consisting of BRPF1, BRPF2/BRD1, are important epigenetic proteins in the assembly of the MYST-family histone acetyltransferase complexes MOZ and MORF[1] (**Figure 1**). These chromatin-modifying complexes can lead to altered expression of genes that drive innate immunity, inflammation, cancer and neurological disorders[2]. Histone Acetyltransferase (HAT) complexes are associated with chromosomal translocations known to contribute to the development of Acute Myeloid Leukemia (AML) and the leukemia-derived fusion proteins such as MOZ-TIF2 are known to promote the self-renewal of leukemic stem cells[3].

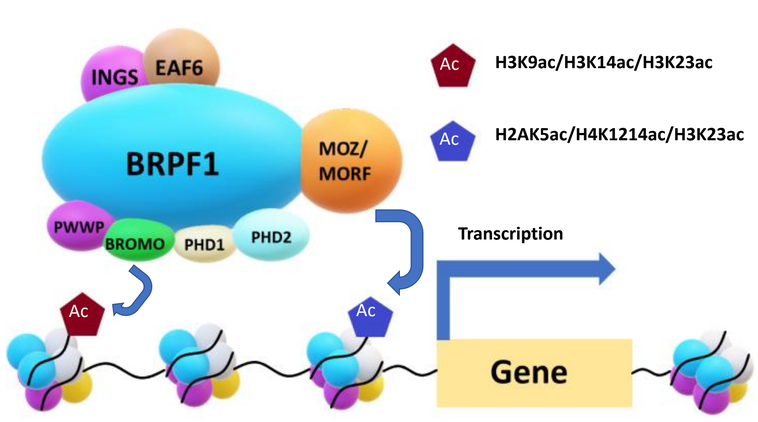

Figure 1. MOZ/MORF histone acetyltransferase complex mediates H3K9, H3K14, and H3K23 acetylation for gene activation. BRPF1 contains acetyl reader domains of two plant homeodomain (PHD) fingers separated by a zinc knuckle (PZP domain), a bromodomain, and a proline-tryptophan-tryptophan-proline (PWWP) domain.

PROTAC Design and Synthesis

A PROTAC toolkit was designed so that degrader molecules could rapidly be prepared with a high level of compatible chemistries (**Figure 2**). It consists of binding moieties for an E3 ubiquitin ligase, with multiple points of attachment joined by linkers of varying lengths. The linkers are typically PEG chains however bespoke variations can be prepared. The three E3 ligases of choice are Cereblon (CRBN), Inhibitor of Apoptosis (cIAP), and Von Hippel-Lindau (VHL).

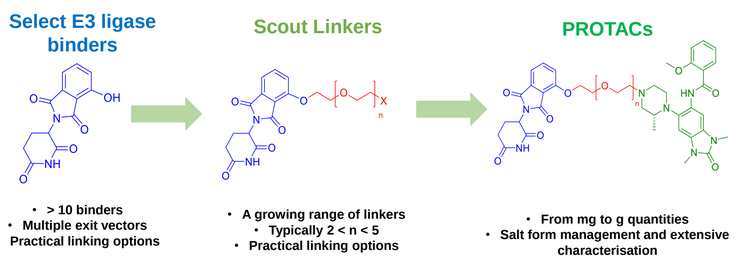

Figure 2. PROTAC elaboration, as exemplified from the Cereblon E3 ligase recruiter to bifunctional BRPF1 degrader

Chemistry covers a diverse range of linking methodologies including direct alkylation, Mitsunobu coupling, amination, and peptide bond formation.

Cereblon Based BRPF1 Degraders

To aid in the synthesis and design of PROTAC degraders of BRPF1 a literature search highlighted the chemical probe **GSK6853**. The ligand has excellent BRPF1 biochemical potency with an IC_{50} of 8 nM, good pharmacokinetic parameters along with an exquisite selectivity profile[4]. Upon initiation of this project, no antiproliferative data was available for this BRPF1 chemical probe in AML or any additional indications. EC_{50} values obtained after 72 hours in THP-1 and Jurkat cells were 420 nM and 1095 nM respectively (**Figure 3**). More recently thorough *in-vitro* and *in-vivo* data has been disclosed on the role of BRPF1 in Hepatocellular carcinoma (HCC) including the effects of small-molecule inhibition[5].

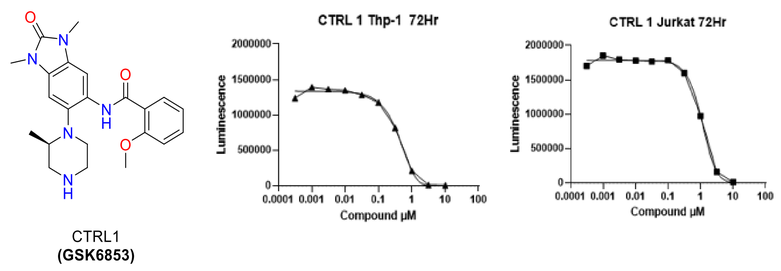

Figure 3. THP-1 and Jurkat cell proliferation of GSK6853 after 72 Hours, Cell Titre Glo (Luminescence), 5000 cells in standard culture medium were treated with GSK6853 and incubated at 37°C for up to 72 hours prior to the addition of Cell Titre Glo reagent. Data presented as the relative luminescence +/- S.D. as indirect measure of cell number, EC_{50} was estimated using non-linear regression.

Four Cereblon based PROTACs (**Figure 4**) were synthesised using two alternate exit vectors and two alternate linker lengths. Structural information was derived from the X-ray crystal structure of BRPF1 (PDB: 4UYE). PROTACs were screened against THP-1 cells harbouring MLL-AF4 translocations and Jurkat cells frequently used as a model cell line for T cell Lymphoma.

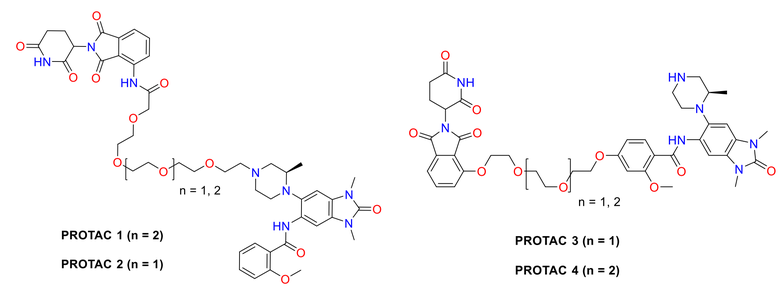

Figure 4: Structures of Cereblon based PROTACs

Data	PROTAC 1	PROTAC 2	PROTAC 3	PROTAC 4
THP-1 72 Hr EC_{50} [nM]	299	606	320	320
Jurkat 72 Hr EC_{50} [nM]	11880	9457	1861	2281

Table 1. THP-1 Jurkat and THP-1 cell proliferation of PROTACs, after 72 Hours, Cell Titre Glo (Luminescence), 5000 THP-1 cells in standard culture medium were treated with PROTAC and incubated at 37°C for up to 72 hours prior to the addition of Cell Titre Glo reagent. Data presented as the relative luminescence +/- S.D. as indirect measure of cell number, EC_{50} was estimated using non-linear regression.

Cereblon based degraders showed antiproliferative effects comparable to **GSK6853**. Time dependant potency improvement was observed for **PROTAC 1-4** in the THP-1 cells (~10-fold change), not within the Jurkat cell line. We hypothesise that this is being caused by catalytic ternary structure formation.

VHL Based BRPF1 Degraders

To complement and expand on the Cereblon PROTACs two further VHL based degraders were prepared (**Figure 5**). E3 ligase expression levels within AML cell lines show VHL to be ubiquitously expressed. **PROTACs 5** and **6** both show greater affinity for growth inhibition for the THP-1 cell line.

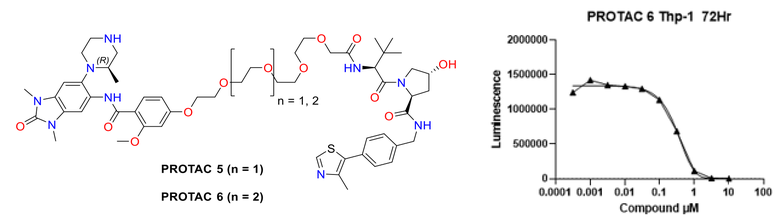

Figure 5: Structure of VHL **PROTAC 5** and **6**, and antiproliferative effect of **PROTAC 6**.

Data	PROTAC 5	PROTAC 6
THP-1 72 Hr EC_{50} [nM]	451	323
Jurkat 72 Hr EC_{50} [nM]	2252	1761

Table 2. THP 1 Jurkat and THP-1 cell proliferation of PROTACs, after 72 Hours, Cell Titre Glo (Luminescence), 5000 THP-1 cells in standard culture medium were treated with **PROTAC** and incubated at 37°C for up to 72 hours prior to the addition of Cell Titre Glo reagent. Data presented as the relative luminescence +/- S.D. as indirect measure of cell number, EC_{50} was estimated using non-linear regression.

ADME Data

A better understanding of the ADME properties of PROTACs represents a high unmet need for their rational design. To date, the evaluation and improvement of the ADME properties of this class of compounds are still poorly understood. There are only a few published studies of their experimental physical-chemical properties[6] and fewer still reporting in-depth analysis of metabolism and design principles[7], we therefore present preliminary studies on a small subset of PROTACs, where logD, solubility, plasma & metabolic stability have been experimentally measured (**Table 3**).

	Data	PROTAC 1	PROTAC 3	PROTAC 5	PROTAC 6
ADME	Kinetic Solubility (µM)	3.7	2.9	3.9	4.6
	LogD pH 7.4	3.0	2.0	1.7	1.8
	MLM (µl/min/mg)	212	64	13.8	5.6
	MHep (µl/min/10^6 cells)	N.D	N.D	38.8	8.1
	HLM (µl/min/mg)	548	86	184	128
	HHep (µl/min/10^6 cells)	22	7.4	4.9	< 3
	MPS (% @120 min)	Low	Low	55	52
	HPPB (% free)	10.2	16.6	32.7	30.7
In-silico	Mw (g/mol)	898	813	1072	1028
	LogP	1.9	2.3	3.5	3.7
	PFI	6.0	5	5.7	5.8

Table 3. Mouse Liver Microsomes (MLM), Mouse Hepatocytes (MHep), Human Liver Microsomes (HLM), Human hepatocytes (HHep), Mouse Plasma Stability (MPS), Human Plasma Protein Binding (HPPB). Metabolic/plasma stability – PROTACs were incubated at 1 µM in either 1 mg/mL microsomes, 0.5 million cells/ml hepatocytes or plasma, with aliquots removed from the incubation mixture at specified time-points and quenched. Following centrifugation, supernatants were taken and analysed by LC-MS/MS to measure test parent compound remaining at each time-point.

An ADME triage was designed; Tier 1 involved testing the suitability of PROTACs for biological screening with a quick readout for solubility and LogD. Tier 2 assays were selected to evaluate the suitability for further in-vivo characterisation. The ADME data for the two Cereblon and two VHL based PROTACs show remarkable differences. Kinetic Solubility and cLogD for all compounds were in a good range for the beyond rule of 5 chemical space (bRo5). Large differences were however observed within metabolic stability (microsomes and hepatocytes) and plasma stability. VHL based PROTACs show much lower intrinsic clearance and greater predicted suitability for *in-vivo* experiments.

Given the promising cellular data, solubility and murine metabolic stability profile **PROTAC 6** has been selected for further *in-vivo* characterisation. Given the large variation in permeability of PROTACs we are developing the SAR around this parameter to promote cellular uptake, in addition to further studies to develop our understanding of the metabolic profile within the series

Future work

Target protein degradation has emerged as one of the most exciting and promising new areas for small molecule drug discovery. As a powerful new modality, we have seen encouraging early results for cell growth inhibition in THP-1 cells harbouring MLL-AF4 translocations. We plan to further explore and validate the role of BRPF1 in AML and neurodevelopmental disorders. The next steps aim to confirm the mechanism of action of the bifunctional small molecules and expand on their therapeutic scope:

- **SPR characterisation of binding and ternary complex formation; E3 ligase-BRPF1-PROTAC**
- **Analysis of ability to degrade BRPF1, DC_{50} and DC_{90} values in AML cell lines and non-AML cells**
- **Growth inhibition in primary AML cell culture**
- **Effects on Stemness**
- **Synergistic studies with DOT1L and KAT6A inhibitors**

[1] Carlson, S.; Glass, K. C. The MOZ Histone Acetyltransferase in Epigenetic Signaling and Disease. J. Cell. Physiol. 2014, 229, 1571– 1574, Ullah, M.; Pelletier, N.; Xiao, L.; Zhao, S. P.; Wang, K.; Degerny, C.; Tahmasebi, S.; Cayrou, C.; Doyon, Y.; Goh, S. L.; Champagne, N.; Cote, J.; Yang, X. J. Molecular architecture of quartet MOZ/MORF histone acetyltransferase complexes. Mol. Cell. Biol. 2008, 28, 6828–6843.

[2] Zhao. W. Isoform-specific involvement of Brpf1 in expansion of adult hematopoietic stem and progenitor cells. Journal of Molecular Cell Biology, Volume 12, Issue 5, May 2020, Pages 359–371. Wang. E. Deficiency of the Chromatin Regulator Brpf1 Causes Abnormal Brain Development. The Journal of Biological Chemistry. 2015. 11. 7114-7129

[3] M. Ullah. MOZ and MORF, two large MYSTic HATs in normal and cancer stem cells. Oncogene. 2007, 26, 5408–5419

[4] Watson. R. J. GSK6853, a Chemical Probe for Inhibition of the BRPF1 Bromodomain. *ACS Med. Chem. Lett.* 2016, 7, 6, 552–557

[5] Cheng, C.LH., Tsang, F.HC., Wei, L. Bromodomain-containing protein BRPF1 is a therapeutic target for liver cancer, *Commun Biol, 2021,* **4,** 888

[6] Maple, H. J. Developing degraders: principles and perspectives on design and chemical space. MedChemComm 2019, 10, 1755– 1764, Steinebach, C . A MedChem toolbox for cereblon-directed PROTACs. MedChemComm 2019, 10, 1037–1041, Steinebach, C. Systematic exploration of different E3 ubiquitin ligases: an approach towards potent and selective CDK6 degraders. Chem. Sci. 2020, 11, 3474– 3486

[7] Zhou, B. Discovery of a small-molecule degrader of bromodomain and extra-terminal (BET) proteins with picomolar cellular potencies and capable of achieving tumor regression. J. Med. Chem. 2018, 61, 462– 481, Pike, A.; Williamson, B.; Harlfinger, S.; Martin, S.; McGinnity D.F., Optimising proteolysis-targeting chimeras (PROTACs) for oral drug delivery: a drug metabolism and pharmacokinetics perspective, Drug Discovery Today, 2020, 25, 1793-1800

www.conceptlifesciences.com

01298 816700

info@conceptlifesciences.com