GENERATING A CHEMICAL TOOLBOX TO SUPPORT PROTAC R&D

TOCRIS a bio-techne brand

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Introduction to PROTACs

PROTACs (PROteolysis TArgeting Chimeras) are bifunctional small molecules that harness the Ubiquitin Proteasome System (UPS) to selectively degrade target proteins within cells. They represent an exciting new modality, repurposing small molecule ligands to achieve selective degradation (knock-down) of target proteins. Moreover, they have the potential to expand the 'druggable proteome', since they can be used to degrade proteins that, although bound, are not effectively inhibited by small molecules.[1,2]

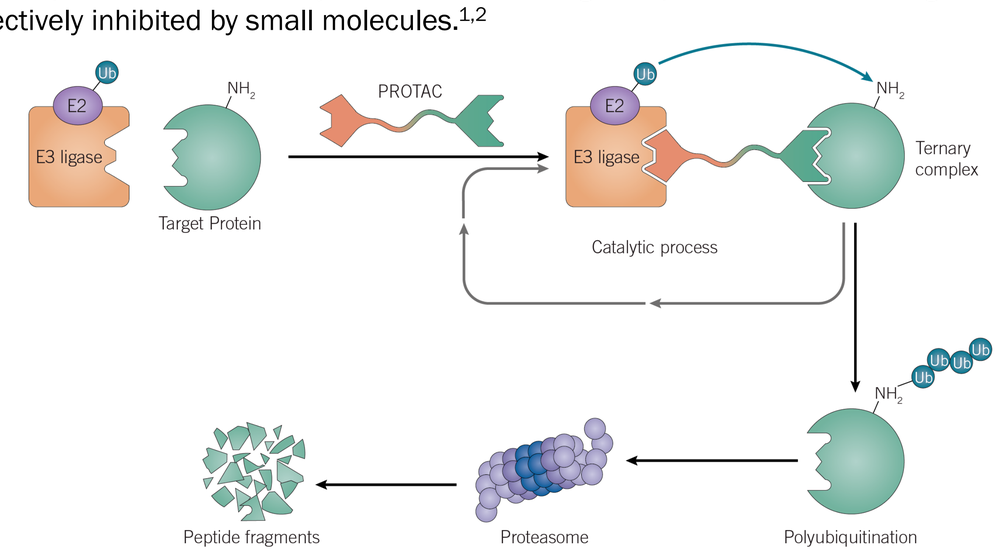


Figure 1. Mechanism of PROTAC action. Adapted from Tinworth *et al.* (2016) Med.Chem.Comm. **7** 2206

PROTACs are modular in design and consist of three, covalently linked components:

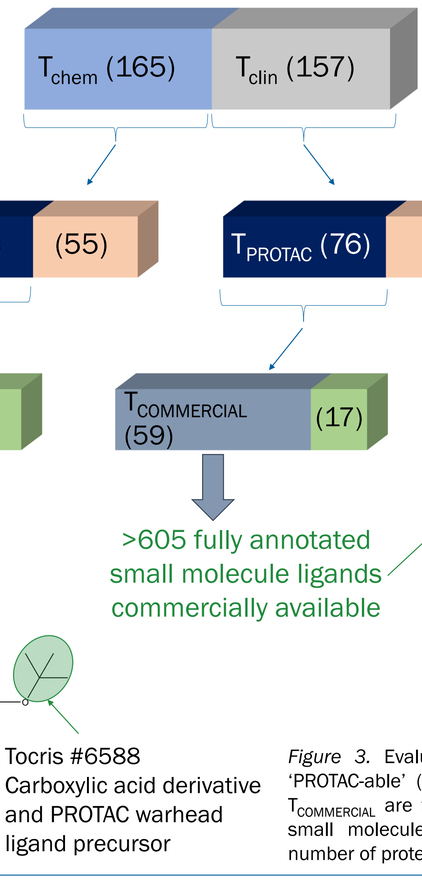
- E3 ubiquitin ligase ligand
- Linker
- Warhead ligand for a target protein of interest

Currently, predictions regarding the optimal nature of each component cannot be done *a priori* and empirical effort is required to guide the development process.[3]

Warhead ligand - expanding the druggable proteome?

A recent review (Oprea *et al.*)[4] examined chemical space to evaluate the state of the targeted human genome. Two classes of target protein were described: T_{chem} and T_{clin} (defined below). Figure 3 presents an analysis of this published data, and further defines the subset of targets that might be 'PROTAC-able' (T_{PROTAC}). We also present an evaluation of the commercial coverage of small molecule ligands within the newly defined T_{PROTAC} target group.

T_{chem} = *Proteins that lack MoA-based links to approved drugs but are known to bind small molecules with high potency*



T_{clin} = *Proteins that are drug targets linked to at least one approved drug*

T_{PROTAC} = *Intracellular proteins (or membrane proteins with ligands targeting the endodomain) with defined small molecule ligands*

Figure 3. Evaluation of druggable (T_{clin}), ligand-able (T_{chem}) and 'PROTAC-able' (T_{PROTAC}) protein targets within the human genome. $T_{COMMERCIAL}$ are targets for which Tocris has commercially available small molecule ligands. Numbers in parenthesis relate to the number of protein targets within each group.

E3 ligase ligands for PROTACs

Despite the human proteome encoding >600 E3 ligases, only a handful have been successfully harnessed for PROTACs, mostly belonging to the RING family of E3 ligases.

This is largely driven by the availability of small molecule ligands to E3 ligases (summarized in Table 1).

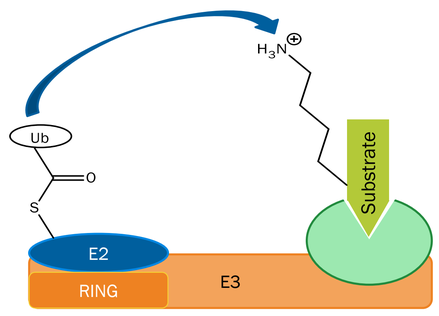
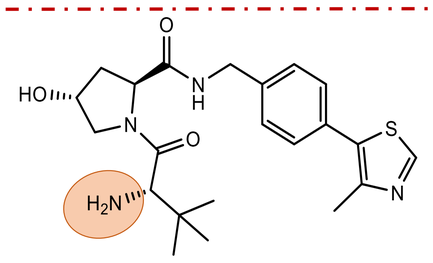


Figure 2. Mechanism of Ubiquitin transfer for monomeric RING E3 ligase

E3	Compound (Tocris cat.no.)	K_d/IC_{50}	nM DC_{50} PROTACs ?	*in vivo* PROTACs ?	MW	HBD /HBA	cLogP	TPSA (Å²)	Pros / Cons
MDM2	Nutlin 3 (#3984)/ Nutlin 3a (#6075), active enantiomer	0.09 µM (IC_{50}) / 90nM (IC_{50})	No	N/D	581.5	1 / 5	4.55	84	Opportunity to explore more recent MDM2 ligands for PROTACs / High MW, poor aq solubility.
c-IAP/XIAP	Bestatin (#1956)	>50 µM (K_d)	No	N/D	308.4	4 / 5	0.66	113	HBD count high. Cell impermeable. Causes proteasomal degradation of c-IAP.
	Methyl Bestatin	>50 µM (K_d)	No	N/D	322.4	3 / 5	1.54	102	Cell permeable version of Bestastin / Causes proteasomal degradation of c-IAP.
	MV1	Low nM	Yes	N/D	576.7	3 / 6	3.44	117	High MW. Causes proteasomal degradation of c-IAP.
	LCL-161	35 nM (XIAP) 0.4 nM (cIAP1) (IC_{50})	Yes	Yes	500.6	2 / 6	3.66	120	Potent and effective E3 ligand for PROTACs / High MW.
	A 410099.1 (#6470)	16 nM (XIAP) (K_d)	Yes	Yes	468.6	3 / 4	2.83	91	Potent and effective ligand. High MW. Causes proteasomal degradation of c-IAP but not c-IAP2 or XIAP.
VHL	VH 032	185 nM (K_d)	Yes	Yes	472.6	3 / 5	2.27	140	Potent and effective E3 ligand for PROTACs / High MW and TPSA.
Cereblon	Thalidomide (#0652)	250 nM (K_d)	Yes	Yes	258.2	1 / 4	0.65	84	Low MW and HBD count. Potent and effective E3 ligand for PROTACs / Known off-targets and stability issues.
	Lenalidomide (#6305)	178 nM (K_d)	Yes	N/D	258.3	2 / 3	0.09	93	Low MW and HBD count. Potent and effective E3 ligand for PROTACs / Known off-targets and stability issues.
	Pomalidomide (#6302)	157 nM (K_d)	Yes	N/D	273.2	2 / 4	0.06	110	Low MW and HBD count. Potent and effective E3 ligand for PROTACs / Known off-targets and stability issues.

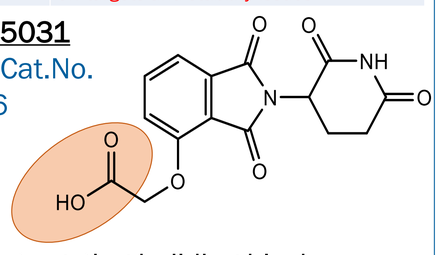
Table 1. Summary of small molecule E3 ligase ligands used in PROTAC R&D

VH 032, amine
Tocris Cat.No. #6462



- VHL-targeting building block
- VH 032 functionalized with a primary amine for easy conjugation to linkers/ligands
- Positioning of amine does not significantly interfere with binding affinity

TC E3 5031
Tocris Cat.No. #6466



- Cereblon-targeting building block
- Thalidomide functionalized with a carboxylic acid for easy conjugation to linkers/ligands
- Positioning of acid does not significantly interfere with binding affinity
- Click (azide) version also available

PROTAC Linkers

Controlled, PROTAC-mediated, ubiquitination of proteins requires the formation of a ternary complex between the E3 ligase, PROTAC and target protein.

The choice of linker drives/impacts several determinants of the final compound's activity and mechanism of action, factors including: conformation and binding orientation, cooperativity of ternary complex formation[5], selectivity[6] and physicochemical properties.

We present an initial set of E3 ligase-linker conjugates, functionalized with primary amine groups conjugated to target ligands (Figure 4). This 'toolbox' contains the most commonly used E3 ligase ligand/linker combinations to provide a starting point for PROTAC development.

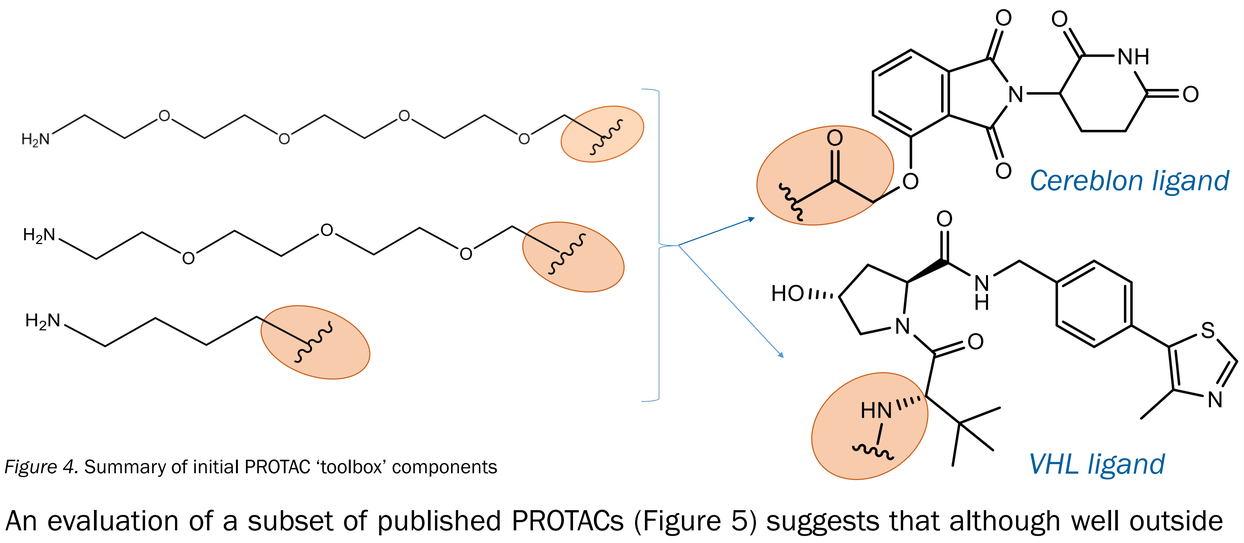


Figure 4. Summary of initial PROTAC 'toolbox' components

An evaluation of a subset of published PROTACs (Figure 5) suggests that although well outside the traditional 'rule of 5' space, active, cell permeable PROTACs almost always fall within the criteria for <5 H bond donors (HBD). Additionally, 60% of cell permeable PROTACs (from the subset analysed) comply with Lipinski's rule for cLogP<5. Given the type and length of standard PROTAC linkers, it is likely that in addition to the standard 2D measures, 3D descriptors such as 3D PSA and radius of gyration could be important predictors of PROTAC cell permeability.

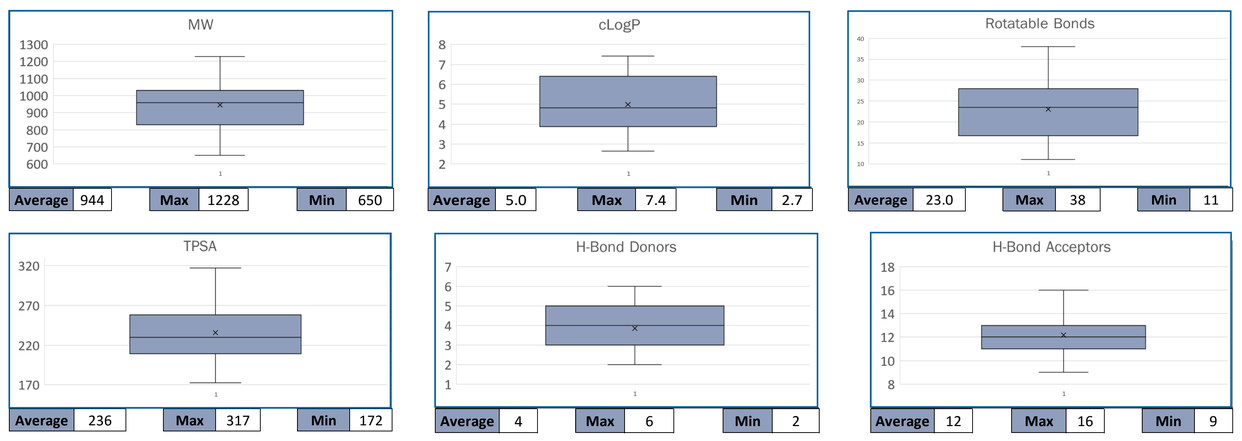


Figure 5. Summary of physicochemical properties for a subset of 77 published PROTACs. Lipinski (or 'lead-like') compliance is defined as MW<500, cLogP<5, HBD<5, HBA<10 and additionally TPSA<140 Å². Values for TPSA, clogP, rotable bonds, HBD and HBA were calculated using http://www.swissadme.ch/

1. Neklesa, T. K. *et al.* Targeted protein degradation by PROTACs. *Pharmacol. Ther.* **174**, 138-144 (2017).
2. Churcher, I. Protac-induced protein degradation in drug discovery: breaking the rules or just making new ones? *J. Med. Chem.* **61**, 444-452 (2018).
3. Fisher, S. L. *et al.* Targeted protein degradation and the enzymology of degraders. *Curr. Opin. Chem. Biol.* **44**, 47-55 (2018).
4. Oprea, T. I. *et al.* Unexplored therapeutic opportunities in the human genome. *Nat. Rev. Drug. Disc.* **17**, 317-332 (2018).
5. Gadd, M. S. *et al.* Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat. Chem. Biol.* **13,** 514-521 (2017).
6. Nowak, R. P. *et al.* Plasticity in binding confers selectivity in ligand-induced protein degradation. *Nat. Chem. Biol.* **14,** 706-714 (2018).

tocris.com/protac-toolbox

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