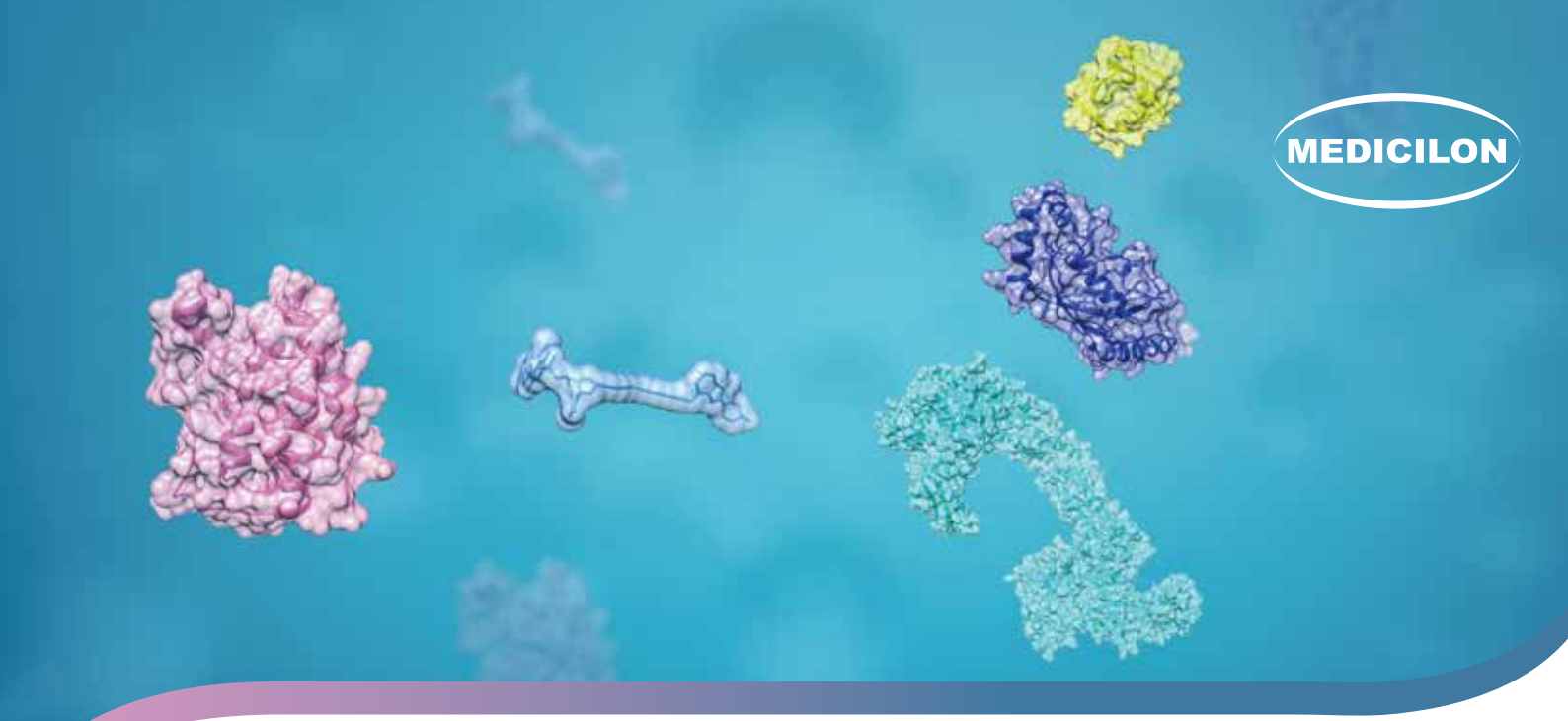


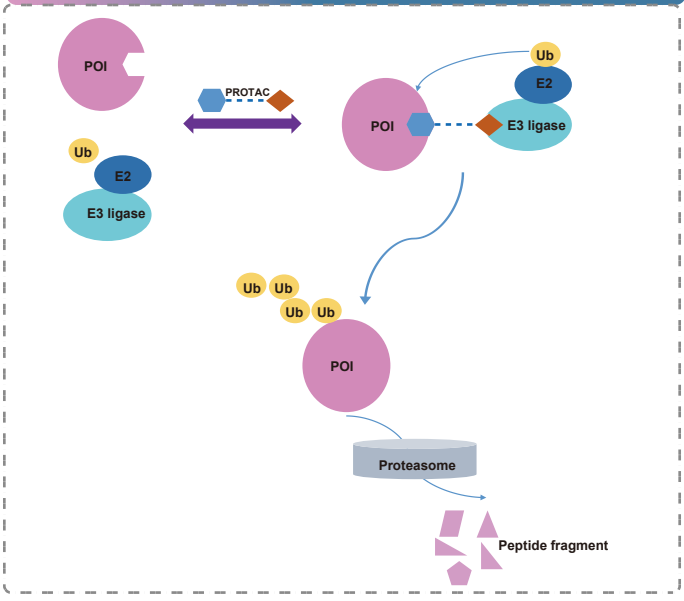
# Medicilon PROTAC R&D Service Platform

Proteolysis targeting chimeras (PROTACs) offer a fast and reversible chemical knock-down approach to control protein function. PROTAC technology takes advantage of a moiety of targeted protein and a moiety of recognizing E3 ubiquitin ligase and produces a hybrid molecule to specifically knock down a targeted protein.

Medicilon provides PROTAC drug discovery, CMC research (API + formulation), pharmacodynamics research, PK study, safety evaluation and other services. 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.

As of the end of June 2024, Medicilon has successfully assisted in the clinical approval of 5 PROTAC drugs (3 approved by FDA and NMPA) and has 20+ PROTAC projects under development.

## Mechanism of PROTAC Technology




- The PROTAC molecule specifically recognizes and binds to the target protein through the protein of interest (POI) Ligand at one end and the E3 Ligase through the E3 Ligase Ligand at the other end.
- Ternary Complex (POI-PROTAC-E3 ligase)
- In this ternary complex, the target protein POI is ubiquitinated by E3 ligase, and the ubiquitinated POI is subsequently recognized and degraded by the proteasome, thereby eliminating the target proteins.

## Advantages of PROTAC Technology

### Make undruggable targets druggable

PROTACs latch on to proteins which lack the active sites typically required to bind small molecule drugs. This enables ubiquitin-mediated degradation and switches a target from undruggable to druggable.

### Recyclable and highly effective

A PROTAC molecule can bring the POI and the E3 ligase in close proximity resulting in (poly)ubiquitination of POI. The polyubiquitinated POI is then degraded by proteasome while the PROTAC is released and recycled for another round of POI degradation. Thus, PROTACs can be effectively employed at much lower doses.

### Non-immunogenic

Compared with biotechnology drugs, PROTAC does not trigger the production of anti-drug antibodies.

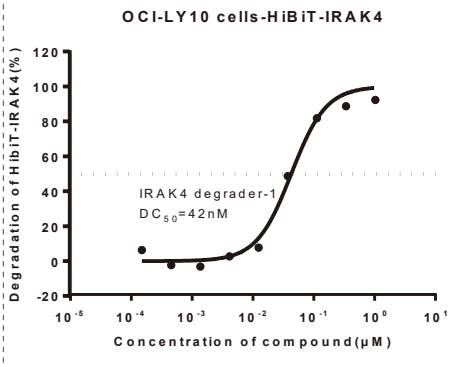
## PROTACs Drug Discovery

Medicilon has access to many of the popular POI ligands and multiple tissue types of E3 ligase ligands, in addition to having established a linker library containing hundreds of diverse molecules.Moreover, Medicilon's CADD technology platform has greatly improved the quality of PROTAC-POI design and synthesis.

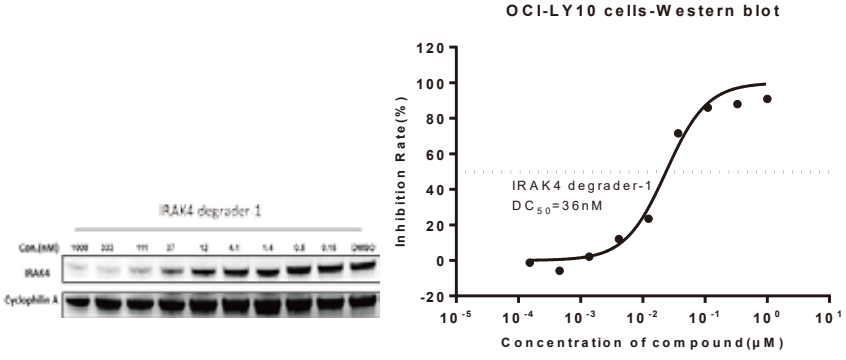
**Validated PROTAC targets in Medicilon:** IRAK4, AR, ER, IKZF1, IKZF2, IKZF3, BTK, EGFR, BRD4, BCL-xL, CDK4, CDK6, SMARCA2, SMARCA4, AKT, ALK, STAT3, SHP2, FGFR1, FGFR2, KRAS G12C, KRAS G12D, etc.

## *In Vitro* Studies of PROTACs

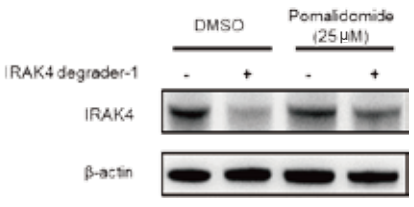
### Medicilon Cases



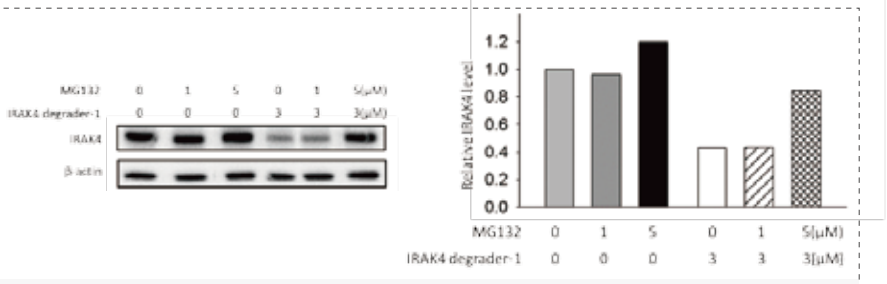

Percentage degradation of HiBiT-IRAK4 in HiBiT-IRAK4 overexpression OCI-LY10 cells.



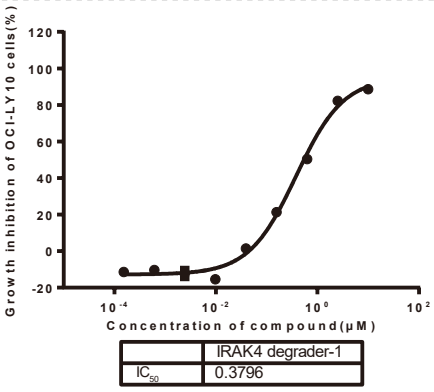

Western blot of anti-IRAK4 on OCI-LY10 cells treated with IRAK4 degrader-1.




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

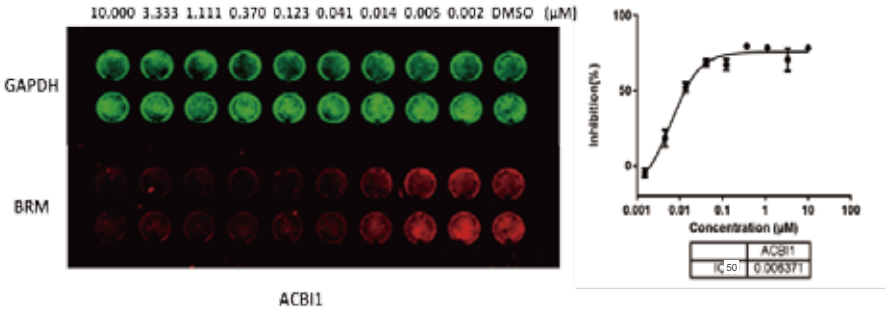



MG132 inhibition experiment show that IRAK4 degrader-1 degrades IRAK4 through proteasome pathway.




| | IRAK4 degrader-1 |
|---|---|
| $IC_{50}$ | 0.3796 |

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

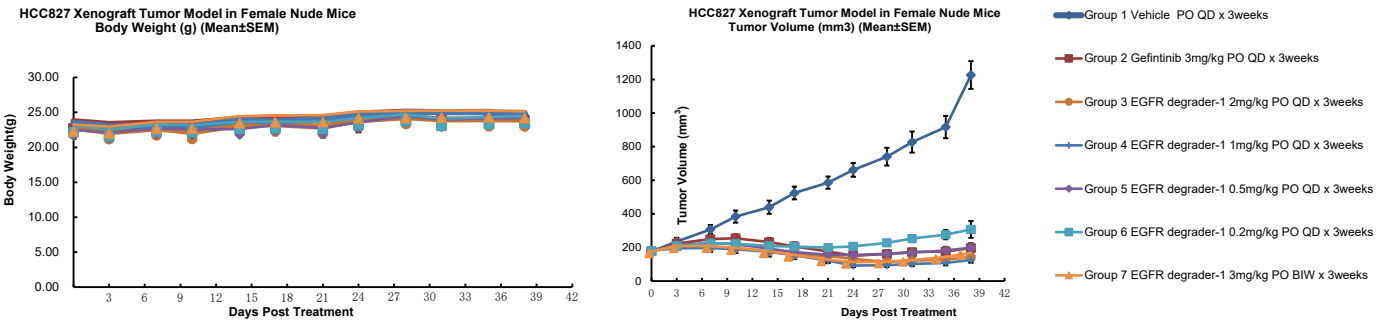



| | ACBI1 |
|---|---|
| $IC_{50}$ | 0.006371 |

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

## Pharmacology Studies of PROTACs

Medcilon provides mature models for evaluating the efficacy of PROTACs *in vivo*. Our animal models are all established and maintained under the regulation of AAALAC. Pharmacology studies are conducted according to GLP-like standards. At present, more than **400** tumor evaluation models in six categories have been established by Medicilon.




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

## Pharmacokinetic (PK) Studies of PROTACs

Medcilon delivers accurate quantification assays for key PROTAC PK parameters.

### Medicilon Cases

The pharmacokinetics (PK) of five highly potent PROTAC AR degraders (compounds 26, 27, 28, 33, and 34) are evaluated in mice with both intravenous and oral administration. The pharmacokinetics (PK) data show that five highly potent androgen receptor (AR) degraders achieve good to excellent overall PK profiles with ARD-2128 being the best compound. ARD-2128 has low clearance (1.2 mL/min/kg) and a moderate to high steady-state volume of distribution (Vss) of 2.7 L/kg. ARD-2128 (2 mg/kg, i.v.) and ARD-2128 (5 mg/kg, p.o.) both have long half-lives following intravenous and oral administration with the T1/2s of 27.6 h and 18.8 h, respectively. ARD-2128 (5 mg/kg) achieves 67% oral bioavailability in mice, effectively reduces AR protein and suppresses AR-regulated genes in tumor tissues with oral administration, leading to the effective inhibition of tumor growth in mice without signs of toxicity. This study was conducted by **Medicilon**.

| 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[1]

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)[1]

## Medicilon Assisted Projects

### CG001419

In August 2022, Cullgen Inc announced the approval of the IND application for a TRK degrader (CG001419) for the treatment of advanced solid tumors. CG001419 is the world's first in class TRK (neurotrophic factor receptor tyrosine kinase) protein degrader. **As the collaborative CRO of Cullgen, Medicilon relies on the PROTAC technology platform to provide comprehensive preclinical research services that comply with both China and US GLP standards for the R&D of CG001419 (including pharmacokinetic research and safety evaluation) and helped CG001419 to successfully get approved.**

### GT919

In June 2023, Gluetacs Therapeutics (Gluetacs)'s first class 1 new drug, GT919 Capsules, a molecular glue degradation agent pipeline, was approved by FDA to enter clinical trials. It is for the treatment of malignant hematological tumors. The pipeline was previously approved by NMPA for clinical trials on December 20, 2022, and is currently undergoing Phase I clinical trials in China. This is the first product pipeline of Gluetacs that has been approved for clinical trials in both China and the United States, and it is another milestone in the globalization process of Gluetacs. **For the research and development of GT919, Medicilon relied on its solid R&D strength to efficiently complete the drug discovery to clinical application for GT919, including drug discovery, pharmaceutical research, and preclinical research based on Medicilon's one-stop preclinical research services platform.**

### GT929

In October 2023, Gluetacs Therapeutics's GT929 capsules, a molecular glue degradation agent pipeline, was approved by FDA to enter clinical trials for the treatment of malignant hematological tumors. GT929 is an IKZF1/3 targeted immunomodulatory drug candidate. The pipeline was previously approved by NMPA for clinical trials on July 2023. **As a strategic partner of Gluetacs Therapeutics, Medicilon provided formulation research and preclinical research (including PD, PK, and safety evaluation) for the development of GT929, as well as preparing filing application materials.**

## References:

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

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