

Research Center for Molecular Recognition and Synthesis

Department of Chemistry, Fudan University



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# 溴端结构蛋白4-靶向降解剂 (BRD4-PROTACs) 的理性设计 及在肿瘤治疗上的应用

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Reporter: Wenqing Li

Supervisor: Prof. Shengming Ma

Date: 2024.01.05

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## 1.1

# 靶向蛋白嵌合体 Proteolysis Targeting Chimera (PROTAC) 研究背景

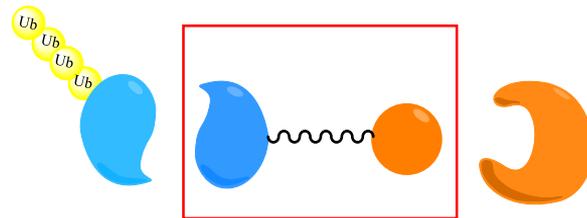
- The proposal of PROTACs



**Craig Crews**

Yale University

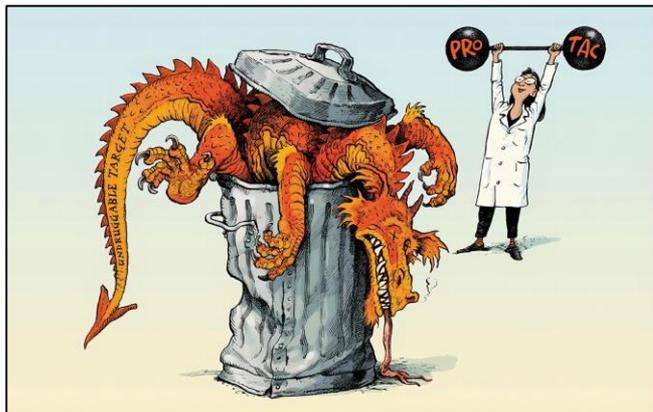
First proposed in 2001



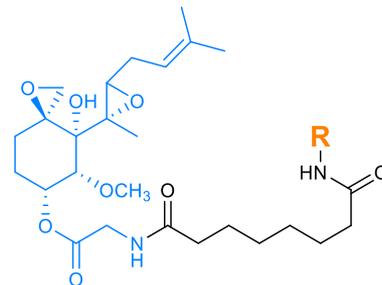
E3 ligase

PROTAC

Protein of Interest(POI)



首个PROTAC 降解剂:



R=GGGGGGRAEDS\*GNES\*EGE-COOH  
or GGGGGGDRHDS\*GLDS\*M-COOH

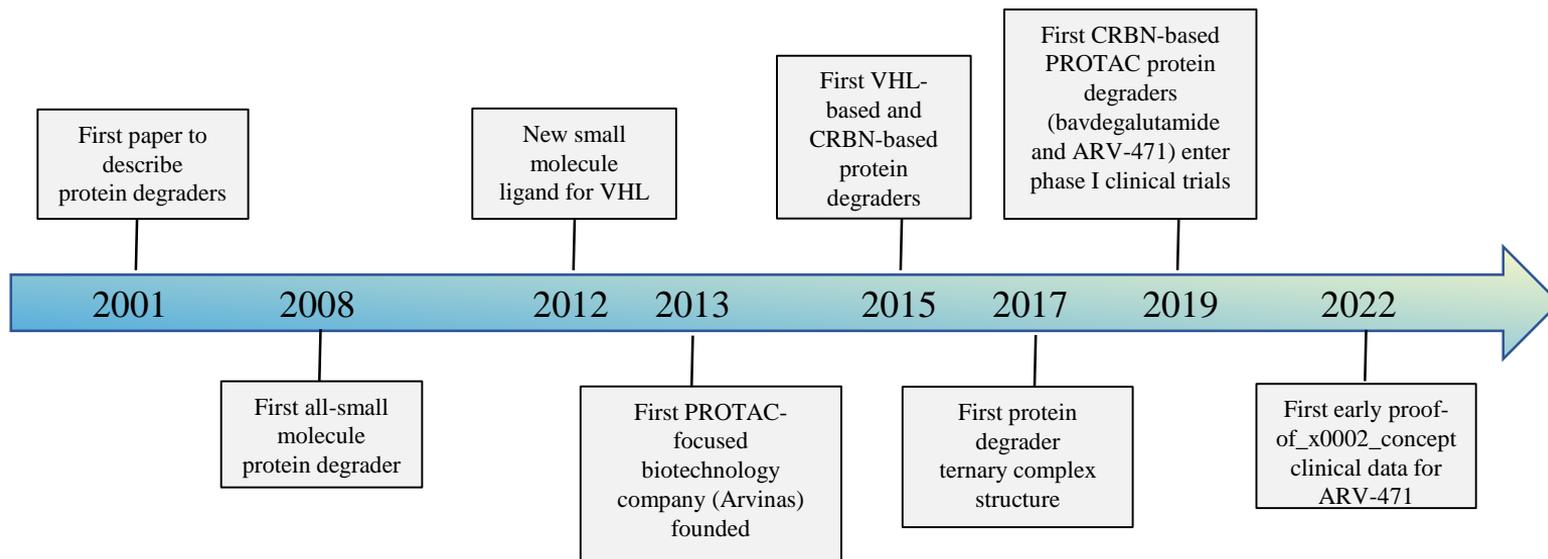
Protac-1

Protein-slaying drugs could be the next **blockbuster** therapies!

## 1.1

# PROTACs研究背景

## ➤ PROTACs发展史



- ❑ Crews, C. et al. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, 98, 8554-8559.
- ❑ Schneekloth, A. R. et al. *Bioorg. Med. Chem. Lett.* **2008**, 18, 5904-5908.
- ❑ Crews, C.; Ciulli, A. et al. *Angew. Chem. Int. Ed.* **2012**, 51, 11463-11467.
- ❑ Crews, C. et al. *J. Chembio.* **2015**, 22, 755-763.
- ❑ Ciulli, A. et al. *ACS Chem. Biol.* **2015**, 10, 1770-1777.
- ❑ Ciulli, A. et al. *Nat. Chem. Biol.* **2017**, 13, 514-521.
- ❑ Crews, C. et al. *Nat. Rev. Clin. Oncol.* **2023**, 20, 265-278.



Craig M. Crews



Alessio Ciulli



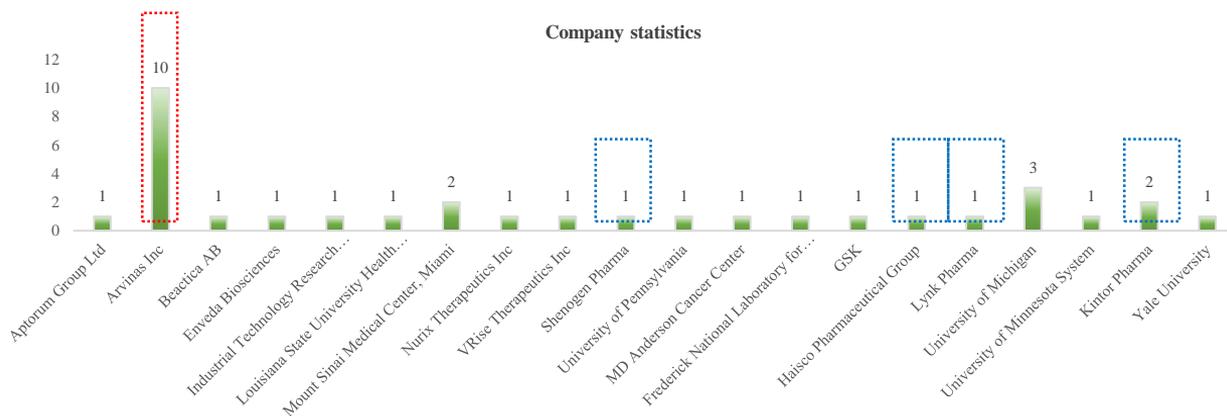
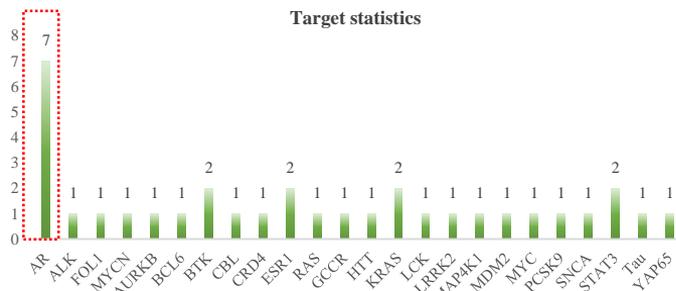
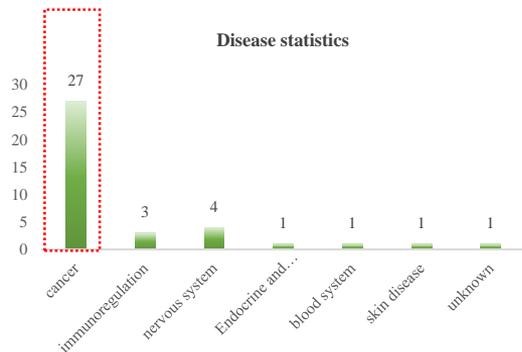
Shaomeng Wang



Ju Rao

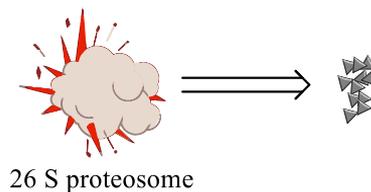
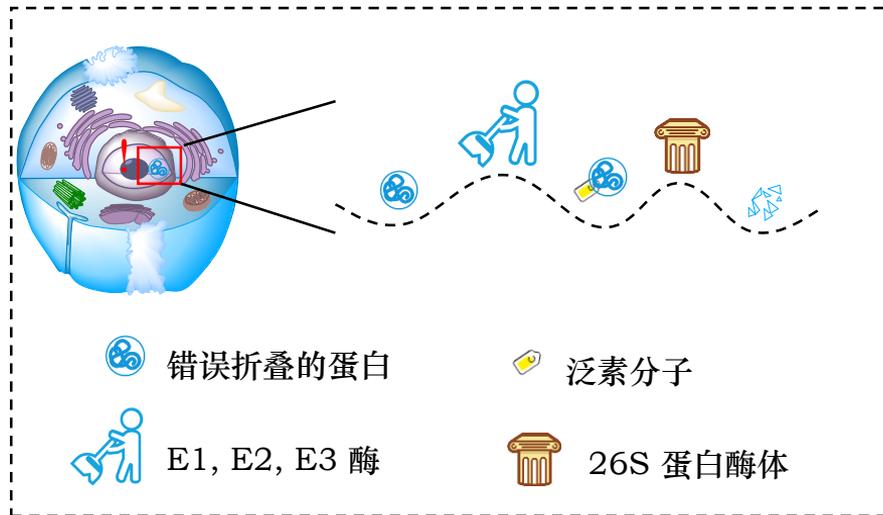
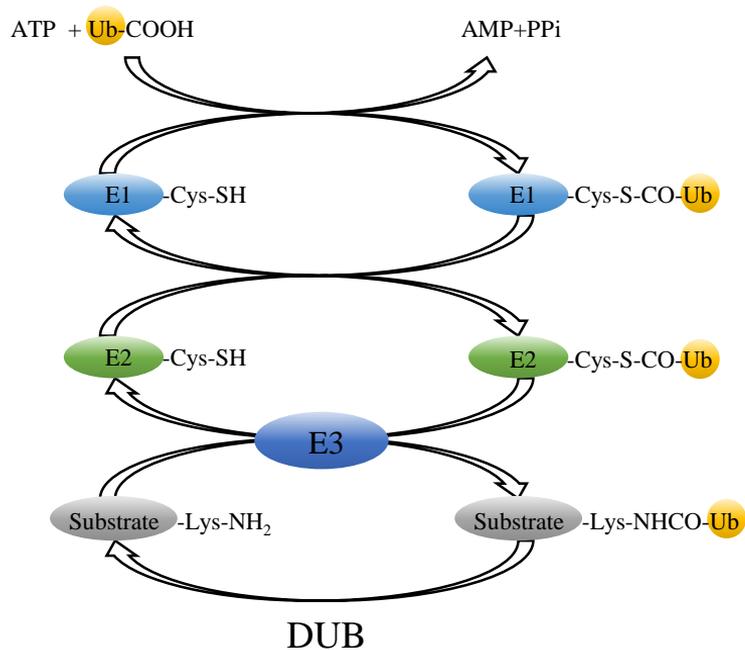
# PROTACs研究背景

## ➤ PROTACs临床数据分析



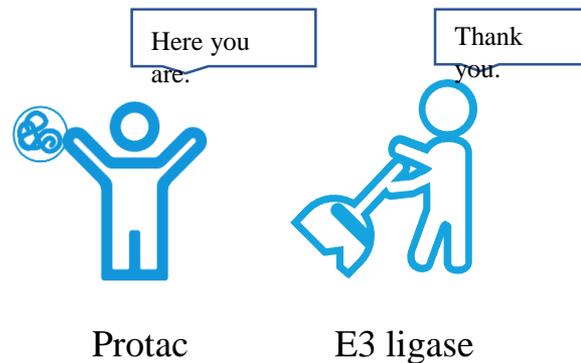
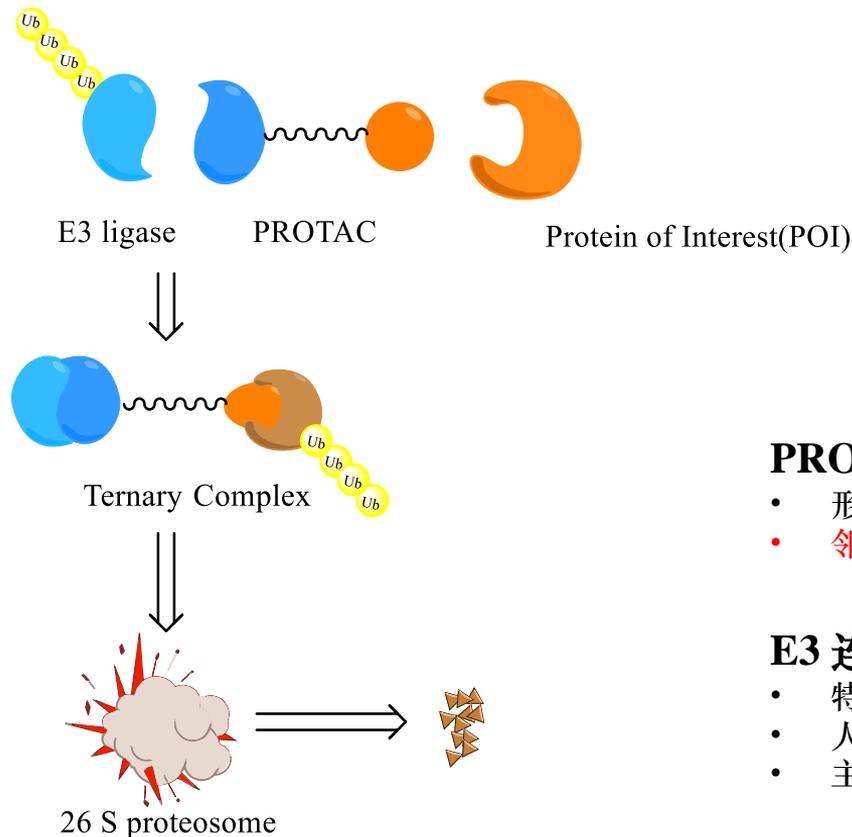
# PROTACs研究背景

➤ 细胞中蛋白降解途径——泛素-蛋白酶体系统UPS



# PROTACs研究背景

## ➤ PROTACs作用机制



### PROTACs特点:

- 形成三元复合物同时靶向靶蛋白和E3酶.
- 邻近诱导和事件驱动.

### E3 连接酶:

- 特异性识别底物
- 人基因组中目前600多种E3酶.
- 主要用于PROTAC设计的E3酶: MDM2、IAPs、VHL、CRBN....

## PROTACs研究背景

➤ PROTAC VS 小分子抑制剂

### Advantages

- 降解不可成药靶点
- 克服耐药性
- 高选择性和降解效率

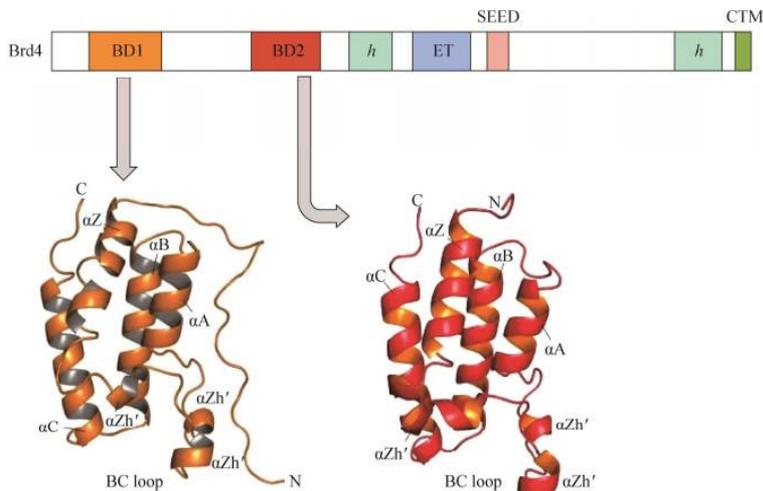
### Disadvantages

- 水溶性、渗透性差
- 不可预料的副作用及毒性
- 对高度同源蛋白的选择性低
- E3 酶工具箱有待开发

## BRD4研究背景

### ➤ BRD4的结构和功能

#### • BRD4的结构



**BRD4:** 含溴结构域蛋白家族中的一员 (Bet proteins: BRD2、BRD3、BRD4 and BRDT).

下游基因: MYC, CDK, BCL2, etc.

#### 功能:

- 在多种癌细胞中过表达;
- 影响细胞周期、增殖和凋亡;
- 在癌细胞的迁移、代谢和恶性发展中发挥重要作用

#### 小分子抑制剂开发现状:

- 泛BRD4小分子抑制剂因其选择性差而导致的不良反应等问题, 最终多数临床研究被终止

**BRD4-PROTAC有望成为泛癌种的治疗药物.**

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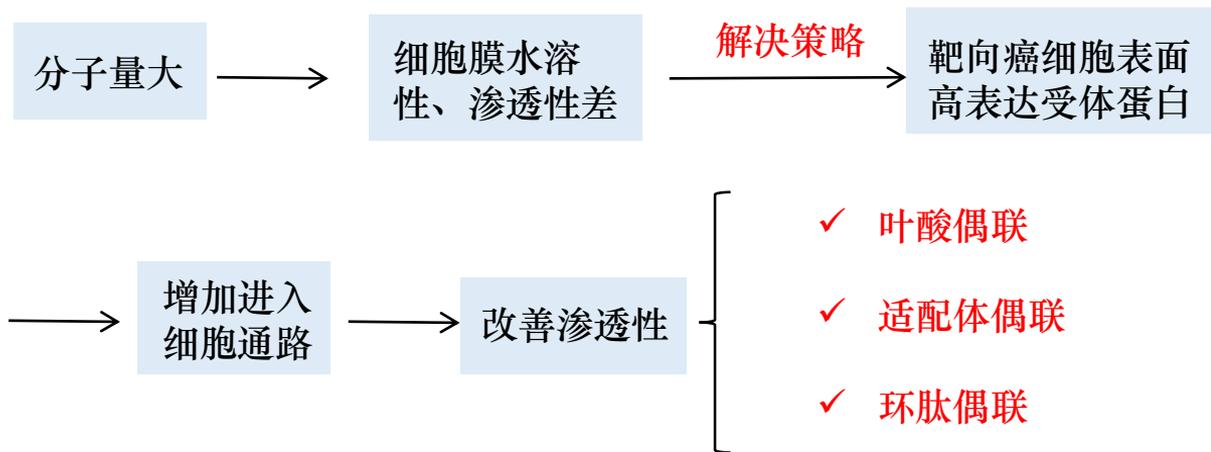
2.2 减小副作用的策略

2.3 提高选择性的策略

2.4 扩展E3连接酶工具

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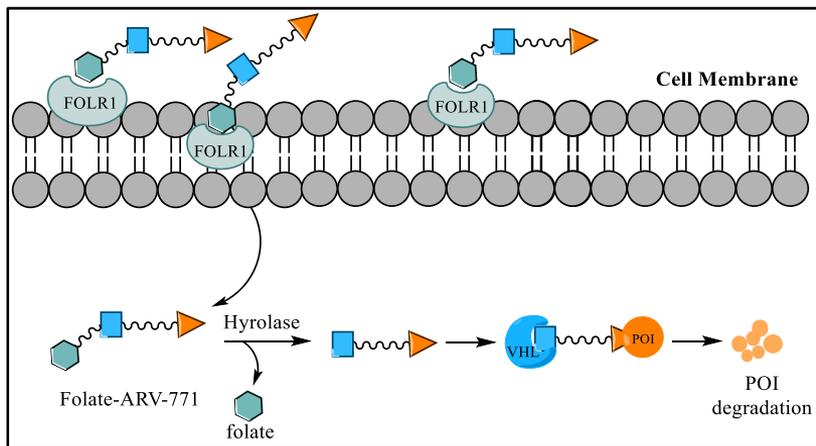
- 总设计思路



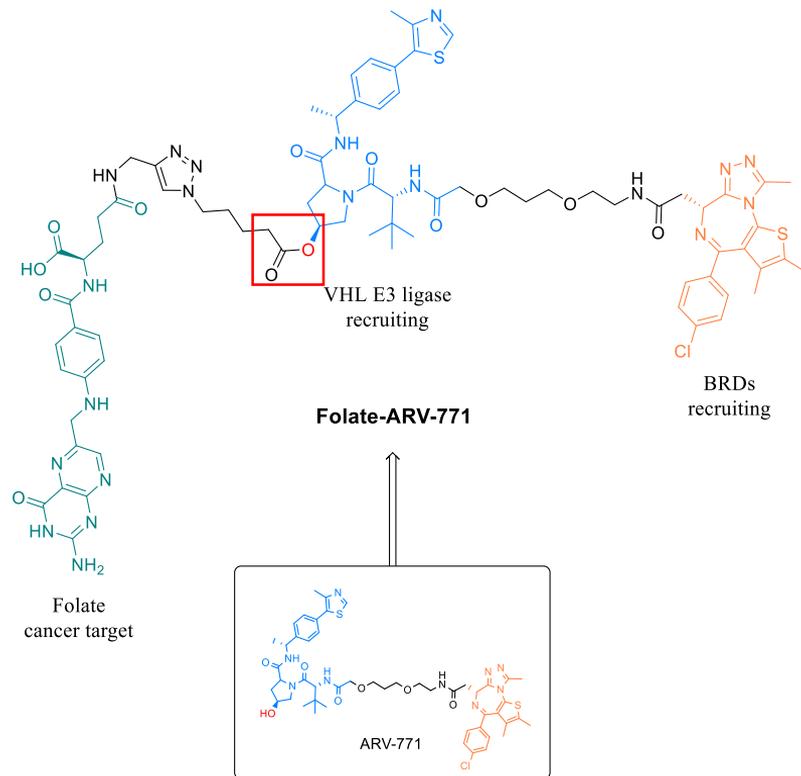
## 2.1.1

## 叶酸偶联策略

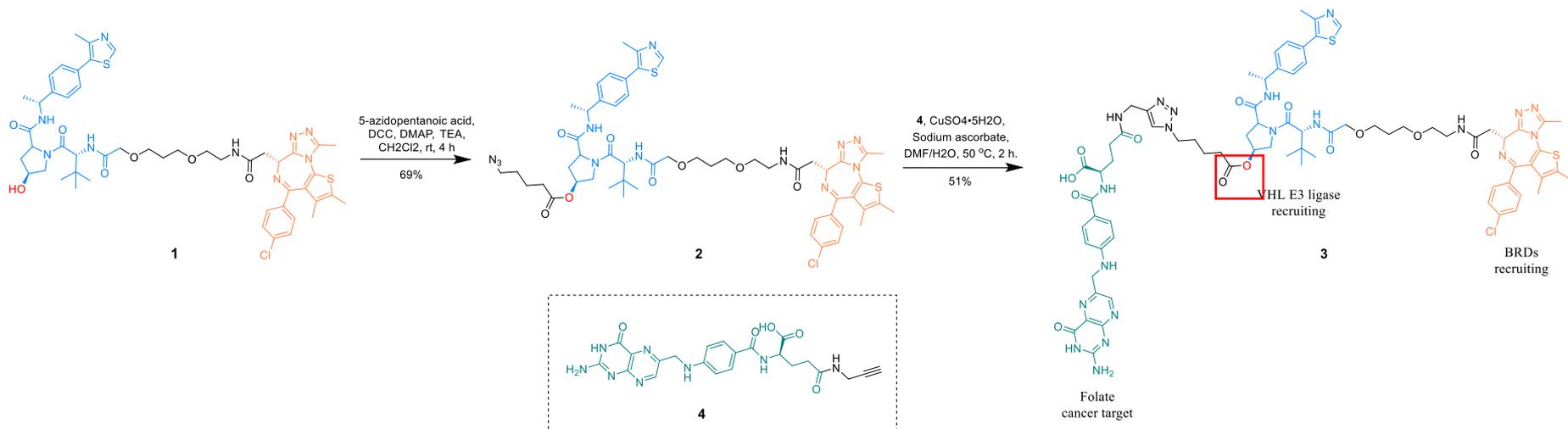
## ➤ 叶酸偶联降解剂 (Folate-ARV-771)

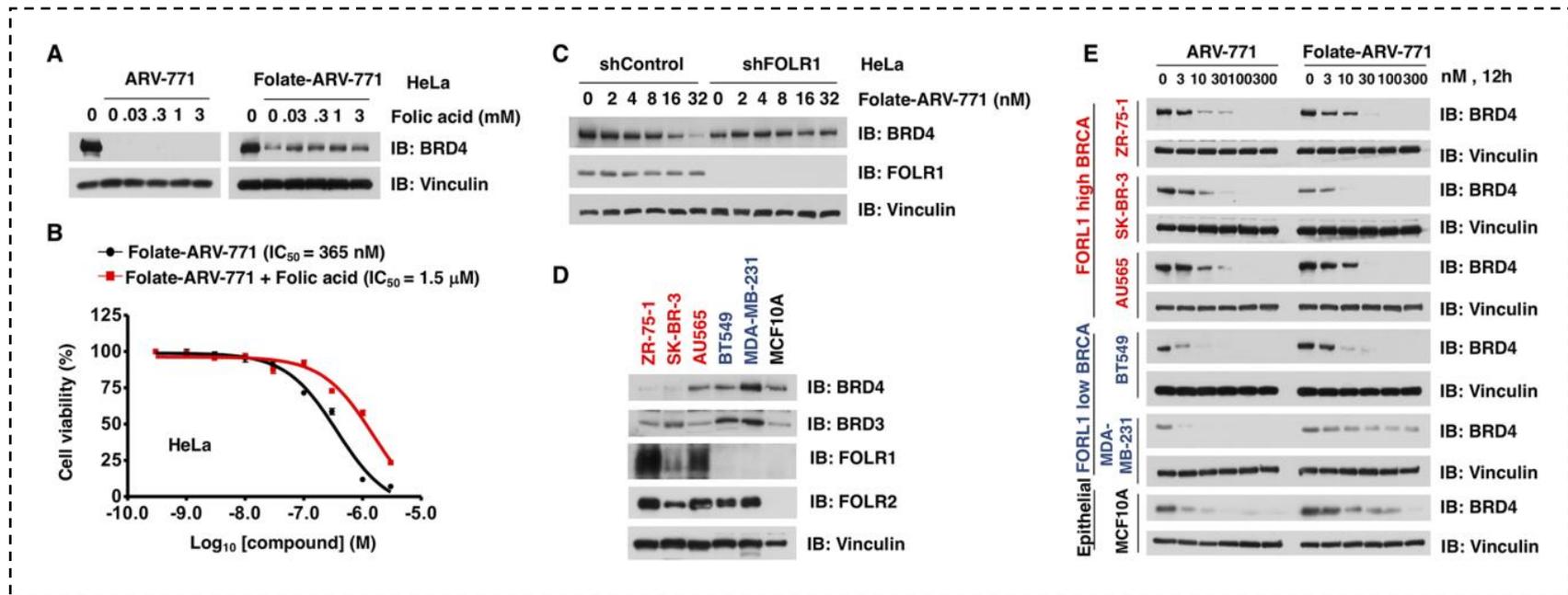
**FOLR1: 叶酸受体  $\alpha$** 

- 在多种癌细胞中高表达
- 在正常细胞中低表达或不表达
- 转运叶酸进入细胞



- The synthesis of Folate-ARV-771



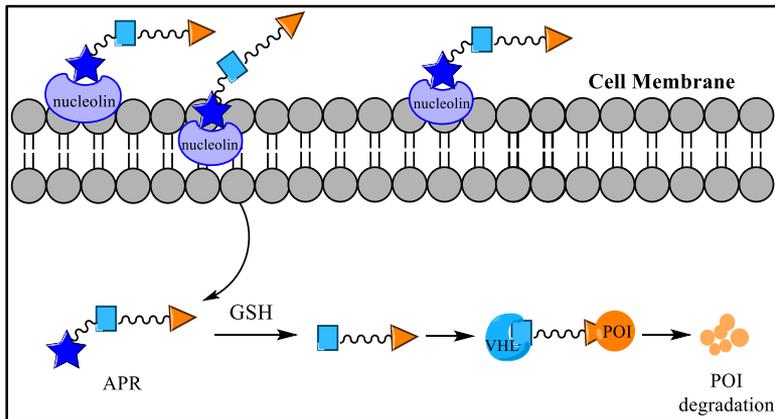


- 叶酸竞争实验及敲除FOLR1实验：降解剂通过FOLR1被转运进细胞内
- 免疫蛋白印迹实验：降解剂可以剂量依赖性地发挥降解作用

## 2.1.2

## 适配体偶联策略

## ➤ Aptamer-PROTAC(APR)



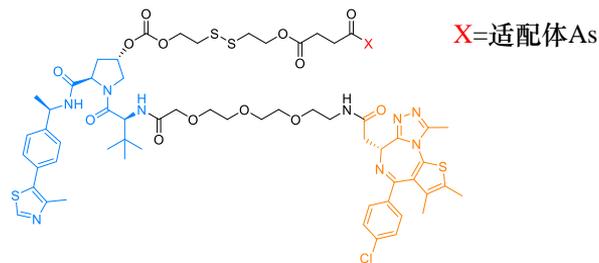
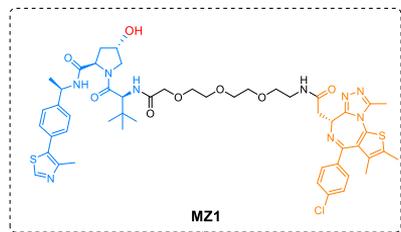
## 适配体AS1411(As):

- 单链核酸
- 特异性识别并结合核仁蛋白 (nucleolin)

**CRO:** 无特异性的 DNA 序列, 阴性对照

**Fam:** Fluorescein amidate, 荧光标记试剂

**Cy3:** Cyanine3, 荧光标记试剂



	x		x
APR	★	CPR	★
APR-Fam	★●	CPR-Fam	★●
APR-Cy3	★●	CPR-Cy3	★●

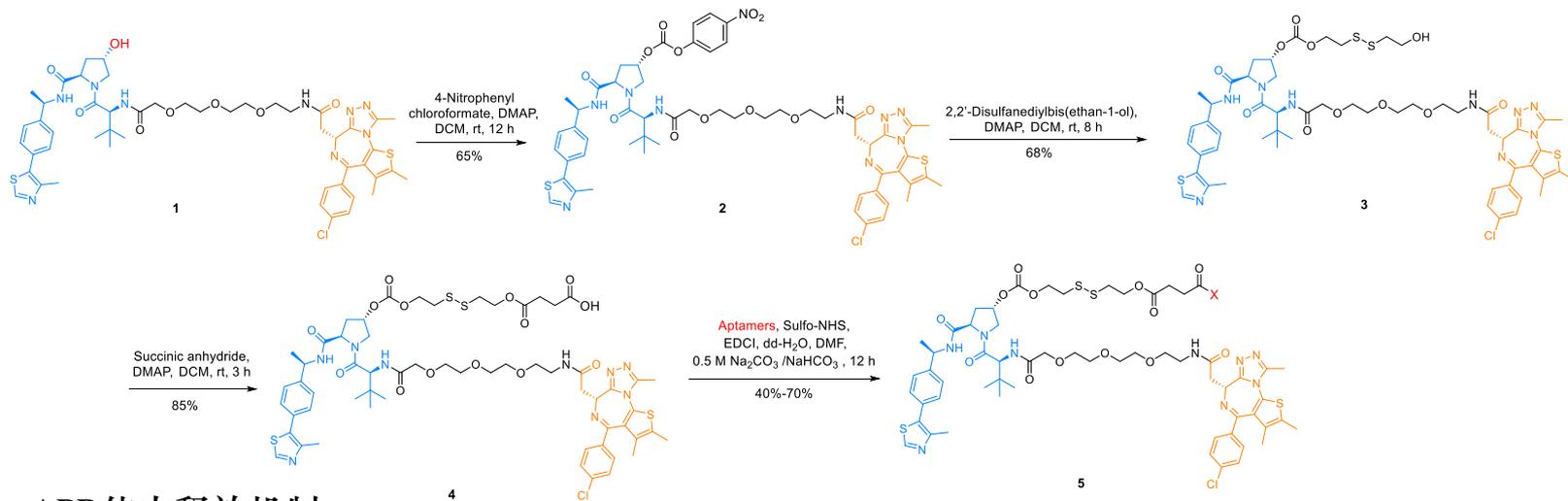
□ Ciulli, A. et al. *ACS Chem. Biol.* **2015**, *10*, 1770–1777.

□ Sheng, C. et al. *Angew. Chem. Int. Ed.* **2021**, *60*, 23299–23305.

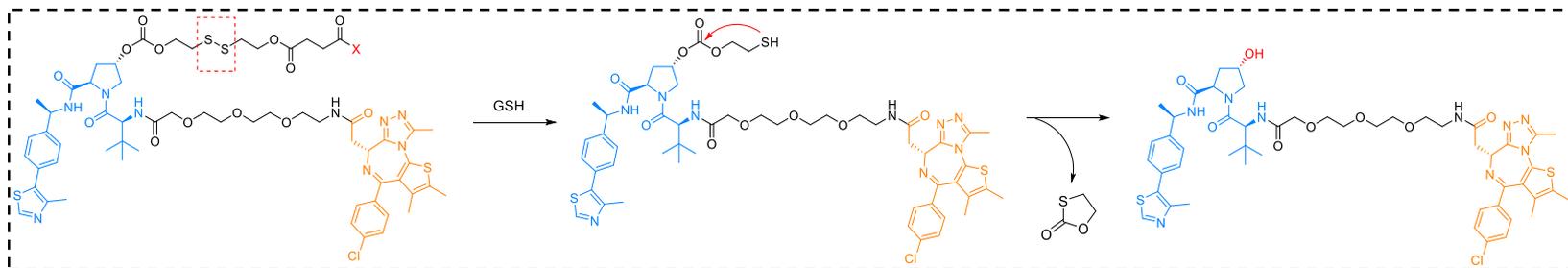
## 2.1.2

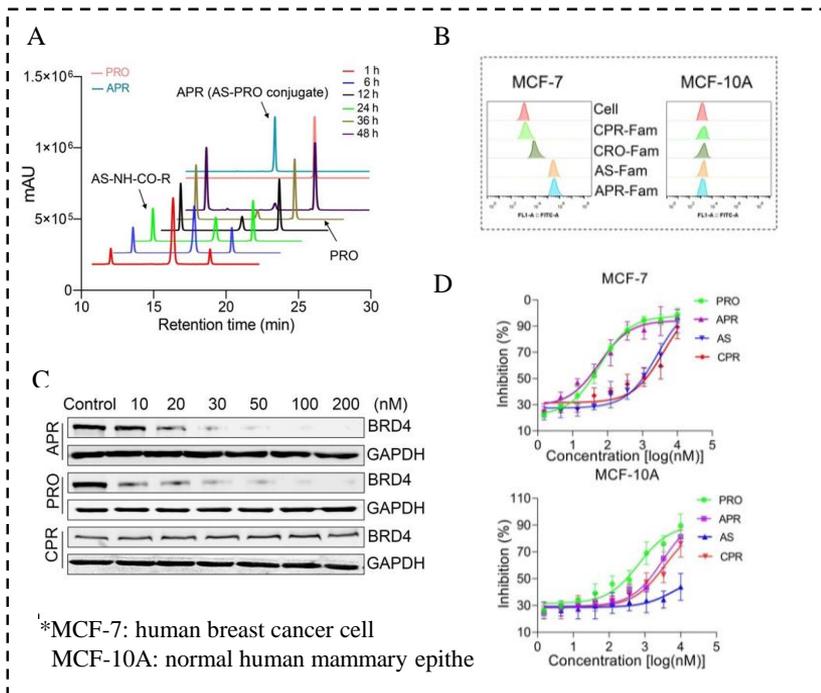
## 适配体偶联策略

## • APR的合成



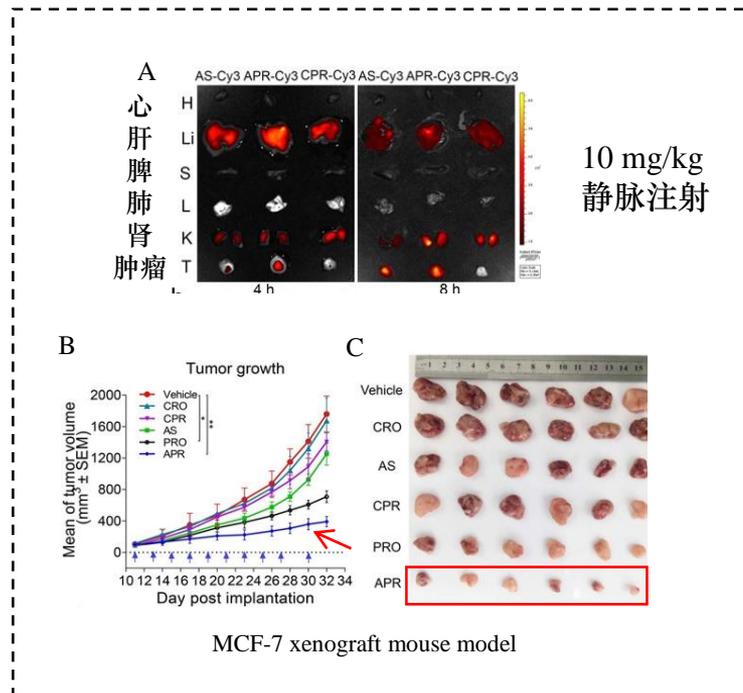
## • APR体内释放机制





### 细胞水平:

- APR可以释放PRO (母核降解剂) ;
- 过表达核仁蛋白的细胞可以促进对APR的摄取, 抑制肿瘤细胞增殖;
- APR可以剂量依赖性降解BRD4



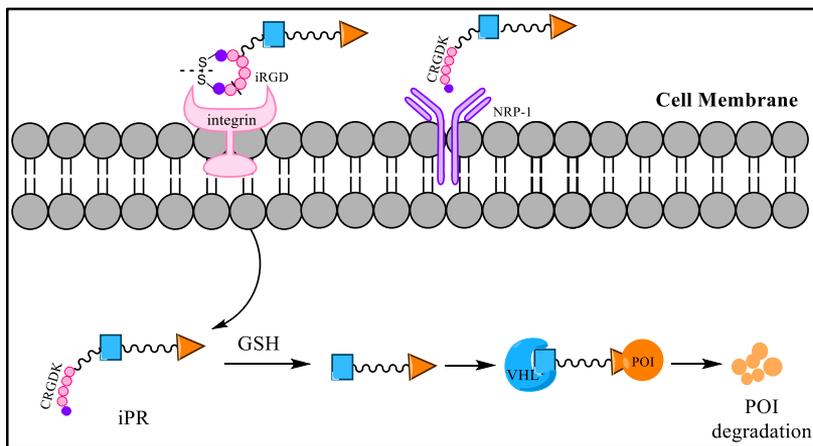
### 动物水平

- APR可以靶向肿瘤组织
- APR可以显著抑制肿瘤生长

## 2.1.3

## 环肽偶联策略

## ➤ iRGD Peptide-PROTACs (iPR)

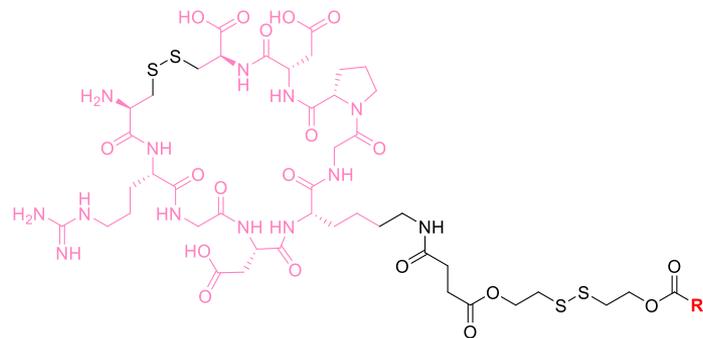


## 整合素Integrin

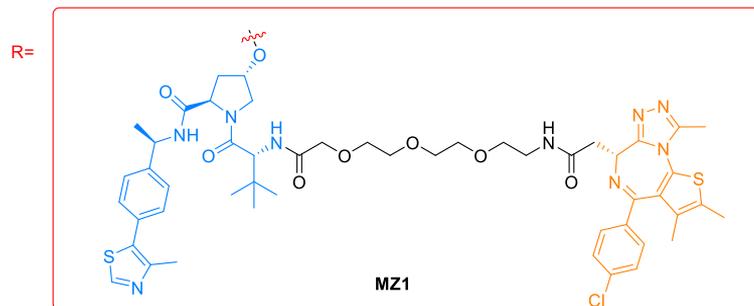
- 在肿瘤细胞表面高表达，特异性识别**RGD**序列多肽

## NRP-1: neuropilin-1

- 一种多功能受体通道蛋白
- 在肿瘤细胞中高表达
- 特异性识**C端-RGDK**序列多肽，增强细胞内化



iPR

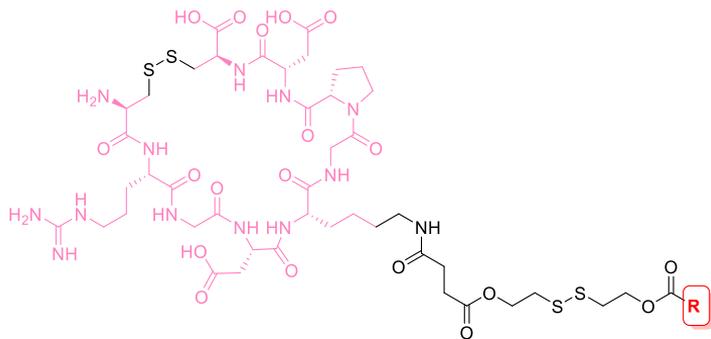


MZ1

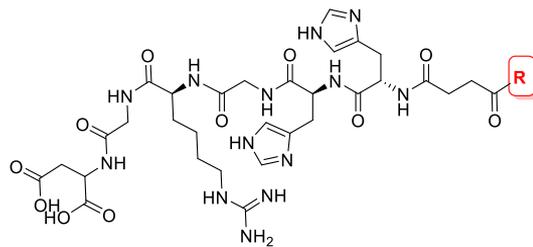
## 2.1.3

## 环肽偶联策略

- iPR及对照化合物的设计

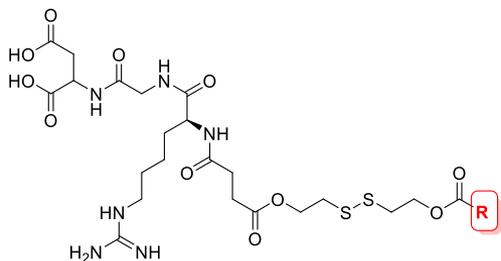


iPR



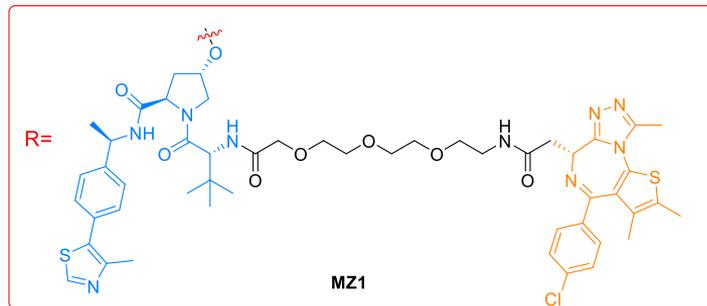
L-RPR

(NO -S-S-)



S-RPR

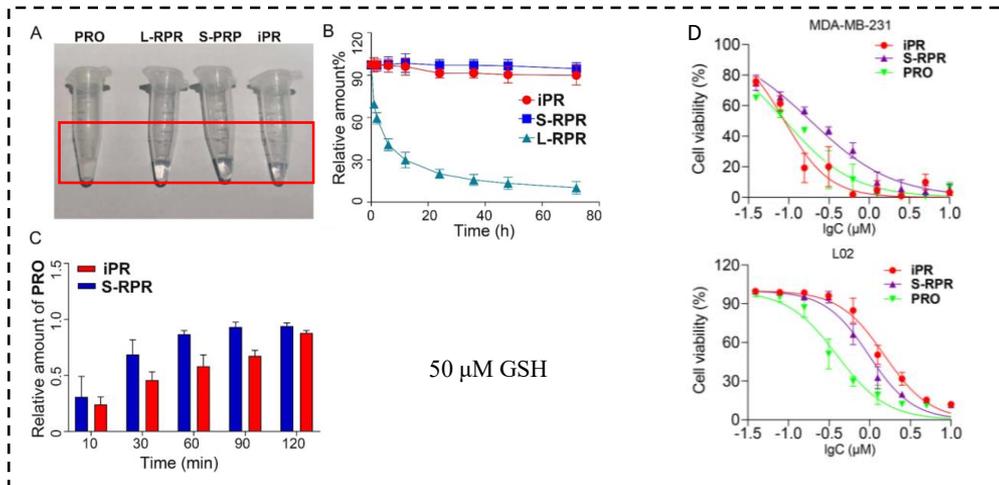
(NO RGD)



MZ1

## 2.1.3

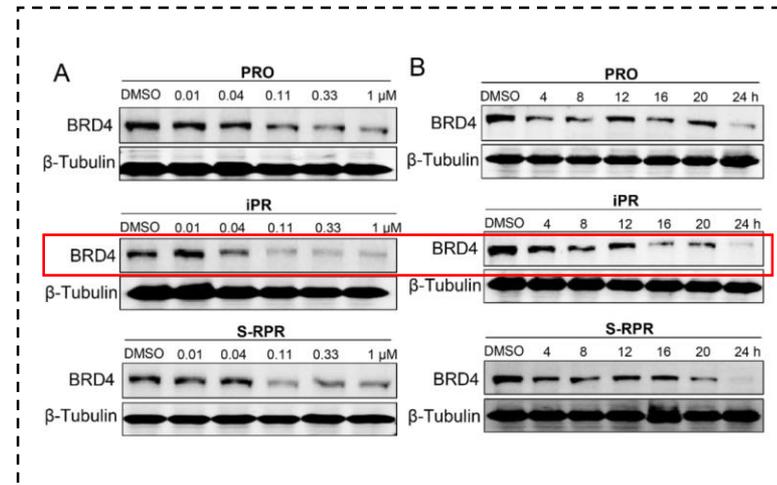
## 环肽偶联策略——细胞水平



MDA-MB-231: triple-negative breast cancer cells

L02: human normal hepical cells

- iPR 和 S-RPR 具有良好的水溶性及体内稳定性
- iPR可以有效释放PRO（母核降解剂）
- 在高表达整合素的MDA-MB-231中对肿瘤细胞增殖抑制增强

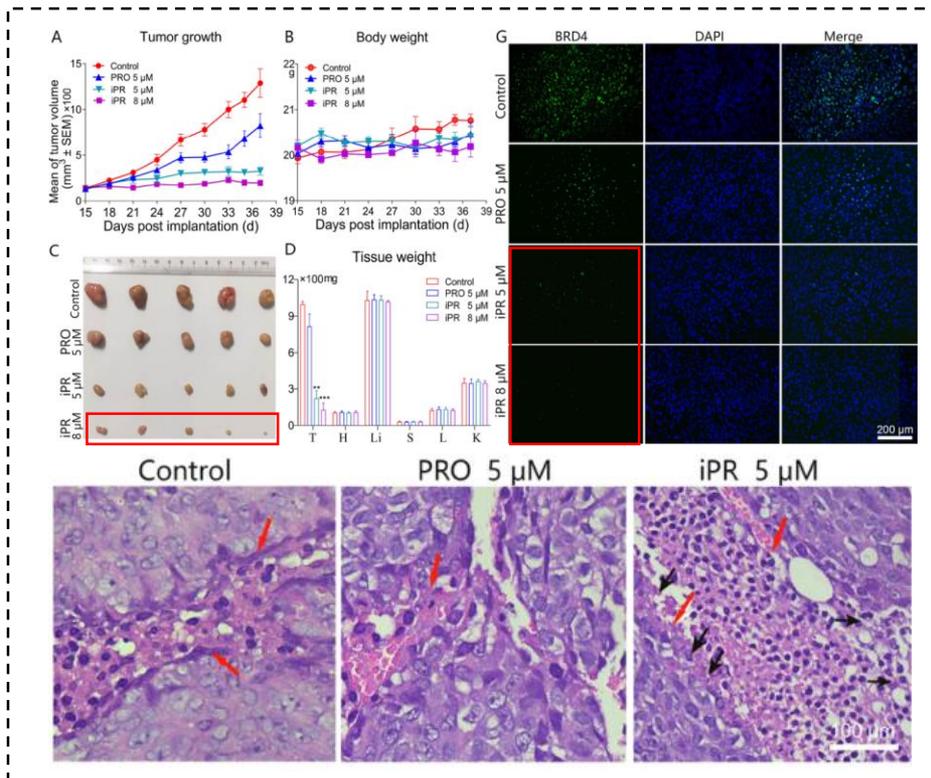


MDA-MB-231 cells

- iPR 可以剂量依赖性和时间依赖性地降解BRD4
- iPR降解效果优于PRO（母核降解剂）

## MDA-MB-231 移植瘤模型

静脉注射



红色箭头表示血管壁，  
黑色箭头表示血管壁的塌陷

• iPR 可以显著抑制肿瘤细胞增殖，降解BRD4蛋白

• iPR改变了肿瘤血管的通透性，增强了在肿瘤组织中的渗透和扩散。

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2.2 减小副作用的策略

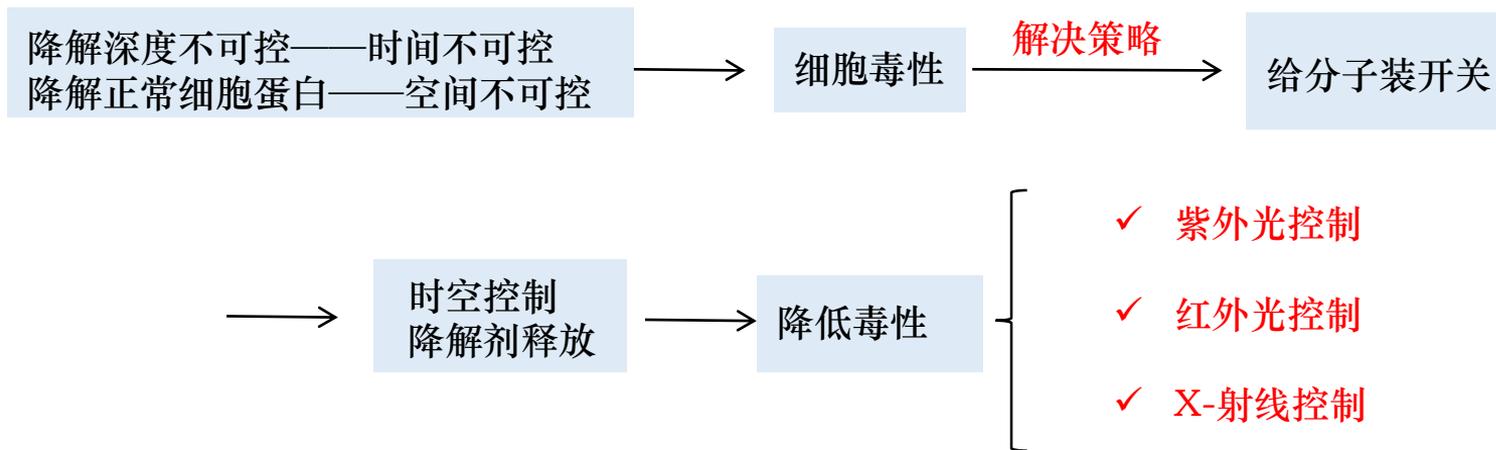
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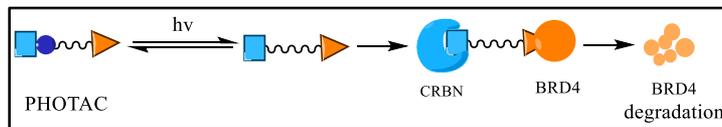
## 降低毒副作用的策略

- 总设计思路

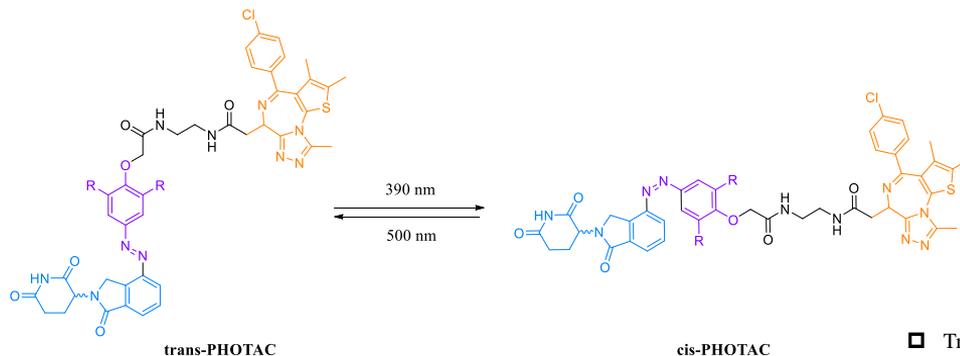


## 2.2.1

## 紫外光控策略

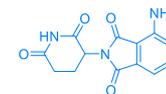
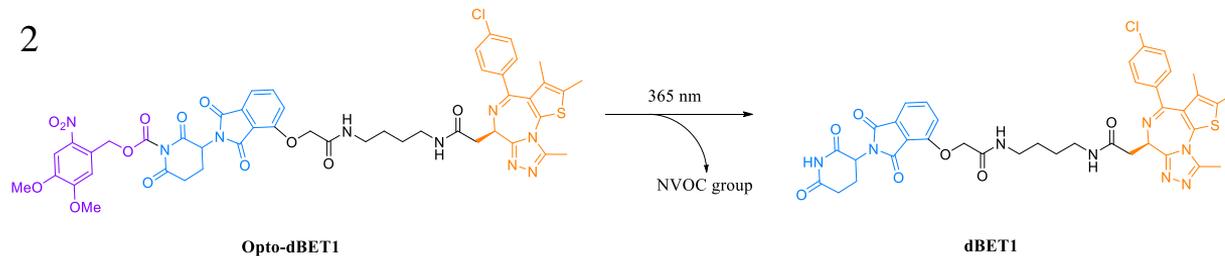


1



□ Trauner, D. et al. *Sci. Adv.* **2020**, *6*, eaay5064.

2



Pomalidomide

□ Wei, W. et al. *Sci. Adv.* **2020**, *6*, eaay5154.

## Similarity

- light induced
- use pomalidomide as the ligand of CRBN E3 ligase
- use dBET1 as the ligand of BRDs

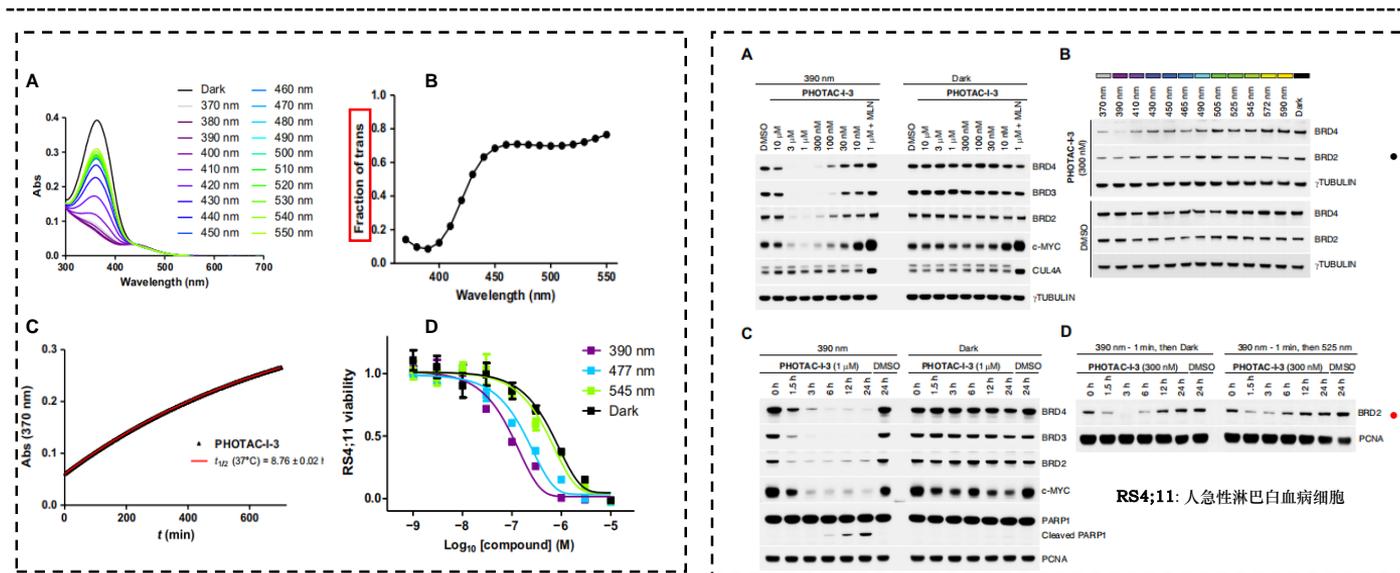
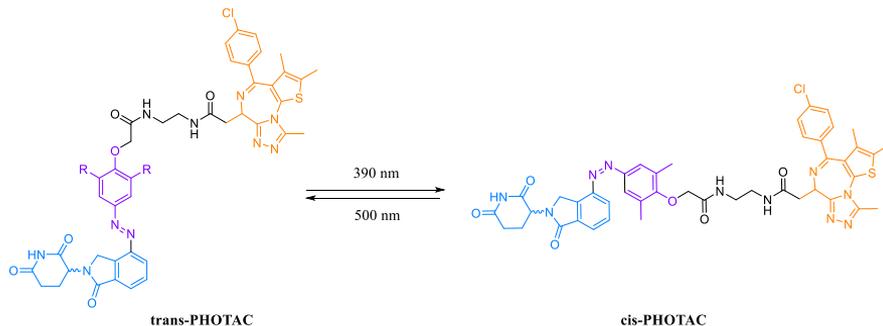
## Difference

- 1 uses azobenzene as switch
- 2 uses NVOC group
- 1 released by cis-trans isomerism
- 2 released by the leave of NVOC group

## 2.2.1

## 紫外光控策略——PHOTAC

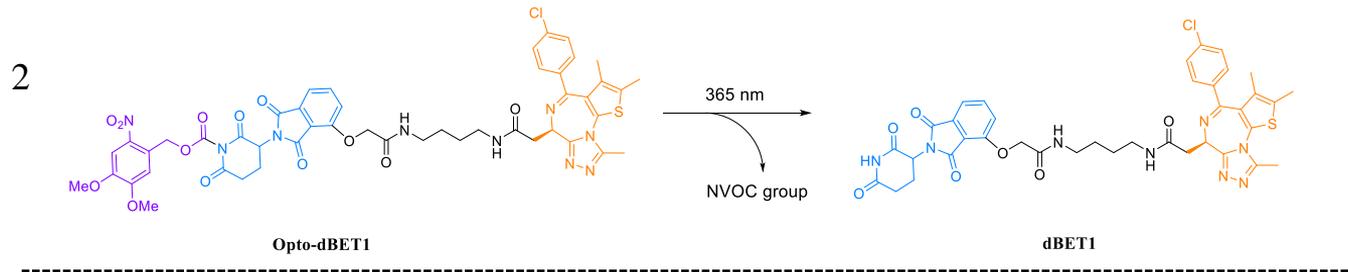
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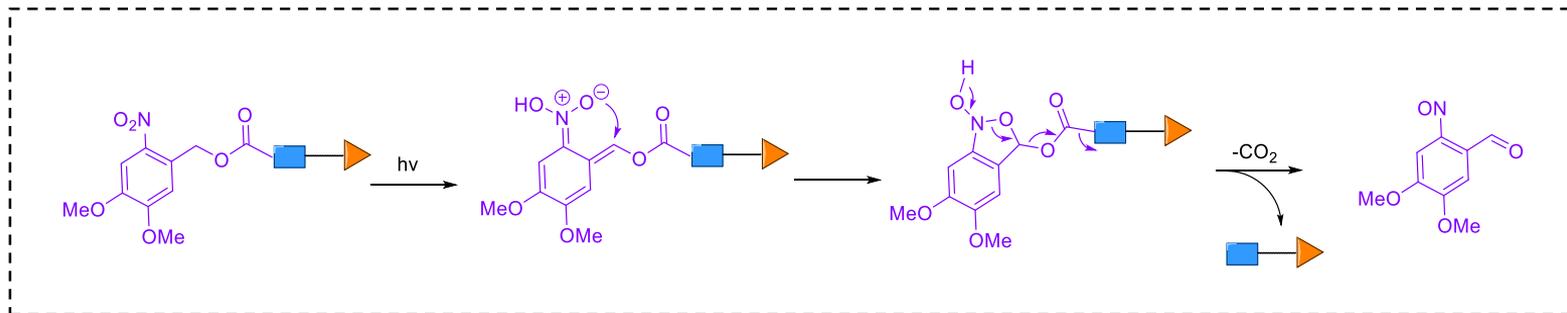
- 390 nm, > 90% cis
- > 450 nm, > 70% trans
- 黑暗条件下cis缓慢异构成trans

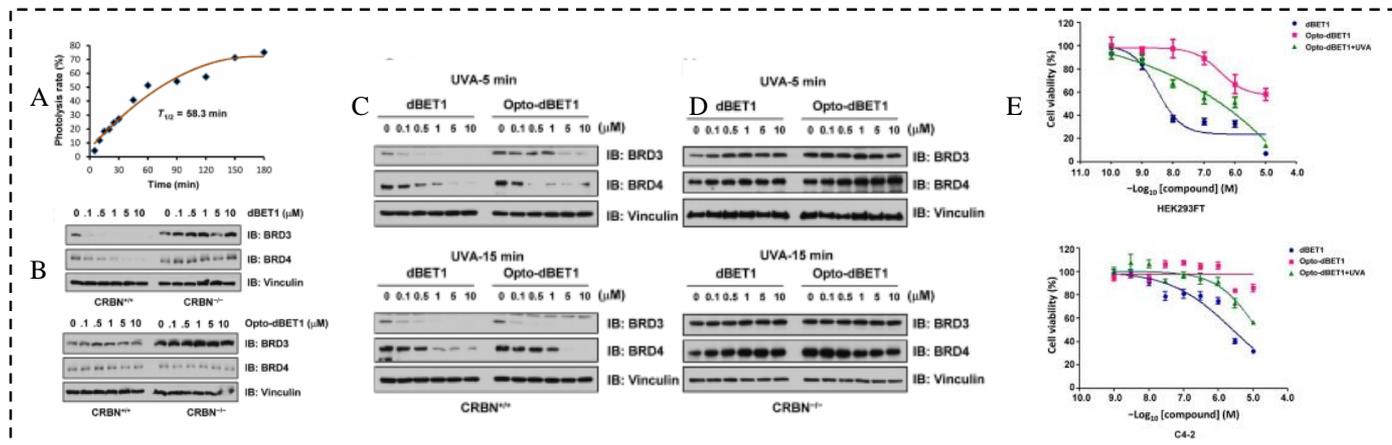
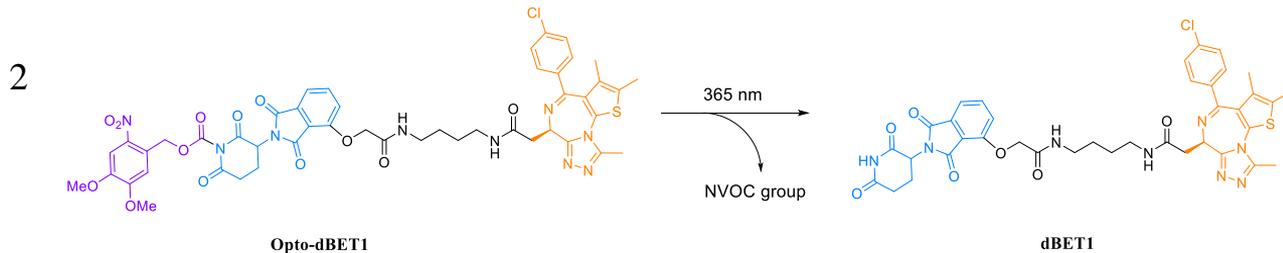
- 390 nm具有最高降解活性, 且具有剂量依赖性

- 拯救实验进一步验证光控可逆性



- 光控释放机制:



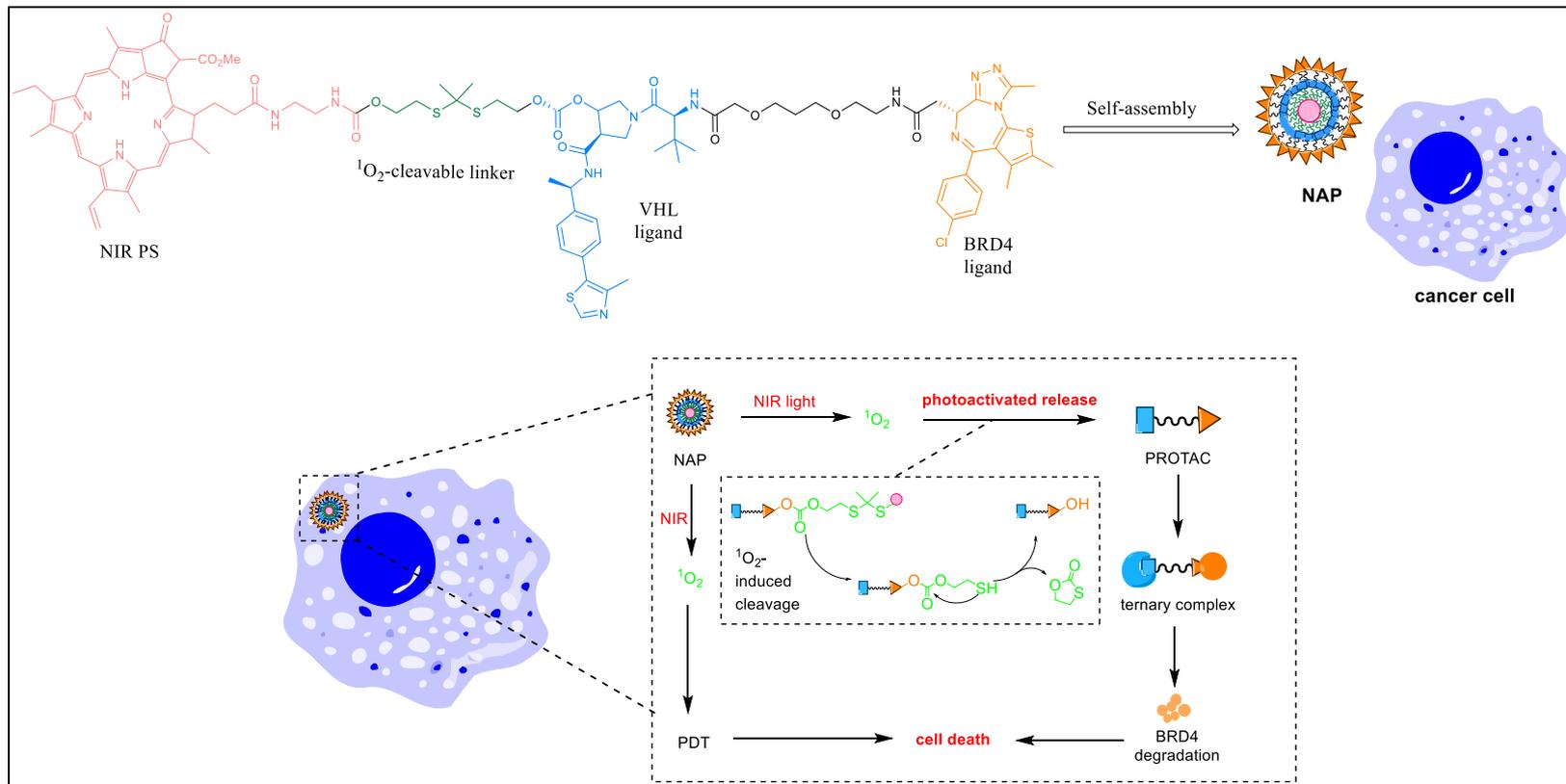


- opto-protac 黑暗条件下无活性，可以通过紫外光控释放，并剂量依赖性降解BRD4，抑制肿瘤细胞增殖

## 2.2.2

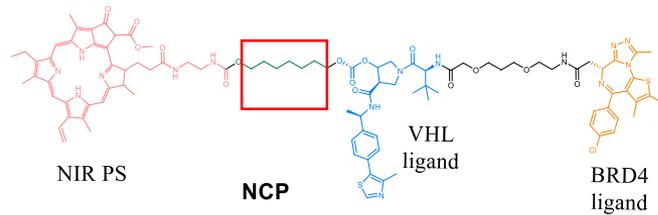
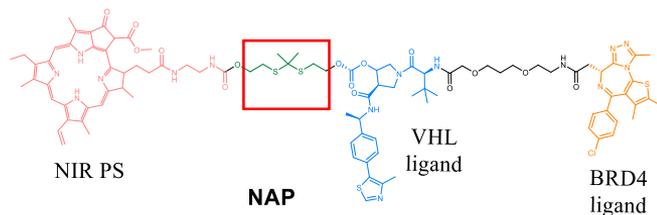
## 近红外光控策略

➤ NIR-Induced control of nano-PROTAC

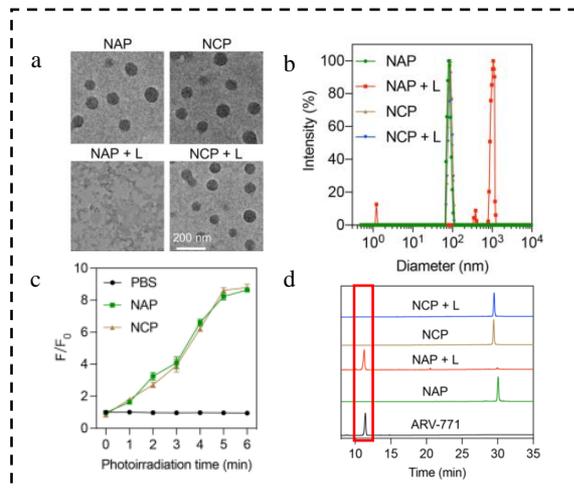


## 2.2.2

## 近红外光控策略——细胞水平

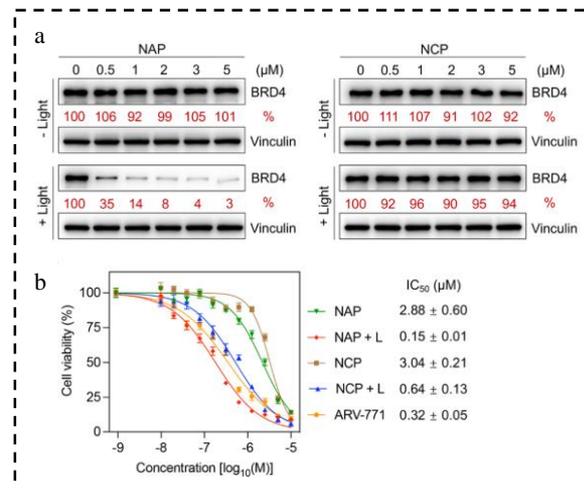


negative control

 $^1\text{O}_2$  sensor green (SOSG)

HPLC

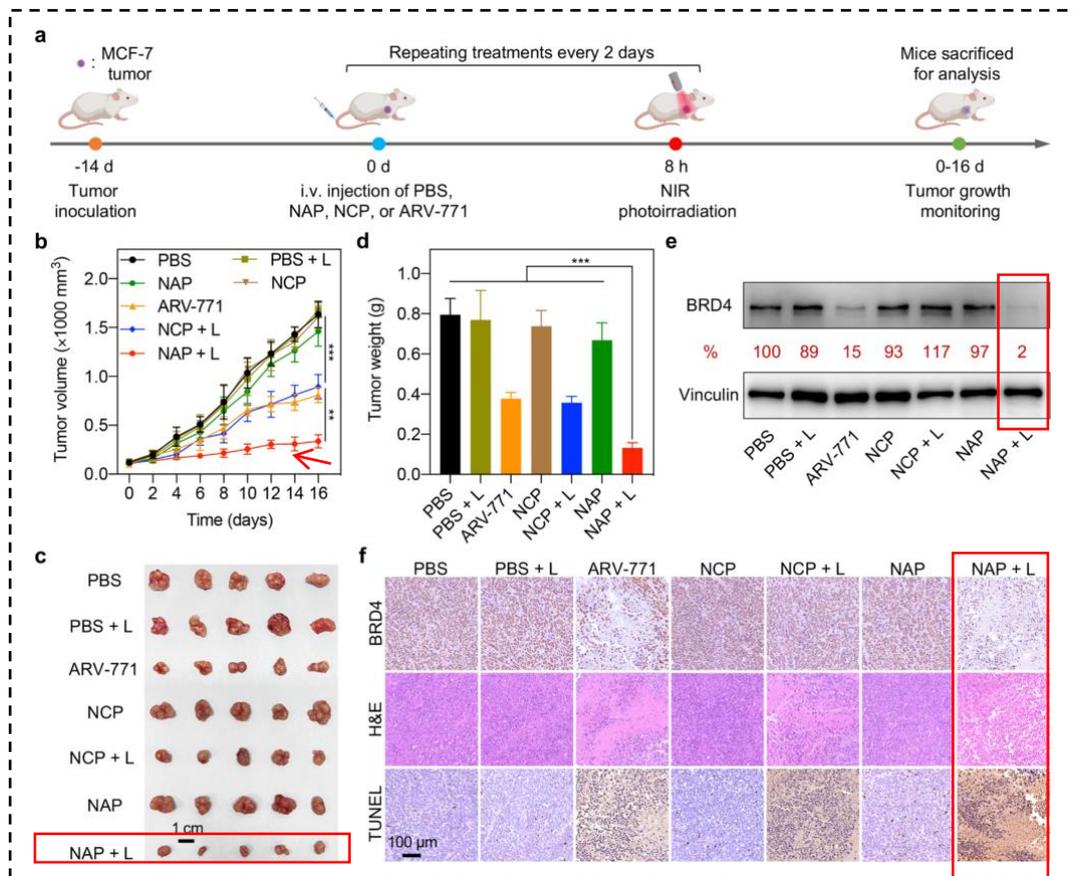
- 粒径为80 nm的球状纳米颗粒，可产生单线态氧，通过680 nm光照可控制ARV-771（母核protac）的释放



## • 细胞水平

只有光照条件下可以剂量依赖性降解BRD4，抑制肿瘤细胞增殖

- NAP只有光照条件下可以发挥抗肿瘤作用

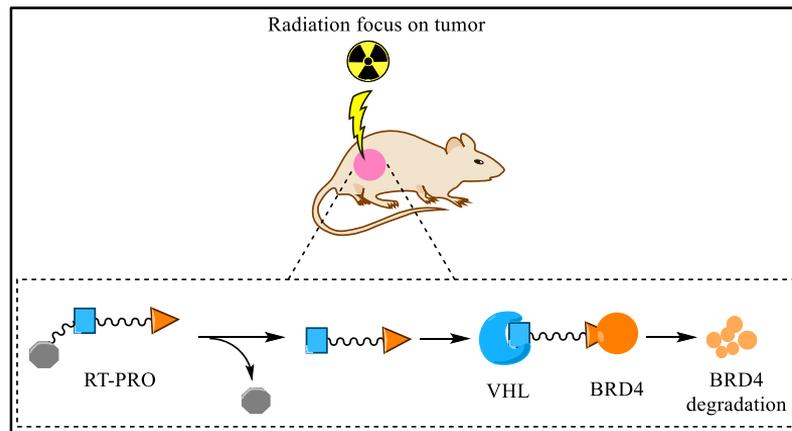
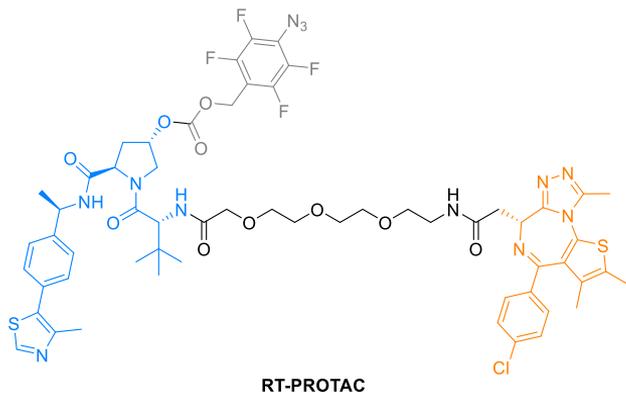


- NAP+light可以显著抑制肿瘤细胞增殖，降解BRD4蛋白

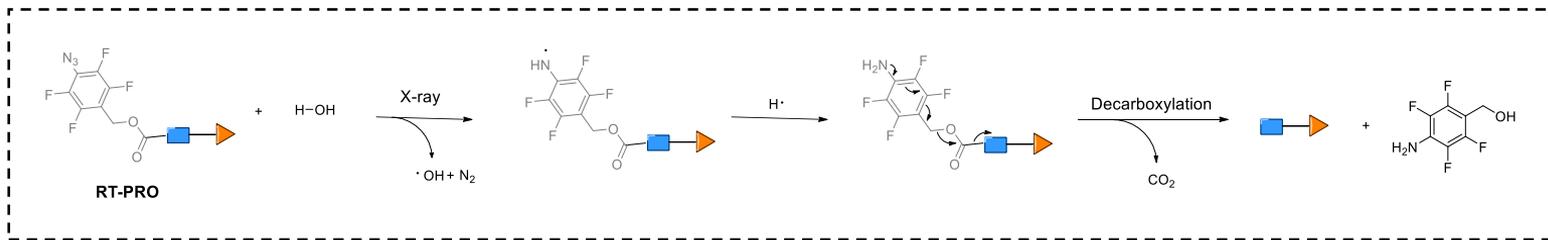
- Tunel染色NAP+light组凋亡肿瘤细胞最多

# X-ray控制释放策略

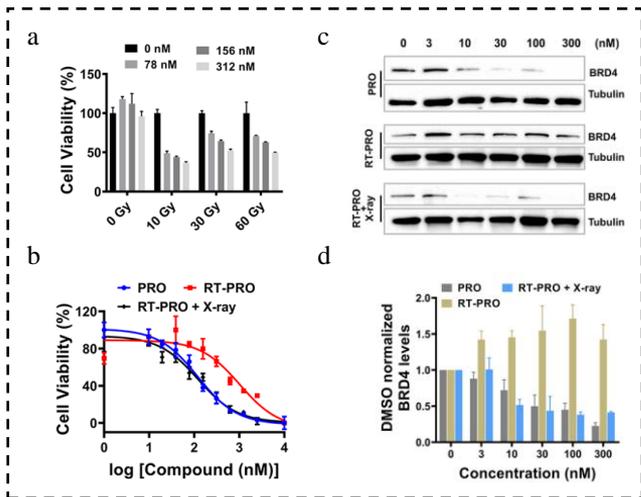
## ➤ Radiation-induced control of PROTAC (RT-PRO)



### • X-ray释放机制:



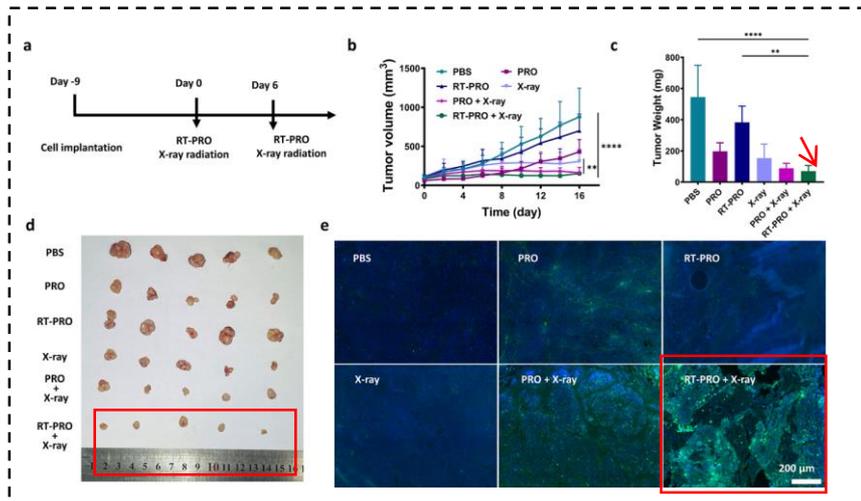
- 细胞水平



(MCF-7人乳腺癌细胞)

- RT-PRO可被射线激发释放，抑制肿瘤细胞增殖
- RT-PRO 以射线依赖的方式降解 BRD4

- 动物水平



(MCF-7小鼠移植瘤模型)

- RT-PRO+X-ray 可以显著抑制肿瘤体积与重量，促进肿瘤细胞凋亡

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1.2 BRD4研究背景

## 2. BRD4-PROTACs的理性设计

2.1 提高渗透性的策略

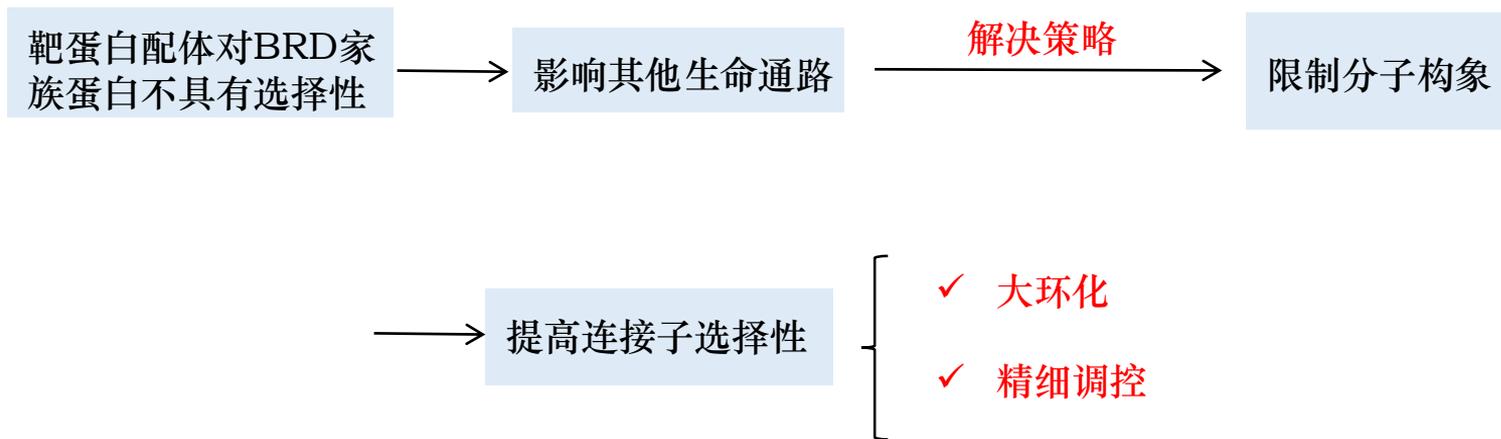
2.2 减小副作用的策略

2.3 提高选择性的策略

2.4 扩展E3连接酶工具

## 3. 总结展望

- 总设计思路

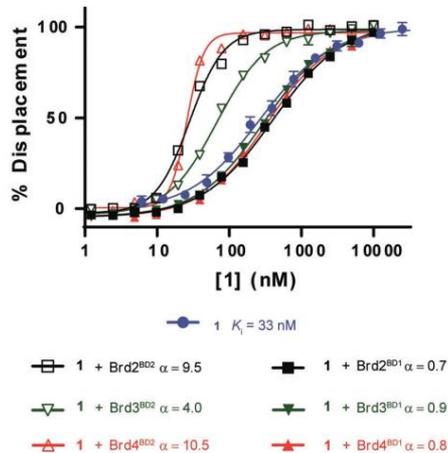




## 2.3.1

# 大环化策略——选择性研究

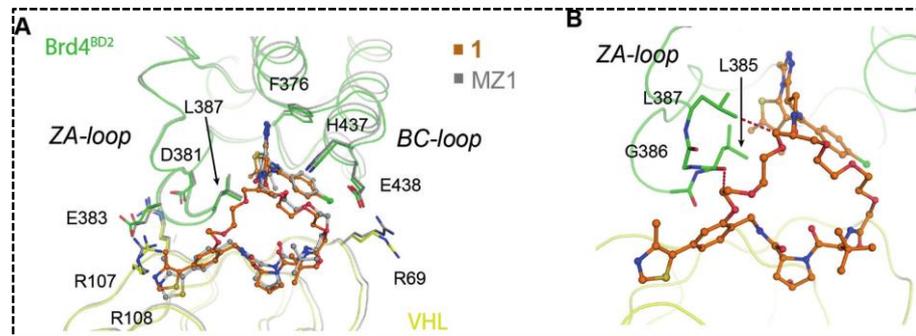
- 荧光偏振实验:



- 等温滴定量热实验:

Protein in syringe	Species in cell	Kd [nM]
BRD2 <sup>BD1</sup>	1	743±202
BRD4 <sup>BD2</sup>	1	180±42
VCB	1	47±9
BRD2 <sup>BD1</sup>	MZ1	62±6
BRD4 <sup>BD2</sup>	MZ1	15±1
VCB	MZ1	66±6

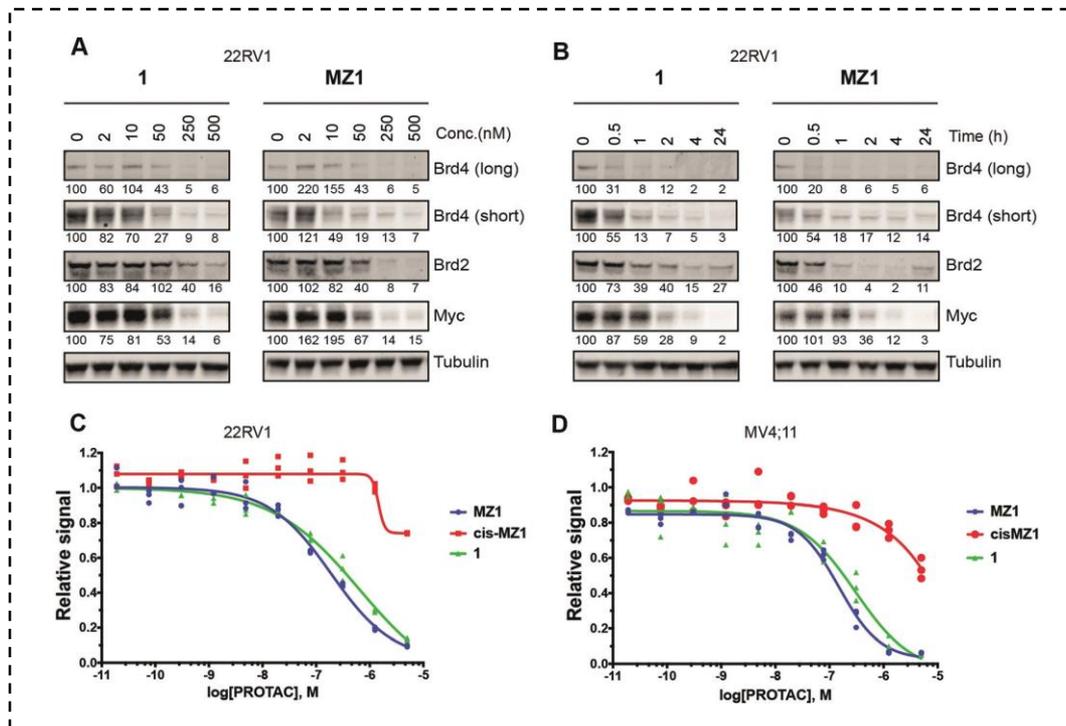
- 蛋白-降解剂共晶结构



$\alpha$ : 协同结合因子, 越大, 结合力越强

- macro-1对BD2结合选择性远大于BD1
- macro-1对BRD4BD2的亲合力低于MZ1

Kd: Kd值越小, 亲和力越强



- MacroPROTAC-1 显著降解 Brd4，抑制肿瘤细胞增殖，活性与MZ1相似。

## 2.3.2

## 精细调控策略——逐步调控

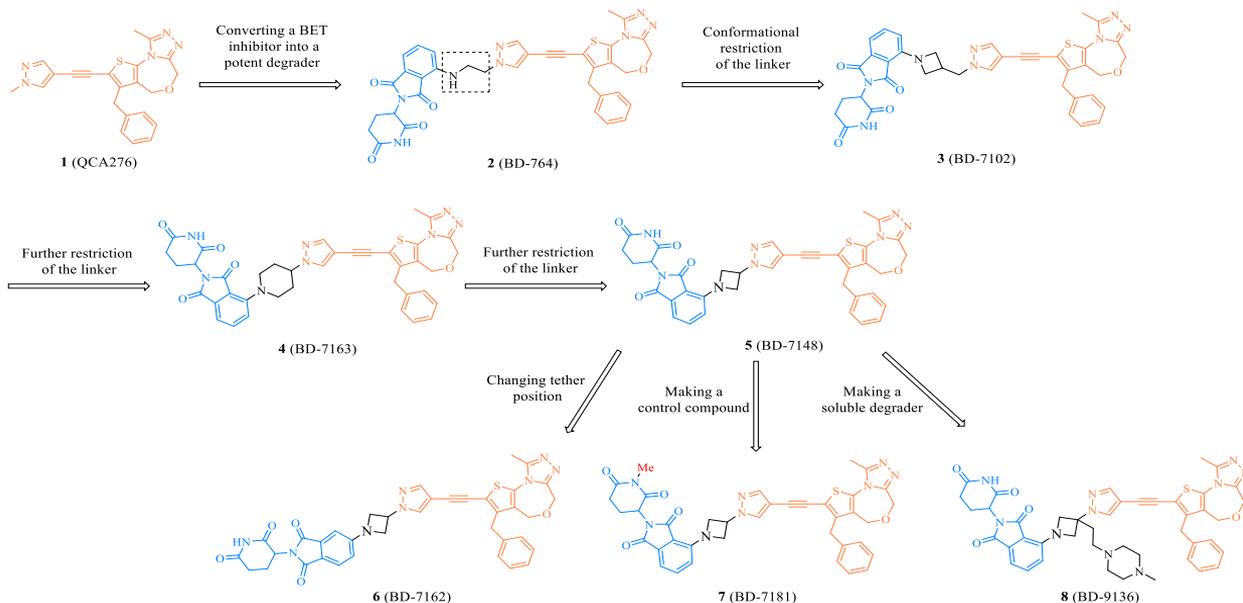


Table 1 Degradation Potency and Selectivity of Compound 1-8

ID	MV4;11 cell (4 h treatment time)					
	BRD2		BRD3		BRD4	
	DC <sub>50</sub> (nm)	D <sub>max</sub> (%)	DC <sub>50</sub> (nm)	D <sub>max</sub> (%)	DC <sub>50</sub> (nm)	D <sub>max</sub> (%)
1 (QCA276)	>1000	0	>1000	0	>1000	0
2 (BD-764)	0.5	99	0.5	98	0.2	98
3 (BD-7102)	0.3	97	0.4	98	0.1	98
4 (BD-7163)	50	68	>100	56	0.4	98
5 (BD-7148)	>1000	0	>1000	0	0.9	99
6 (BD-7162)	0.2	99	0.7	99	0.3	98
7 (BD-7181)	>1000	5	>1000	12	>1000	2
8 (BD-9136)	>1000	0	>1000	0	1.2	99

DC<sub>50</sub>: 半降解活性浓度。值越小，降解能力越高

- 生物膜干涉 (BLI) 实验

Table 2 Binding affinities of BET inhibitor and degraders

Protein	binding affinities Kd (nM)			
	1 (QCA276)	2 (QCA764)	5 (BD-7148)	6 (BD-7162)
	BRD2 BD1	120±19	140±11	39±7.5
BRD2 BD2	680±210	350±15	230±25	110±19
BRD3 BD1	73±8.5	93±14	46±6.3	14±2.6
BRD3 BD2	180±37	160±56	26±4.1	26±8
BRD4 BD1	35±4.3	32±8.4	66±11	170±41
BRD4 BD2	94±31	71±9.5	240±38	210±8.5

数值越小，对蛋白亲和力越强

**RS4;11:** Human acute lymphocytic leukemia cells

**HL60:** Human promyelocytic leukemia cells

**MOLM13:** Human acute myeloid leukemia cells

在多种细胞系中都可以选择性降解BRD4

Table 3 Degradation Potency and Selectivity of 5 (BD-7148)

cells	BD-7148					
	BRD2		BRD3		BRD4	
	DC <sub>50</sub> (nm)	D <sub>max</sub> (%)	DC <sub>50</sub> (nm)	D <sub>max</sub> (%)	DC <sub>50</sub> (nm)	D <sub>max</sub> (%)
RS4;11	>1000	<10	>1000	6	0.2	93
HL60	>1000	10	>1000	18	3.6	93
MOLM13	>1000	27	>1000	13	13	92
MDA-MB-231	>1000	0	>1000	19	1.0	93
MDA-MB-453	>1000	18	>1000	5	5.6	95
MCF-7	>1000	0	>1000	34	3.5	90
T47D	>1000	0	>1000	22	0.2	95

数值越小，降解活性越强

**MDA-MB-231:** Human breast cancer cells

**MDA-MB-453:** Human breast cancer cells

**MCF-7:** Human breast cancer cells

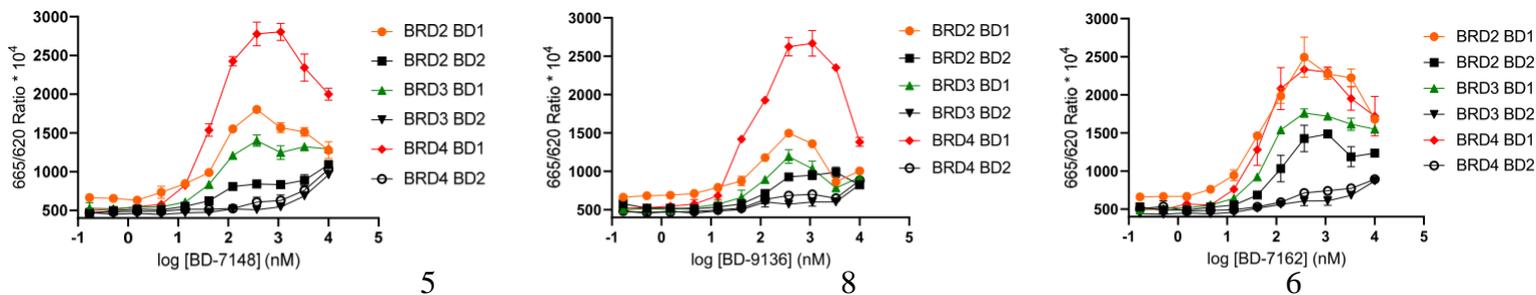
**T47D:** Human duct cancer cells

- 对BRD4的选择性与亲和性无直接联系

## 2.3.2

## 精细调控策略——选择性探究

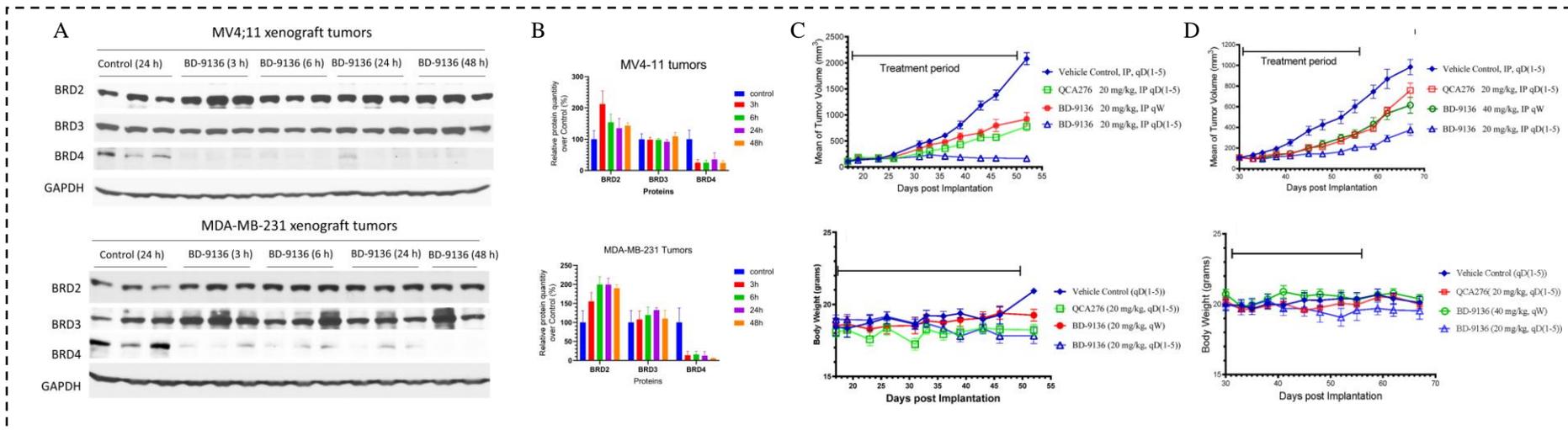
- 均质时间分辨荧光 (HTRF) 测定法



	BD-7148						BD-9136						BD-7162					
	BRD2 BD1	BRD3 BD1	BRD4 BD1	BRD2 BD2	BRD3 BD2	BRD4 BD2	BRD2 BD1	BRD3 BD1	BRD4 BD1	BRD2 BD2	BRD3 BD2	BRD4 BD2	BRD2 BD1	BRD3 BD1	BRD4 BD1	BRD2 BD2	BRD3 BD2	BRD4 BD2
Max Signal	1800	1400	2777	1091	959	1031	1498	1191	2667	987	830	882	2494	1761	2334	1487	871	893
Min Signal	664	527	500	457	473	471	663	503	520	520	458	463	660	493	494	487	484	476
$\Delta(\text{Max}-\text{Min})$	1136	873	2277	634	486	560	835	688	2147	467	372	419	1834	1268	1840	1000	387	417
Relative Amount of Complex (%)	50	38	100	28	21	25	39	32	100	22	17	20	100	69	100	54	21	23

- 对BRD4的降解选择性部分来自于形成三元复合物的优先性

- Pharmacodynamic analysis of BRDs proteins in the MV4;11 and MDA-MB-231 xenograft tumors in SCID mice



- BD-9136可以在两种移植瘤模型中选择性降解BRD4，抑制肿瘤增殖

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2.2 减小副作用的策略

2.3 提高选择性的策略

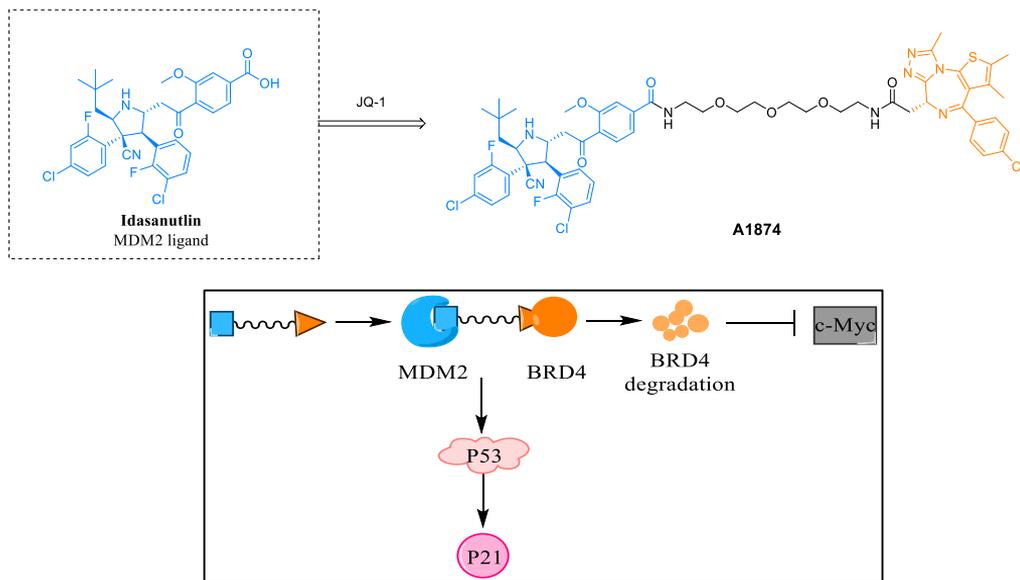
2.4 扩展E3连接酶工具

## 3. 总结展望

## 2.4.1

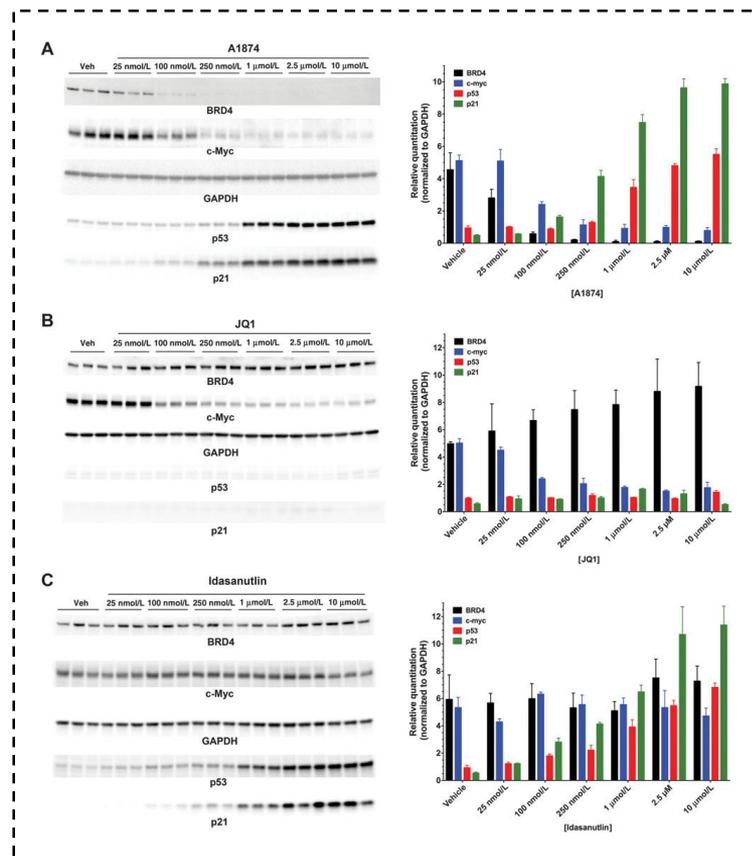
## 基于MDM2的BRD4-PROTAC

- Screening and evaluation of BRD4 degrader library



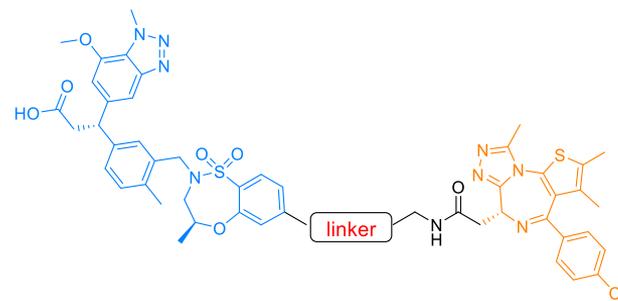
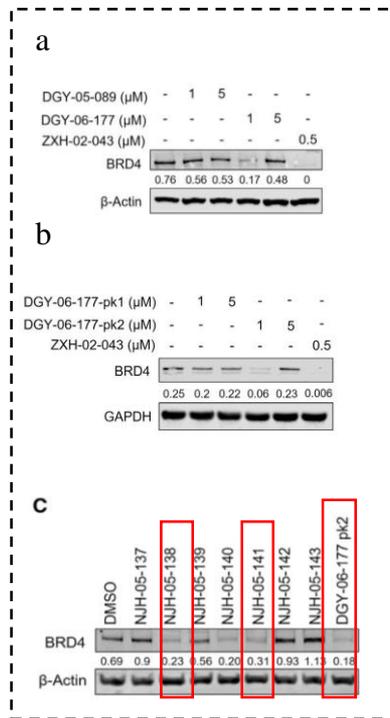
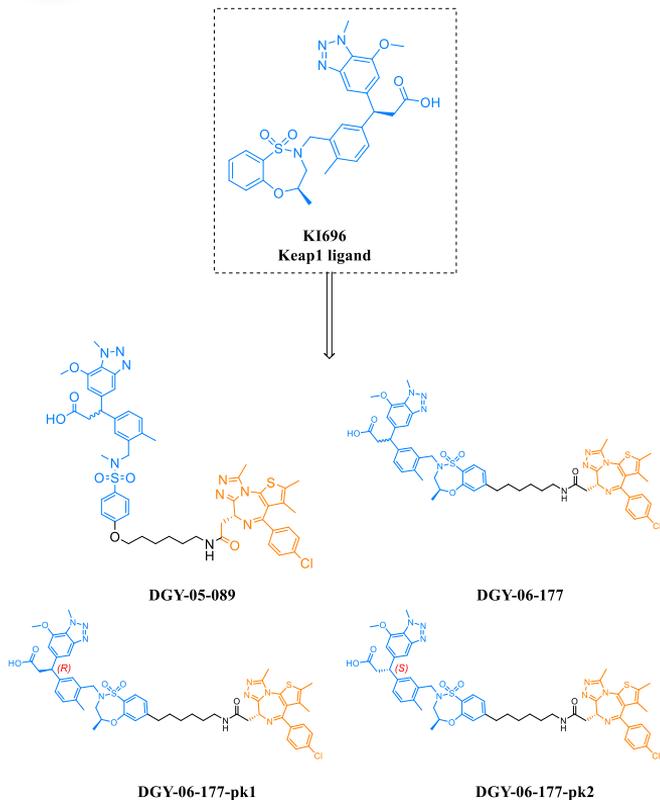
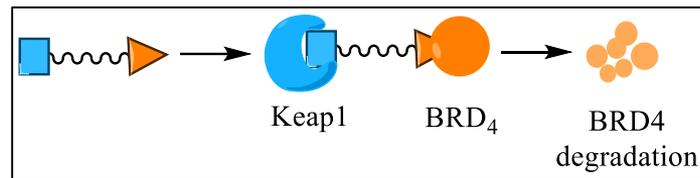
**MDM2:** mousedouble minute 2, is a E3 ligase and key negative regulator of p53  
**c-Myc:** c-Myc, a transcription factor, drives cell proliferation, downstream for MDM2  
**p53:** tumor suppressor p53, plays a crucial tumor suppressor role  
**p21:** the downstream gene for p53, a cell cycle suppressor protein

- 基于MDM2的A1874抗癌效果优于基于VHL and CRBN的PROTAC.



## 2.4.2

## 基于KEAP1的BRD4-PROTAC



	linker	R
NJH-05-137		H
NJH-05-138		H
NJH-05-139		H
DGY-06-177-pk2		H
NJH-05-141		H
NJH-05-142		H
NJH-05-143		H
NJH-05-146		Et

**KEAP1:** Kelch-like ECH-associated protein 1

a key regulator of the cellular response to oxidative and electrophilic stress 45

- Davies, T. et al. *J. Med. Chem.* **2016**, *59*, 3991–4006.
- Nathanael S. et al. *Cell Chem. Bio.* **2022**, *29*, 1470–1481.

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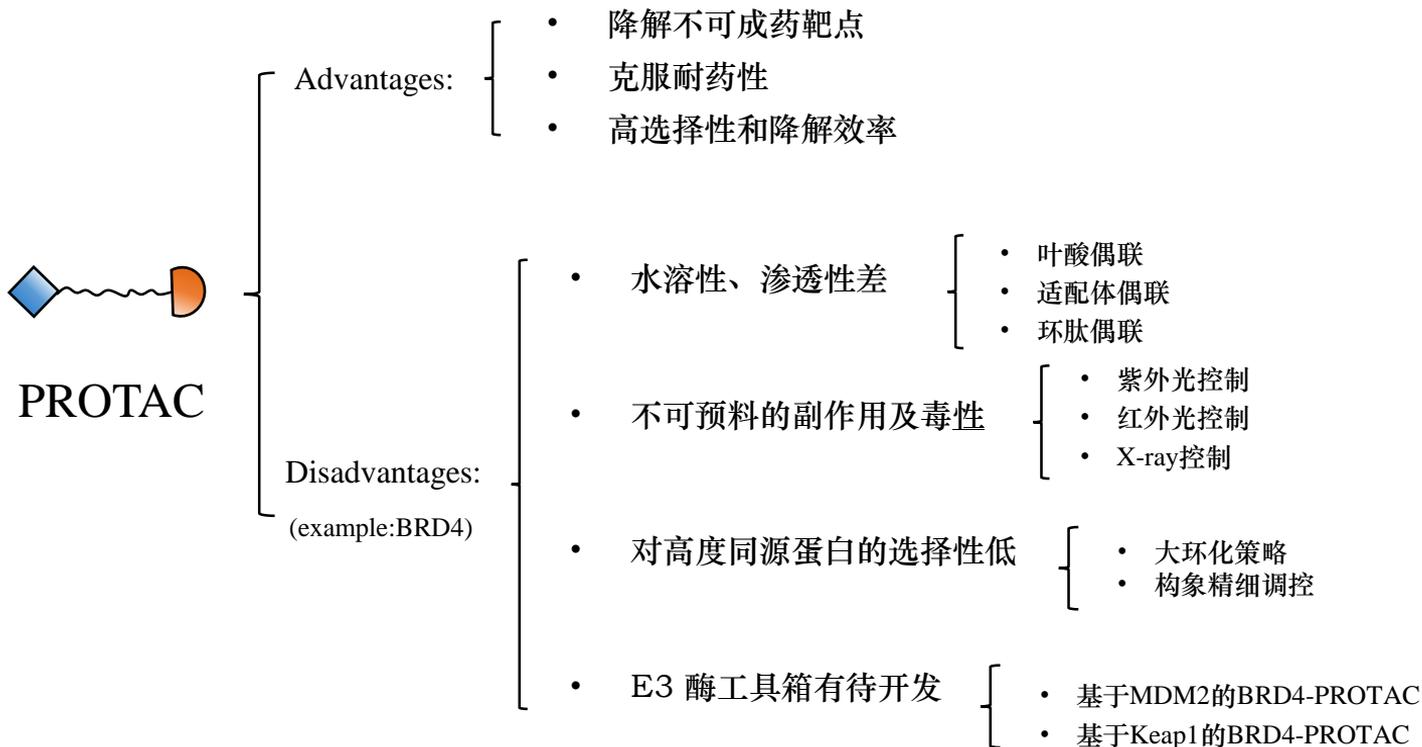
2.1 提高渗透性的策略

2.2 减小副作用的策略

2.3 提高选择性的策略

2.4 扩展E3连接酶工具

## 3. 总结展望



## 1 Protac的设计

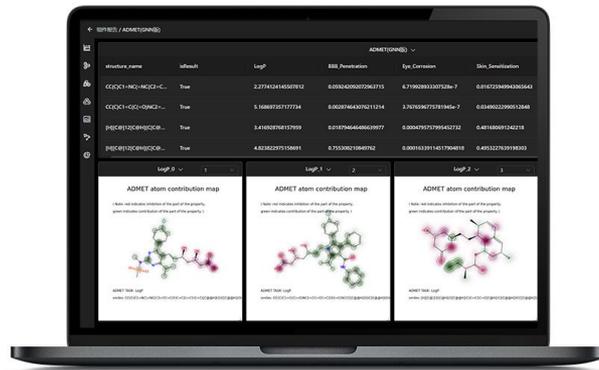
AIDD: Intelligent screening of linker's length, rigidity, binding sites, and affinity between ligands and different E3 ligases. (eg: X-Degrader™)

## 2 邻近诱导策略

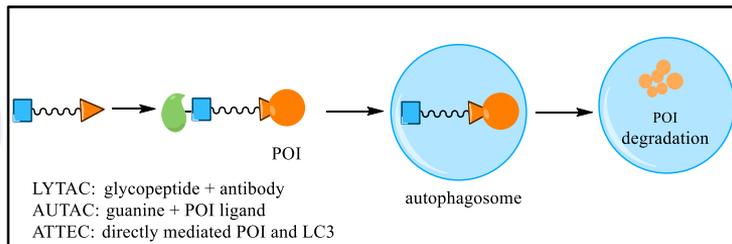
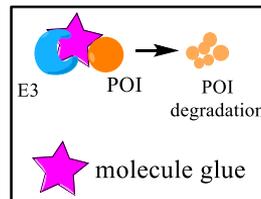
Molecular glue has a smaller molecular weight and better druggability, but it cannot be rationally designed for the target.

## 3 靶向溶酶体降解策略

Lysosome targeting chimeras (LYTACs, AUTACs, ATTECs, etc.) are already in development, but have not yet entered the clinical pipeline



- ✓ workflow引擎
- ✓ 微观结构搭建
- ✓ 分子模拟
- ✓ 机器学习-深度学习算法
- ✓ 实验设计
- ✓ APPs
- ✓ SaaS部署

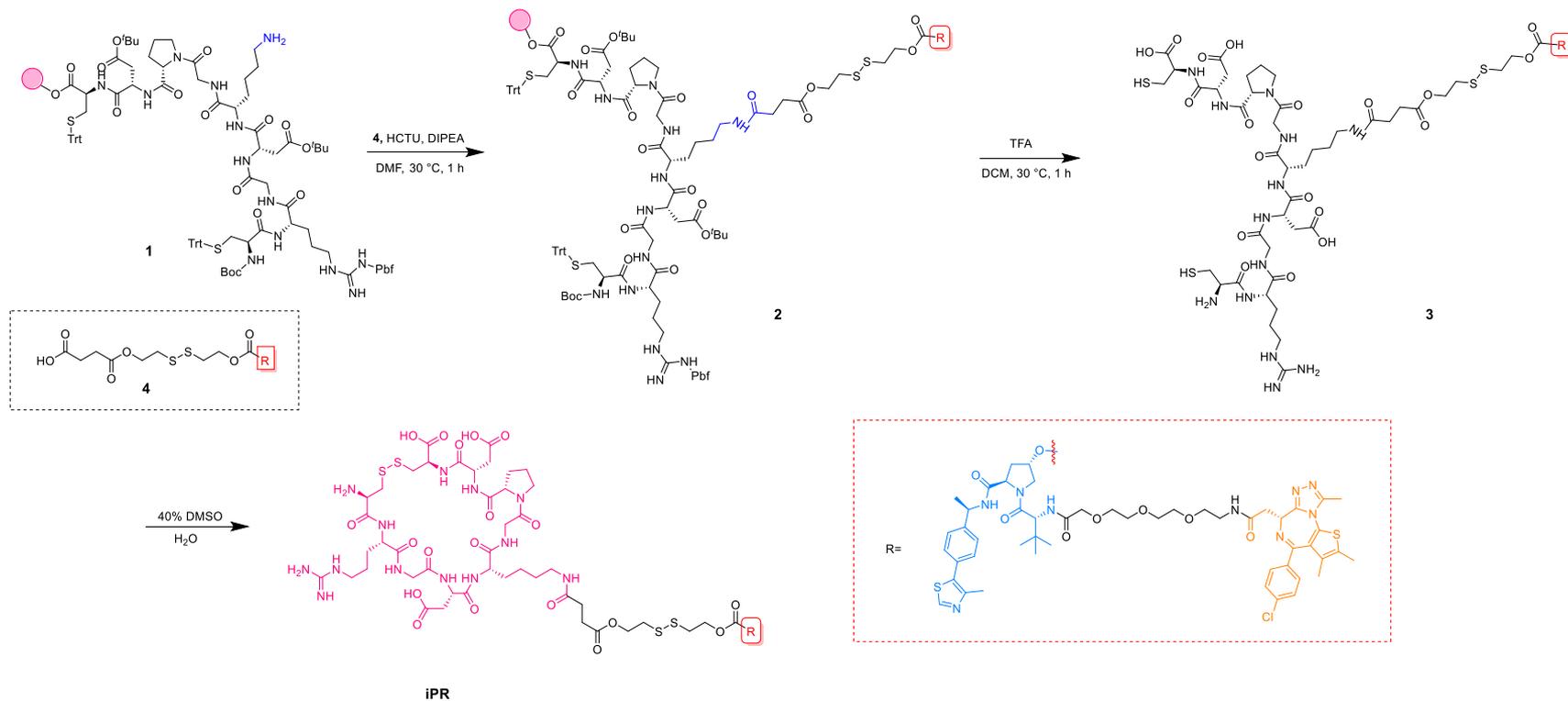




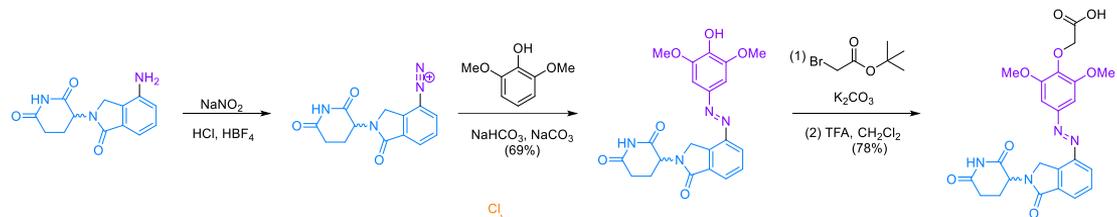
Thanks For Your Attention!

Wenqing Li  
2024.01.05

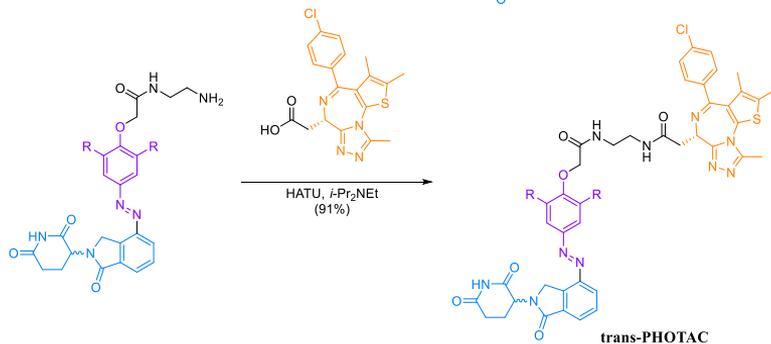
- The synthesis of iPR



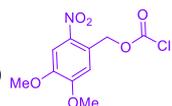
- The synthesis of PHOTAC and Opto-dBET1

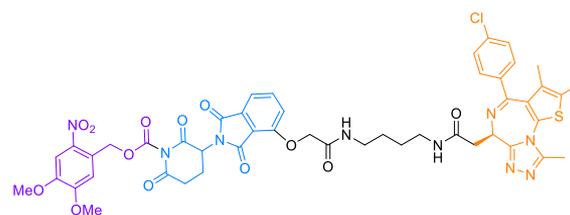
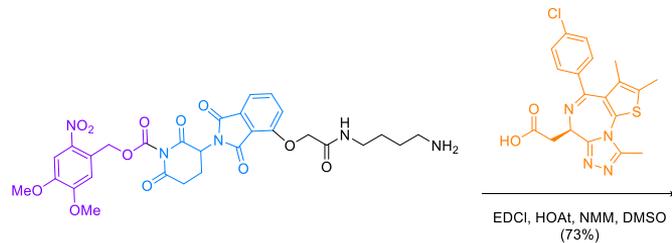


(1) HATU, *i*-Pr<sub>2</sub>NEt  
 $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH-Boc}$   
 (2) TFA  
 75%



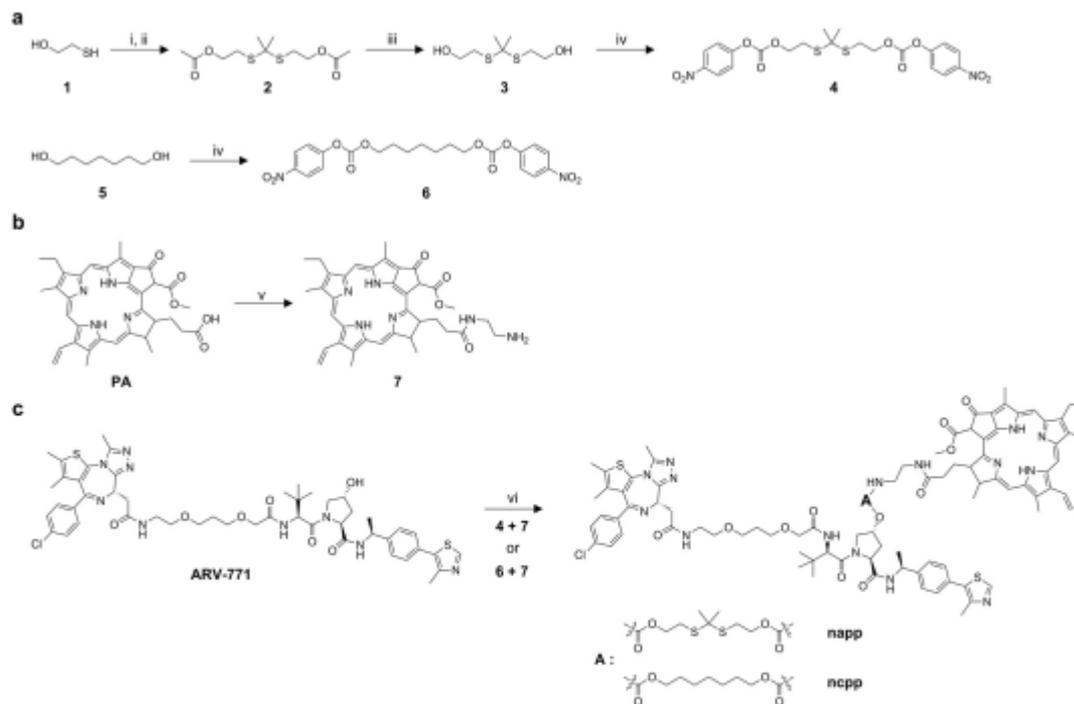
trans-PHOTAC

(1)   
 $\text{NaH}$ , DMF (47%)  
 (2) TFA,  $\text{CH}_2\text{Cl}_2$  (96%)



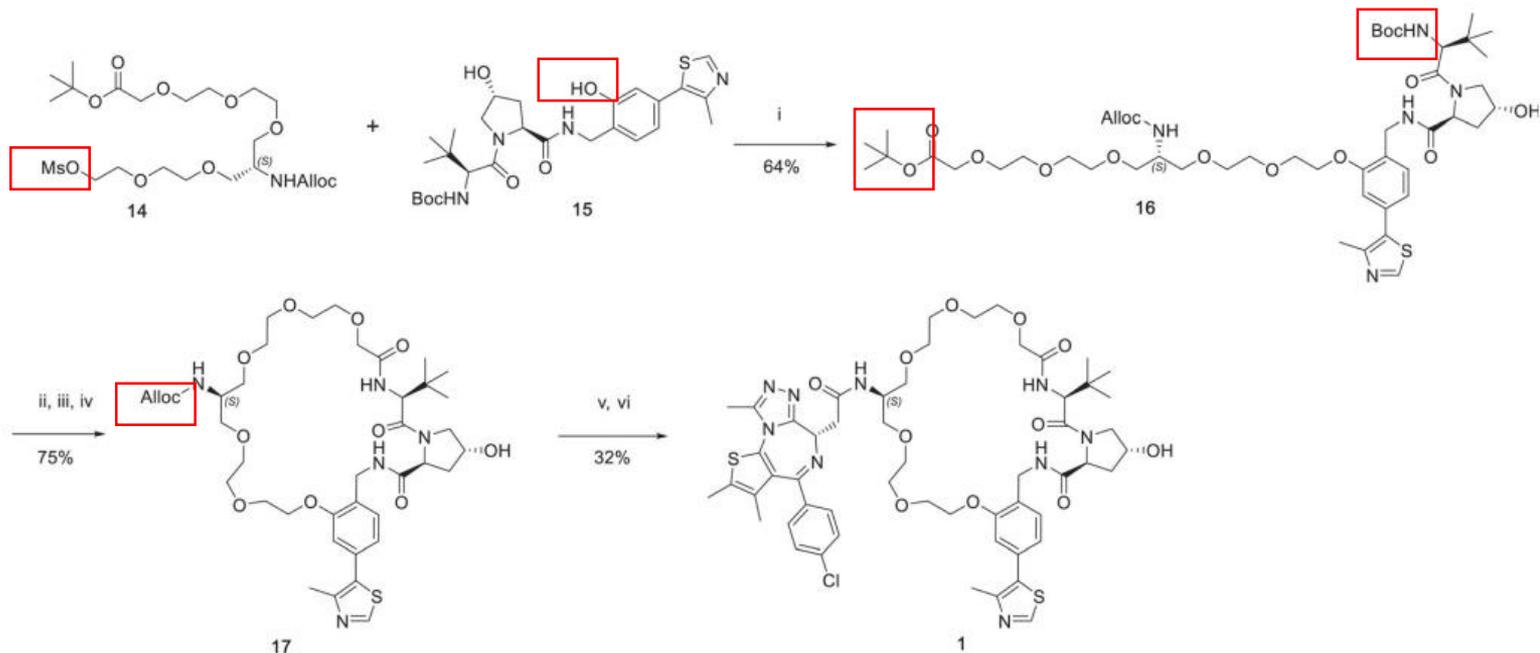
Opto-dBET1

- The synthesis of NAP and NCP



**Figure S1.** Synthesis of napp and ncpp. (a) i: KF, CH<sub>3</sub>COOH, 80 °C, 18 h; ii: acetone, TFA, room temperature, 24 h; iii: KOH, MeOH, room temperature, 16 h; iv: 4-nitrophenyl chloroformate, TEA, DCM, room temperature, 12 h; (b) v: N-Boc-ethylenediamine, HBTU, DIEA, DMF, room temperature, 12 h; 20% TFA/DCM, room temperature, 2 h; (c) vi: DMAP, TEA, DCM, room temperature, 24 h.

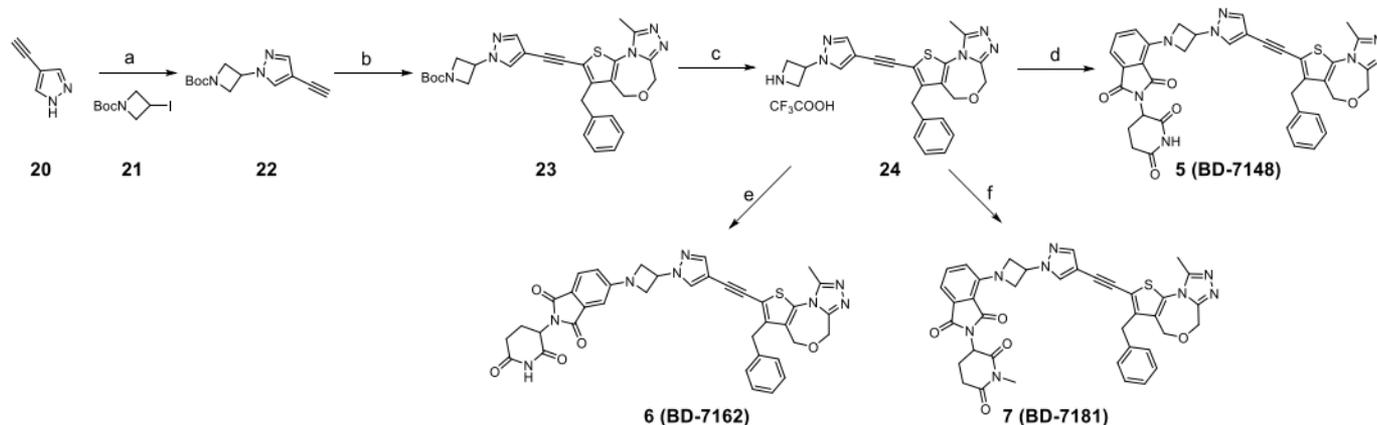
- The synthesis of macro-PROTAC



**Scheme 2.** Macrolactamization and completion of the synthesis of macroPROTAC-1. i)  $K_2CO_3$ , DMF; ii) TFA, DCM; iii) HCl,  $H_2O$ ; iv) HATU, DIPEA, DMF; v)  $Pd(PPh_3)_4$ ,  $PhSiH_3$ , THF; vi) JQ1-COOH, COMU, DIPEA, DMF.

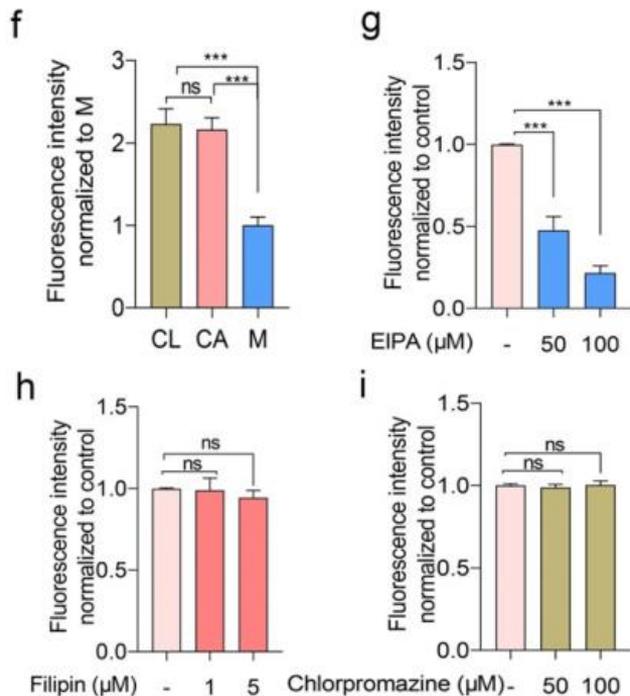
- The synthesis of BD-7158 BD-7162 and BD-7181.

Scheme 2. Synthesis of Degradar Compounds **5** (BD-7148), **6** (BD-7162), and **7** (BD-7181)<sup>a</sup>



<sup>a</sup>Reagents and conditions: (a)  $\text{Cs}_2\text{CO}_3$ , DMF, 80 °C, 12 h, 89%; (b) **19**,  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ , CuI, DMF/trimethylamine (TEA) (v/v 1:1), 80 °C, 5 h, 85%; (c) DCM/TFA (v/v 2:1), rt, 1 h, 82%; (d) 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione, DIPEA, DMF, 90 °C, 12 h, 75%; (e) 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione, DIPEA, DMF, 90 °C, 12 h, 79%; (f) 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione, DIPEA, DMF, 90 °C, 12 h, 83%.

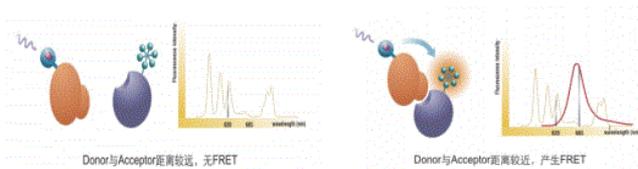
- 适配体策略——大胞饮



- 均质时间分辨荧光（HTRF）测定法

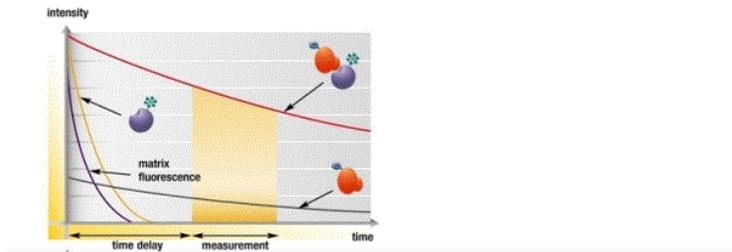
**荧光共振能量转移（FRET）**

利用两种荧光基团的能量转移，这两种荧光基团分别称为能量供体（Donor）和能量受体（Acceptor）。Donor 被外来光源激发（例如氙灯或激光），如果它与 Acceptor 比较接近，可以将能量共振转移到在 Acceptor 上，使其受到激发，发出特定波长的发射光。将 Donor 和 Acceptor 分别与相互作用的两个生物分子结合，生物分子的结合可以将 Donor 和 Acceptor 拉到足够近的距离，产生能量转移，由于 Acceptor 的发射光来自能量转移，所以实验中不需要将未结合与已结合的分子分开，即不需要洗涤步骤。

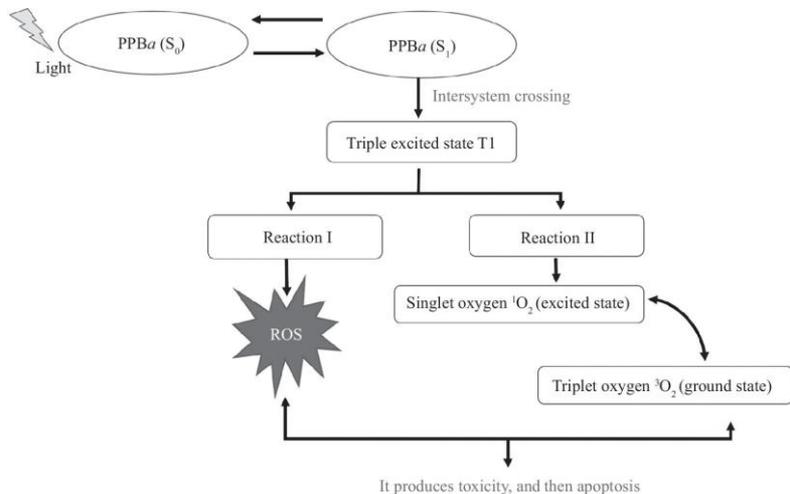


**时间分辨荧光（TRF）**

TRF 利用稀土元素中镧系元素的独特性质，它们与普通荧光的主要区别是荧光的持续时间不同。普通荧光的半衰期为纳秒级。镧系元素的半衰期为毫秒级，有 6 个数量级的差别。所以，在检测室，TRF 有一个时间延迟---50us，经过这个时间延迟，普通荧光的信号几乎为零，所以，TRF 的背景非常低，反映样品实际情况。



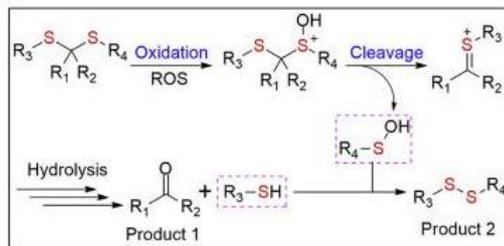
• 单线态氧的产生



适当波长光照下，光敏剂从单重态 $S_0$ 转变成激发态 $S_1$ ，通过系间窜跃形成激发三重态 $T_1$ 。三重激发态条件下，能量直接转移至基本态氧分子中，产生单线态氧。单线态氧具有极强的氧化性，氧化生物大分子引起脂质过氧化物酶失活、蛋白质变性、交联和结构变化，造成靶向组织或部位损伤甚至直接死亡。

• 缩硫醇活性氧响应机理

Oxidative cleavage mechanism for thio-acetals/-ketals



**ROS sensitive thio-acetals/-ketals**

- Simple structure for facile synthesis
- Functional groups for materials construction
- Tunable sensitivity by substituent selection