Efficacy Studies and PK/PD of PROTAC Drugs *In Vitro* &*Vivo*

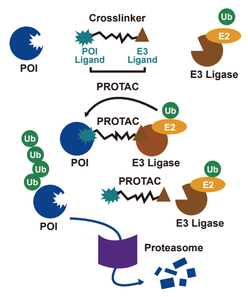
MEDICILON

Abstract

Proteolysis targeting chimeras (PROTACs) offer a fast and reversible chemical knock-down approach to control protein function. The impact of PROTAC platform has changed the landscape of drug discovery and development[1-3]. Medicilon's PROTAC drug discovery technology platform covers the currently popular target protein ligands. We have established a linker system with an extensive collection of bifunctional linkers. Together with our expanding E3 ubiquitin ligase binder library, we can efficiently synthesize a substantial amount of highly active PROTAC bispecific small molecules, which would have the potential to significantly facilitate the drug discovery and development process. In addition, Medicilon has established as well as improved the PROTAC biological screening and testing platform throughout the pre-clinical stages. Medicilon's strong technical expertise and flexible service models allow individualized and customized projects ranging from sole chemical synthesis to *in vitro* and/or *in vivo* service, and to more comprehensive integrated package support. Our laboratories are US FDA and China NMPA accredited, and we will soon receive the European OECD GLP accreditation as well. We have successfully filled over 150 IND submissions worldwide. Medicilon is confident in providing efficient, cost-effective, and professional services to support our clients in reaching their drug development milestones.

Background

Proteolysis targeting chimeras (PROTACs), also known as bivalent chemical protein degraders, are heterobifunctional molecules that degrade specific endogenous proteins through the E3 ubiquitin ligase
pathway. A PROTAC molecule structurally connects the protein of interest (POI)-binding ligand and the E3 ubiquitin ligase (E3) ligand through an appropriate linker.
PROTAC technology is an effective tool to degrade endogenous target proteins through the ubiquitin-proteasome system (UPS)[1-3].



Medicilon PROTAC Technology Platform

- **Know-how:** > 6 years PROTACs experience
- **Comprehensiveness:** > 20 ongoing projects
- **Team:** > 300 dedicated well-trained chemists
- **Building Block:** > 300 advanced linkers Available
- **Scaffold:** > 150 E3 ligands available including Cereblon, VHL, MDM2, IAP, etc.

Method

Biochemical assays

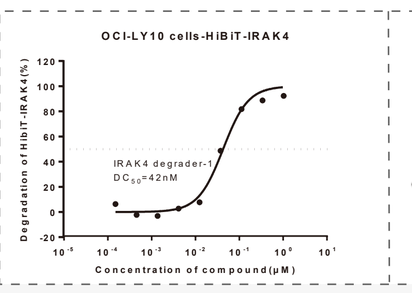
- Study binary/ternary complex formation
- Target ubiquitination & degradation assay
- Biophysics approach with SPR

Cellular Assays

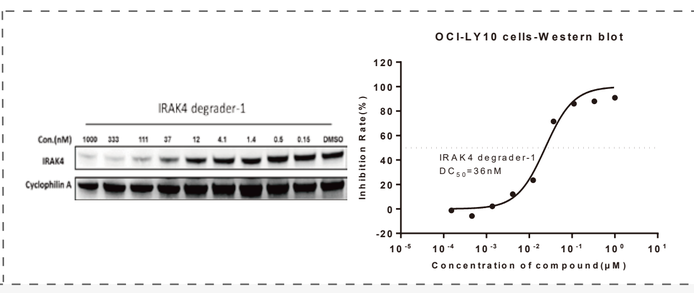
- Target-E3 ligase interaction assay
- Target degradation assay(DC_{50})

Results

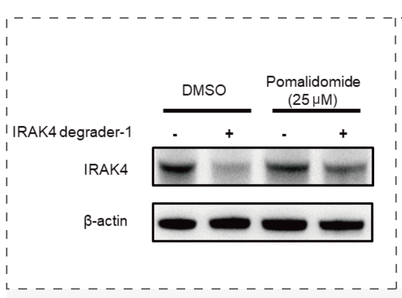
Case: PROTAC *In Vitro* Evaluation



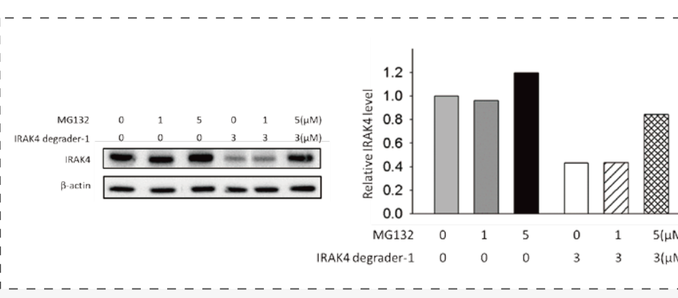
Percentage degradation of HiBiT-IRAK4in HiBiT-IRAK4 overexpression OCI-LY10 cells.



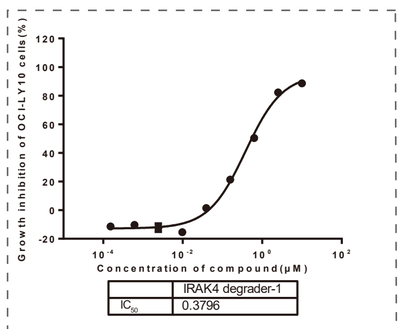
Western blot of anti-IRAK4 on OCI-LY10 cells treated with IRAK4 degrader-1.



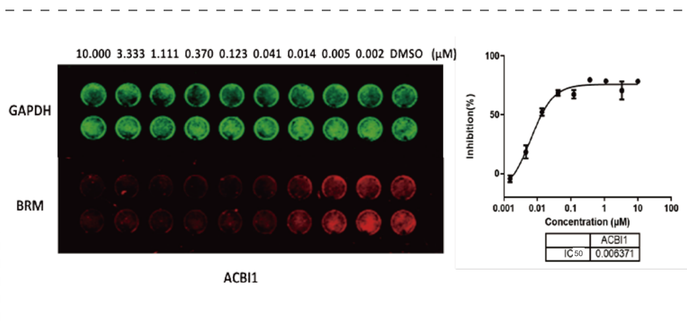
Pomalidomide(CRBN binder) competition experiment show that IRAK4 degrader-1 degrades IRAK4 through CRBN.



MG132 inhibition experiment show that IRAK4 degrader-1 degrades IRAK4 through proteasome pathway.



Growth inhibition effect of IRAK4 degrader-1 on OCI-LY10 cells.

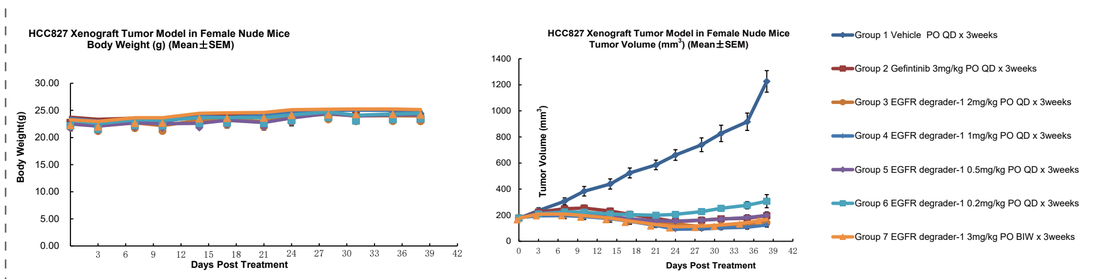


In cell Western of BRM show that ACBI1(BRM degrader) degrades BRM in dose response manner.

Case: PROTAC *In Vivo* Evaluation

Pharmacology Models

- 50 CNS disease models
- 20 Cardiovascular & metabolic disease models
- 10 Inflammatory & immune diseases models
- 10 Digestive system disease models
- 5 Ocular diseases models
- 5 Other disease models
- 150 Xenograft models
- 35 Orthotopic models
- 30 Syngeneic models
- 30 Humanized models
- 5 GEM models



In vivo efficacy study of HCC827 model (EGFR degrader-1 & positive control Gefitinib)

Case: Plasma Stability Studies of PROTAC

Evaluation of ARD-2128 for Its Plasma Stability: PROTAC AR degrader ARD-2128 was evaluated for its plasma stability in mouse, rat, dog, monkey, and humans. ARD-2128 has excellent plasma stability in all the five species.

species	plasma stability ($T_{1/2}$, min)
mouse	>120
rat	>120
dog	>120
monkey	>120
human	>120

Plasma Stability of ARD-2128 in Five Species (Human, Mouse, Rat, Dog, and Monkey)

Case: PK Studies of PROTAC

The pharmacokinetics (PK) of five highly potent AR degraders (compounds 26, 27, 28, 33, and 34) are evaluated in mice with both intravenous and oral administration.

compound	route	dose (mg/kg)	$T_{1/2}$ (h)	AUC_{0-t} (h·ng/ml)	Cl (ml/min/kg)	V_{ss} (L/kg)	route	dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-t} (h·ng/ml)	F (%)
26	IV	2	17.8	11,035	1.9	2.7	PO	5	12.0	4.0	1389	20,600	75
27	IV	2	11.5	15,759	1.7	1.5	PO	5	11.2	4.0	980	14,588	37
28 (ARD-2128)	IV	2	27.6	13,299	1.2	2.7	PO	5	18.8	4.7	1304	22,361	67
33	IV	1	21.0	4334	2.2	3.8	PO	3	12.4	6.0	207	3127	24
34	IV	1	25.5	2565	3.2	6.8	PO	3	67.8	4.7	134	2550	33

Summary of PK Data for Compounds 26, 27, 28, 33, and 34 in Male ICR Mice

Summary

PROTACs offer a fast and reversible chemical knock-down approach to control protein function in the cell. The impactof the PROTAC platform has changed the landscape of drug discovery and development. Medicilon's PROTAC drug discovery technology platform covers many of the popular target protein ligands. We have established an extensive collection of bifunctional linkers. Together with our expanding E3 ubiquitin ligase binder library, we can efficiently synthesize highly active PROTAC bispecific small molecules, which have the potential to significantly facilitate the drug development process. In addition, Medicilon has established as well as improved the PROTAC biological screening and testing platform throughout the pre-clinical stages. Medicilon's strong technical expertise and flexible service models allow individualized and customized projects ranging from sole chemical synthesis to *in vitro* and/or *in vivo* service, and to more comprehensive integrated package support. As of the end of 2022, Medicilon has successfully assisted in the clinical approval of **3** PROTAC drugs by NMPA and/or FDA and has more than 20 PROTAC projects under development.

Medicilon has accumulated rich PROTAC experience working on a wide range of popular target proteins with high affinity small molecules and small molecule fragment compound libraries, a wide range of E3 ubiquitin ligase binders, and an extensive collection of bifunctional linkers. We leverage our broad chemistry capabilities and capacity with fully integrated biological and pre-clinical validation of the candidate PROTAC molecules.

- We have successfully established a series of *in vitro* protein-based assays for PROTACs screening.
- We also have successfully generated a series of cell lines based on different POI.
- We also have developed a series of cell line-based validation assays.

We hope that our detection system can speed up the development of new drugs and contribute to anti-tumor therapy.

References

[1] Si-Min Qi, et al. PROTAC: An Effective Targeted Protein Degradation Strategy for Cancer Therapy. Front Pharmacol. 2021 May 7;12:692574.

[2] Galen Andrew Collins, et al. The Logic of the 26S Proteasome. Cell. 2017 May 18;169(5):792-806.

[3] Jared A M Bard,et al. Structure and Function of the 26S Proteasome. Annu Rev Biochem. 2018 Jun 20;87:697-724.

[4] Xin Han, et al. Strategies toward Discovery of Potent and Orally Bioavailable Proteolysis Targeting Chimera Degraders of Androgen Receptor for the Treatment of Prostate Cancer. J Med Chem. 2021 Sep 9;64(17):12831-12854. doi: 10.1021/acs.jmedchem.1c00882.