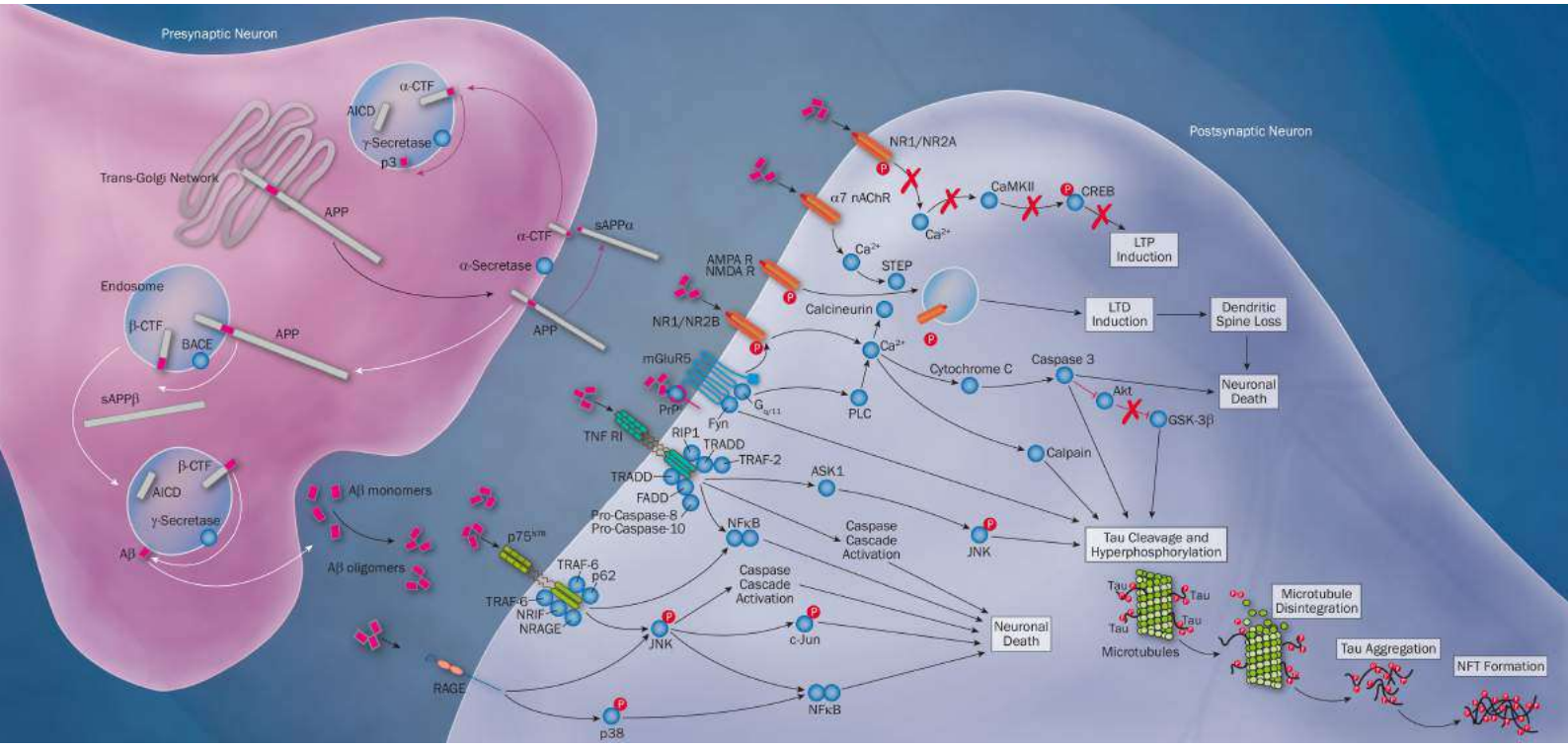
# Alzheimer's disease Research

with biotechne®

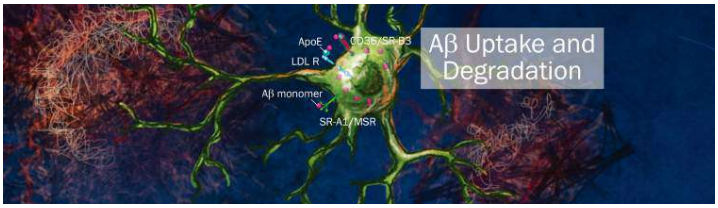



## Alzheimer's disease Research Product

| | R&D | | |
|---|---|---|---|
| | Ab | ELISA | Duoset |
| Apolipoprotein E/ApoE | H | | |
| APP/Protease Nexin II | H | | |
| APP 695+1 | H | | H |
| MMP-9 | H,M,P | H,M,R | H,M,R |
| Presenilin-1 | H | | H |
| Presenilin-2 | H | | |
| TACE/ADAM17 | H | | H |
| ADAM9 | H,M | | H |
| ADAMTS4 | H | | H |
| Cystatin C | H,M,R | H,M,R | H,M |
| DYRK1A | H,R | | |
| DYRK2 | H,M,R | | |
| FPRL1 | H | | |
| LRRTM3 | H,M | | |
| MMP-2 | H,M,R | H,M,R,P,C | H |

| | R&D | | |
|---|---|---|---|
| | Ab | ELISA | Duoset |
| Neprilysin/CD10 | H,M | | H,M |
| RAGE | H,M,R,C | H,M | H,M,R |
| Serpin A3/α-Antichymotrypsin | H | | |
| Serpin A3N | M | | |
| Serpin E2/PN1 | H,M | | |
| TLR4 | H,M,R | | |
| JNK | H,M,R | | H,M,R (p-JNK) : Duoset IC |
| Calcineurin A | H,M,R | | |
| Calcineurin B | H,M,R | | |
| Src | H,M,R | | H(p-Src) : Duoset IC |
| ZO-1 | H,M,R,P | | |
| ZO-2 | H,M,R | | |
| β-catenin | H,M,R | | H : Duoset IC |
| P-selectin/CD62P | H,M,R | H,M | H,M |
| GSK-3β | H,M,R | | |
| β-Amyloid | H | H | |

H: Human, M:Mouse, R:Rat, P:Porcine C:Canine




R&D SYSTEMS a biotechne brand

# Intoduciton 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]

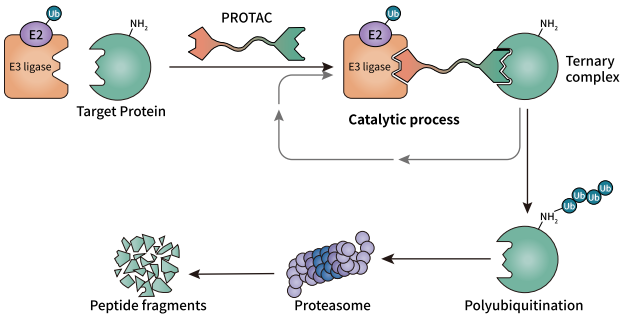



*Figure 1.* Mechanism of PROTAC action. Adapted from Tinworth *et al.* (2016) Med.Chem.Comm. 7 2206

PROTACs are modular in desing 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

# 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).

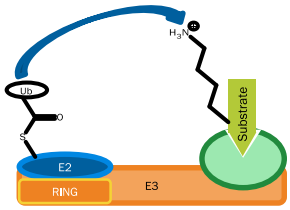


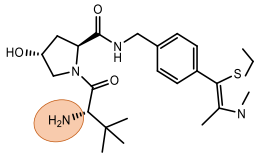

Figure 2. Mechanism of Ubiquitin transfer for monomeric RING E3 ligase

| E3 | Compund (Tocris cat.no.) | $K_d$/$IC_{50}$ | nM $DC_{50}$ PROTACs ? | *in vivo* PROTACs ? | MW | HBD /HBA | cLogP | TPSA (Å²) | Pros / Cons |
|---|---|---|---|---|---|---|---|---|---|
| MDM2 | Nutilin 3 (#3984)/ Nutlin (#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 | >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 | >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 | 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 | 3 / 5 | 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 HBO 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 HBO 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 HBO 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

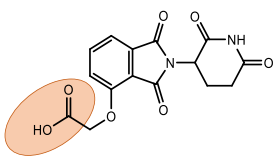
Tocris Cat.No. #6462



- VHL-targeting building block
- VH 032 functionalized with a primary amine for easy conjugation to linkers/ligands
- Positioning of amine does not significantly intefere 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 intergere with binding affinity
- Click (azide) version also available