CYBER AIDD

WEEKLY REPORT

Cyber-AIDD analysis of the of CBPD-268, An Orally Available CBP/p300 PROTAC degrader

(*Reference from: Journal of Medicinal Chemistry, 2024, 67,7,5275-5304*)

CyberAIDD Analysis CBPD-268, an orally available CBP/p300 PROTAC degrader

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Part I

CBP/p300 is a key epigenetic regulator and a promising target for the treatment of castration-resistant prostate cancer and other types of cancer. This article reports the discovery and characterization of **CBPD-268**, an extremely potent and orally active PROTAC degrader of CBP/p300 protein. **CBPD-268** induces CBP/p300 degradation with DC_{50}≤0.03 nM in three androgen receptor-positive prostate cancer cells, D_{max}>95%, resulting in cancer cell growth inhibition. **CBPD-268** has excellent oral bioavailability in mice. Oral administration of 0.3-3 mg/kg of **CBPD- 268** results in sustained degradation of CBP/p300 in tumor tissues and in mouse VCaP and 22Rv1Strong anti-tumor activity in xenograft tumor models, including tumor regression in VCaP tumor models. **CBPD-268** is well tolerated in mice and rats and shows a therapeutic index >10. Taken together, **CBPD-268** is a potent CBP/p300 degrader that could serve as a potential new cancertherapy.

The CyberSAR System provides in-depth elucidation of CBP/p300 target molecules, and the system demonstrates the active molecules associated with the target through a clustered structure view and a raw structure view, presenting the potential in the form of a timeline of the R&D phase Hit 。 In addition, CyberSAR also provides visual analysis of indications and trial design, helping developers quickly obtain target structure information and develop research ideas. Although CyberSAR has not been used in the initial development of molecules, it has shown great potential for application in the elucidation and optimization of drug molecules.

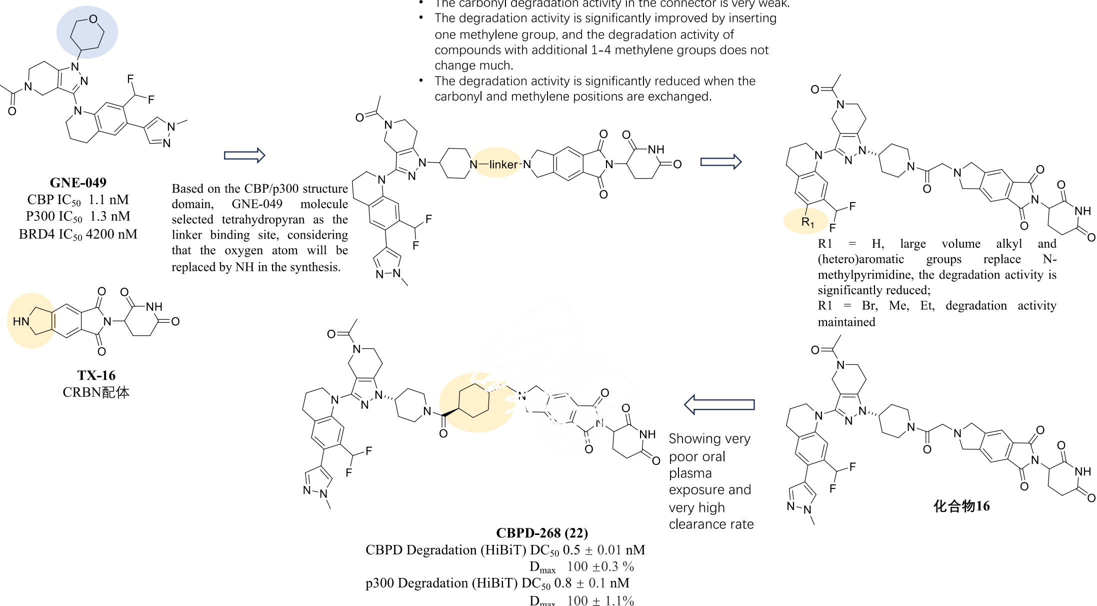

Figure 1CBPD-268 discovery and molecular optimization process

Journal of Medicinal Chemistry > Vol 67/Issue 7 > Article

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FEATURED ARTICLE | March 13, 2024

Discovery of CBPD-268 as an Exceptionally Potent and Orally Efficacious CBP/p300 PROTAC Degrader Capable of Achieving Tumor Regression

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Journal of Medicinal Chemistry
Cite this: J. Med. Chem. 2024, 67, 7, 5275−5304

https://doi.org/10.1021/acs.jmedchem.3c02124
Published March 13, 2024

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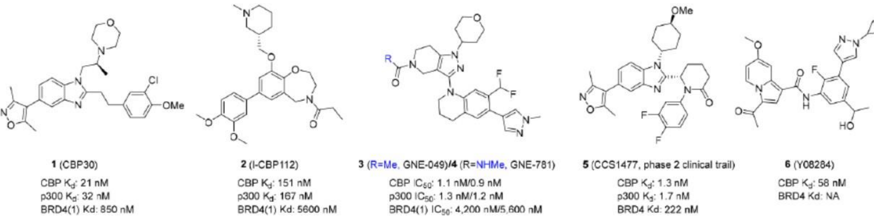
Part II

Prostate cancer remains the leading cause of cancer-related deaths in men worldwide. Activation of androgen receptors (ARs) drives prostate cancer development and growth. Abiraterone, which blocks androgen synthesis, and second-generation AR antagonists (such as enzalutamide and darolutamide) are used to treat prostate cancer, including metastatic trend-resistant prostate cancer (mCRPC). While these therapies are effective in treating mCRPC and improving patient survival, patients typically develop resistance within 18 months. AR signaling continues to play an important role in the development and resistance of patients who develop resistance to AR-targeted therapies. The progression of mCRPC is mainly due to alterations in AR signaling, including AR gene amplification, AR ligand binding domain (LBD). mutations and the expression of AR-V7 and other AR splice variants (AR-SV) are some of the main mechanisms of resistance. Since currently approved AR-targeted therapies are not effective in addressing these resistance mechanisms, there is a need to develop new therapies for prostate cancer, particularly mCRPC.

Histone acetyltransferase-binding protein (CBP) and its closely related homologous protein E1A-binding protein (p300), commonly referred to as CBP/p300, are: Key transcriptional coactivators for AR and other transcription factors. Increased CBP/p300 protein expression has been shown to be strongly associated with poor treatment outcomes and short survival in prostate cancer. It is considered to be another important resistance mechanism against currently approved AR- targeted therapies. Knockdown of CBP/p300 by siRNA or shRNA effectively inhibits AR and AR-SV signaling. With the use of a potent, selective, and orally available CBP/p300 bromodomain inhibitor **CCS1477** inhibition of CBP/p300 has been shown to be effective in reducing AR-, The expression of AR-SV-, c-Myc and their regulatory genes blocks the cell proliferation of AR+ prostate cancer cell lines, and expresses AR-SV Achieved robust in vivo antitumor activity in the 22Rv1 tumor model. CCS1477 is currently in a Phase II clinical trial in prostate cancer. Targeting CBP/p300 represents a new therapeutic strategy for the treatment of prostate cancer, especially mCRPC.

CBP/p300 is a macromolecular protein composed of multiple functional domains. In addition to the bromodomain (BRD), CBP/p300 includes a histone acetyltransferase (HAT) domain, a plant homology domain (PHD) refers to the domain, the nuclear receptor interaction domain (RID), and the kinase-induced CREB interaction domain (KIX), cysteine/histidine regions (TAZ1/CH1 and TAZ2/CH3), and interferon response binding domains (IBiD). A variety of CBP/p300 inhibitors have been identified, such as the bromodomain inhibitors **CBP30**, **ICBP112**, **GNE-049** and their analogues**GNE-781, CCS147717**, and **Y08284**, as well as the HAT domain inhibitor **C464**, **A-485**, **B026**, **DS-9300**, and **CPI-1612** (shown in Figure 1). The combination of bromodomain inhibitors and HAT domain inhibitors can synergistically inhibit the growth of prostate cancer cells. In addition, CBP/p300 mRNA levels in castration-resistant prostate cancer cell lines have been reported to be significantly upregulated after treatment with bromodomain inhibitors after treatment with bromodomain CCS1477 inhibitors, after treatment with bromodomain inhibitors. May affect the efficacy of CBP/p300 bromodomain inhibitors in the treatment of prostate cancer. Therefore, the authors hypothesize that direct degradation of CBP/p300 protein may result in a more complete inhibition of its biologically active function than inhibition of the bromodomain or HAT domain, resulting in stronger therapeutic efficacy against prostate cancer and other cancers.

CBP/p300 Bromodomain Inhibitors



CBP/p300 HAT domain Inhibitors

7 (C646) p300 K_i: 400 nM; 8 (A-485) CBP IC_{50}: 2.6 nM, p300 IC_{50}: 9.8 nM; 9 (B026) CBP IC_{50}: 9.5 nM, p300 IC_{50}: 1.8 nM; 10 (DS-9300) CBP IC_{50}: 22 nM, p300 IC_{50}: 28 nM; 11 (CPI-1612) p300 IC_{50}: 8.0 nM

CBP/p300 PROTAC Degrader

12 (dCBP-1); 13 (JQAD1); 14 (JET-209)

Figure 1. Structures for the representative CBP/p300 bromodomain and HAT domain inhibitors and CBP/p300 PRTOAC degraders.

In recent years, the use of proteolysis-targeting chimera (PROTAC) technology to induce protein degradation has emerged as a very attractive strategy for the development of novel therapeutics for the treatment of cancer. At present, more than 20 PROTAC degraders have entered clinical research and have broad clinical application prospects, such as **ARV-110** (an androgen receptor PROTAC degrader) and **ARV-471**, an estrogen receptor PROTAC degrader. The effective PROTAC degraders of CBP/p300 such as **dCBP-1**, **JQAD1** and **JET-209** (Figure 1) has been previously reported, and none of them are orally biologically active. Since PROTAC degraders are composed of two small molecule ligands linked by linkers, the molecular weight is typically 800-1200, which is much higher than that of traditional oral small molecules. Therefore, the design of orally active PROTAC degraders has been the main focus of pharmacochemistry, and orally bioavailable PROTAC degraders have recently reported a very limited number of targets, including AR and ER, BTK, and IRAK4, there is a strong need to develop potent and orally available CBP/p300 PROTAC degraders for clinical development purposes.

In this paper, we report on the CBP/p300 bromodomain inhibitor **GNE-049** and the new CRBN ligand **TX-16** developed in the author's lab Design, synthesis and biological evaluation of a series of novel CBP/p300 PROTAC degraders. **CBPD-286** was found to be an abnormally effective and orally effective CBP/p300 degrader. **CBPD-268** was able to induce deep degradation of CBP/p300 protein at picomolar concentrations in cells and potently inhibited cell growth in three AR+ advanced prostate cancer cell lines. Excellent oral bioavailability was achieved in mice and rats and exhibited a favorable overall pharmacokinetic (PK) profile. Oral administration of **CBPD-268** effectively abolishes CBP/p300 protein in mouse tumor tissues and achieves potent antitumor efficacy in a prostate cancer xenograft mouse model with a tolerated dose regimen.

Design of effective and orally administerable CBP/p300 degraders

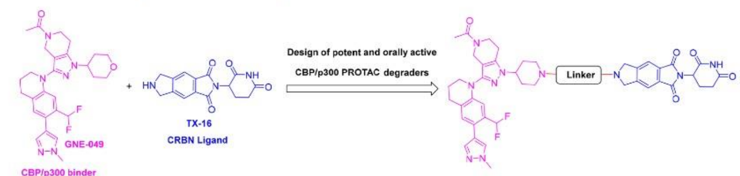
While many PROTAC degraders have been reported to be available for different protein targets, orally active PROTAC degraders are only implemented for a few targets, including ER, AR, BRD9, IRAK4, and BTK. To obtain an effective and orally active CBP/p300 PROTAC degrader, it is critical to select the appropriate E3 ligase ligand CBP/p300 ligand.

Since cereblon (CRBN) ligands have been successfully used in the development of orally active PROTAC degraders, the focus is on the design of oral activity using cereblon ligands PROTAC CBP/p300 degrader. In previous studies, an optimized cereblon ligand, **TX-16**, was developed, which has a similar binding affinity for cereblon compared to tothalidomide and lenalidomide, and exhibited good cell permeability and excellent PK properties. With **TX-16**, potent and orally active ER and AR PROTAC degraders have been successfully developed, which are ideal among different species PK characteristics. Therefore, in this study, the CRBN ligand **TX-16** was used to design an orally bioavailable CBP/p300 PROTAC degrader.

The authors selected the bromodomain ligand **GNE-049** as the CBP/p300 ligand for the design of an effective orally active CBP/p300 degrader for several reasons: **(1) GNE-049 is a highly potent CBP/p300 bromodomain inhibitor with $_{IC50}$ for both proteins= 1.1−1.3 nM； (2) GNE-049 exhibited excellent binding selectivity over other bromodomain proteins, including BRD4 protein, with more than 3,000-fold** selectivity**; (3) GNE-049 has excellent cell permeability and PK properties, including high oral bioavailability.**

The first set of potential CBP/p300 PROTAC degraders was designed and synthesized to determine the optimal joint length (Table 1). Based on the eutectic structure with the CBP bromodomain and **GNE049**, the oxygen in the tetrahydro-2H- pyran ring is exposed to the solvent and at a suitable location with the CRBN The ligand **TX-16** is bound by a linker. To facilitate the synthesis of potential CBP/p300 PROTAC degraders, oxygen pyrranophora was replaced with nitrogen, and a series of potential PROTAC degraders were synthesized using the resulting CBP/p300 ligands (Table 1). ）。

Table 1. Determination of the Optimal Linker Length[a]



Compound	Linker	CBP Degradation (HiBiT)		p300 Degradation (HiBiT)	
		DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)	D_{max} (%)
15 (CBPD-939)		>1000	20 ± 3.3	>1000	44 ± 1.4
16 (CBPD-1264)		20 ± 1.9	94 ± 3.0	7.9 ± 0.5	87 ± 0.0
17 (CBPD-1268)		>1000	32 ± 1.7	>1000	30 ± 2.6
18 (CBPD-1259)		>1000	38 ± 2.3	>1000	41 ± 0.3
19 (CBPD-1260)		368 ± 124	55 ± 3.7	105 ± 21	50 ± 4.9
20 (CBPD-908)		168 ± 44	58 ± 5.5	46 ± 6.7	74 ± 5.3
21 (CBPD-909)		362 ± 8.1	74 ± 1.9	108 ± 5.1	95 ± 4.3

[a]CBP/p300 degradation activity was tested using HiBiT assay with 24 h treatment time. DC_{50}, dose reduced CBP or p300 protein by 50%. D_{max}, maximal reduction of CBP or p300 protein achieved by the drug at up to 1 μM.

To quantitatively determine the degradation potency ($_{DC50}$) and efficiency (D) of the designed CBP/p300 PROTAC degrader $_{max}$), the authors employed CBP and p300 proteins in U2OS cell lines HiBiT degradation assay, in which CBP or p300 proteins are genetically modified by CRISPR/Cas9, will be HiBiT The tag is fused to its carboxyl terminus and processed for 24 h for CBP and p300 proteins in the HiBiT assay, The degradation potency and efficiency of all synthetic compounds were evaluated as shown in Table 1.

Compounds containing only carbonyl groups in the **linker15** have very weak CBP/p300 degradation potency (DC_{50}>1000). nM) and efficiency (D_{max} =20−44%). However, inserting only one methylene group into the linker of compound **15** yields compound **16**, which significantly increases the degradation potency (CBP and p300 with$_{DC50}$ of 20 nM and 7.9 nM, respectively) and efficiency ($_{DMX}$ = 94% and 87% for CBP and p300）。 Inverting the carbonyl group in compound 16 from the left side of the linker to the right side of the linker yields compound **17**, which is much weaker than **16**. Compound **18−21** was obtained by inserting an additional 1−4 methylene into the linker group of compound **16**, which had a positive effect on CBPand p300 were weaker than compound **16**.

The eutectic structure of **GNE-049** compounded with CBP indicates that the N-methylpyrazole group in **GNE-049** is located in CBP on the shallow hydrophobic surface pockets. The authors designed, synthesized, and evaluated a series of degraders in which the N-methylpyrazole group was replaced by various hydrophobic groups, as shown in Table 2。

Table 2. Modifications of the R1 Group[a]

Compound	R1	CBP Degradation (HiBiT)		p300 Degradation (HiBiT)	
		DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)	D_{max} (%)
16 (CBPD-1264)	*N*-Methylpyrazole-3-yl	20 ± 1.9	94 ± 3.0	7.9 ± 0.5	87 ± 0.0
22 (CBPD-236)	H	519 ± 52	67 ± 1.7	204 ± 30	74 ± 0.3
23 (CBPD-279)	Br	64 ± 18	84 ± 1.2	17 ± 0.3	86 ± 0.1
24 (CBPD-274)	Me	55 ± 2.1	91 ± 0.8	9.6 ± 1.2	94 ± 1.5
25 (CBPD-275)	Et	89 ± 4.7	91 ± 4.3	18 ± 7.5	93 ± 2.3
26 (CBPD-276)	*c*-Pr	52 ± 6.0	95 ± 0.5	29 ± 7.4	93 ± 0.1
27 (CBPD-278)	*i*-Pr	57 ± 0.1	85 ± 0.3	40 ± 3.2	89 ± 3.9
28 (CBPD-2117)	*c*-Hexyl	37 ± 5.2	93 ± 4.4	39 ± 2.9	87 ± 1.5
29 (CBPD-2118)	Phenyl	31 ± 0.0	94 ± 0.6	23 ± 2.6	93 ± 0.4
30 (CBPD-2119)	4-F-Phenyl	36 ± 2.0	88 ± 0.5	41 ± 7.2	86 ± 2.1
31 (CBPD-2120)	Thiophen-2-yl	24 ± 0.5	94 ± 0.9	33 ± 10	92 ± 0.6

[a]CBP/p300 degradation activity was tested using HiBiT assay with 24 h treatment time. DC_{50}, dose reduced CBP or p300 protein by 50%. D_{max}, maximal reduction of CBP or p300 protein achieved by the drug at up to 1 μM.

Substitution of the N-methylpyrazole group in compound **16** with hydrogen atoms generates compound **22**, which has a positive effect on CBP and p300 The degradation effect is much weaker and less effective than **16**. Compound **23** is obtained by substituting N-methylpyrazole with bromine atom, which is against

CBP and p300Quite potent degraders ($_{DC50}$ = 64nM for CBP and p300, Respectively and 17 nM, D_{max} = 84−86%)). Changing the bromine atom in compound **23** to methyl or ethyl groups produces compounds **24** and **25**, which are the same as compound **23**In comparison, their potency against CBP and p300 is similar to that of compound **23**, but based on their D_{max}The value is more valid than

23. Further substitution of the N-methylpyrazole group with a larger alkyl group or other (hetero)aryl substituent yields compounds **26-31**, which show DC_{50}Values range from 23 to 57 nM and D $_{max}$ values range from 85 to 95%. However, none of these modifications achieved an improvement in degradation potency compared to compound 16.

Of all these degraders in Table 2, compound **16** still exhibits the best degradation potency and efficiency. Next, the PK profile of compound **16** in rats was evaluated as shown in Table 3. Disappointingly, compound **16** showed very poor oral plasma exposure and very high clearance (Cl = 100.4 mL/min/kg). Since compound **22** has the lowest molecular weight of all compounds in Table 2, its PK in rats is also evaluated as shown in Table 3show. However, compound **22** still exhibits very poor oral plasma exposure and very high clearance (Cl = 194.0 mL/min/kg).

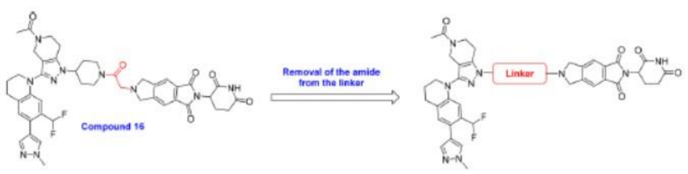
Table 3. Plasma Exposure in Rats and Liver Microsome Stability for Compounds 22 and 23

	Oral Plasma Exposure in Rats at 3 mg/kg (Plasma Drug Concentration at Different Time-Points, ng/mL)								
Compound	5 min	15 min	30 min	1 h	2 h	4 h	6 h	8 h	24 h
16 (CBPD-1264)	3.7	1.9	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	4.6
22 (CBPD-236)	1.0	0.5	0.4	0.3	0.4	0.7	0.9	BLQ	BLQ
	IV PK Profile in Rats at 1mg/kg						Liver Microsome Stability ($T_{1/2}$, min)		
Compound	$T_{1/2}$(min)		$AUC_{(0-t)}$ (h*ng/mL)	V_{ss} (L/kg)		Cl (mL/min/kg)	Human	Rat	Mouse
16 (CBPD-1264)	1.3		165.8	6.3		100.4	9.8	>60	19
22 (CBPD-236)	1.1		88.7	1.7		194.0	>60	>60	>60

After oral exposure, the metabolic stability of compounds **16** and **22** was tested in human, rat, and mouse liver microsomes. As shown in Table 3, compounds **16** and **22** exhibit good metabolic stability in rat liver microsomes ($T_{1/2}$>60 min), suggesting that the poor oral bioavailability of the two compounds was not due to their microsomal instability.

In order to improve the degradation potency of compound **16** and improve oral bioavailability, further modifications were made to the linker moiety. The amide groups in **the PROTAC** molecular linker **have been shown to result in low cell permeability and** poor **ADME properties.** Therefore, it is thought that the removal of amide groups from the linker of compound **16** can significantly improve its degradation potency and oral bioavailability. Therefore, compounds lacking amide groups in a series of linkers were designed, synthesized, and evaluated, as shown in Table 4.

Table 4



Compound	Linker	CBP Degradation (HiBiT)		p300 Degradation (HiBiT)		CBP Binding	p300 Binding	CRBN Binding
		DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (μM)
16 (CBPD-1264)		20 ± 1.9	94 ± 3.0	7.9 ± 0.5	87 ± 0.0	7.1 ± 1.0	7.7 ± 1.1	0.9 ± 0.04
32 (CBPD-290)		7.1 ± 1.3	85 ± 1.4	6.2 ± 0.1	91 ± 0.4	13 ± 1.0	13 ± 1.1	1.3 ± 0.05
33 (**CBPD-268**)		0.5 ± 0.01[b]	100 ± 0.3[b]	0.8 ± 0.1[b]	100 ± 1.1[b]	11 ± 1.1	9.5 ± 1.0	1.8 ± 0.00
34 (CBPD-266)		12 ± 3.5	62 ± 1.1	13 ± 0.3	82 ± 1.3	19 ± 1.1	18 ± 1.0	2.8 ± 0.14
35 (CBPD-289)		>1000	37 ± 0.9	>1000	40 ± 3.6	49 ± 1.1	44 ± 1.1	1.3 ± 0.01
36 (CBPD-240)		46 ± 0.4	67 ± 2.9	41 ± 0.7	72 ± 1.9	52 ± 1.1	35 ± 1.0	1.1 ± 0.01
37 (CBPD-216)		13 ± 0.1	61 ± 1.2	4.9 ± 0.5	77 ± 1.0	15 ± 1.1	13 ± 1.1	1.1 ± 0.03

[a]CBP/p300 degradation activity was tested using HiBiT assay with 24 h treatment time. DC_{50}, dose reduced CBP or p300 protein by 50%. D_{max}, maximal reduction of CBP or p300 protein achieved by the drug at up to 1 μM. IC_{50}, half-maximal inhibitory concentration. [b]Values are shown as mean ± SEM from $n = 3$ independent duplicate assays.

Compound **16** converts the amide group in compound **2** to the $CHCH_2$ group shows CBP3-fold increase in degradation potency (DC_{50}=7.1 nM vs.). 20nM), but the degradation efficiency of CBP decreased slightly (D_{max} = 94% vs. 85%)。 Compared to compound **16**, compound **32** showed a high degree of degradation potency at p300 (DC_{50} = 6.2 nM vs 7.9 nM) and efficiency (D_{max} = 91% vs 87%). Removing a methylene group from the linker of compound **32** yields compound **33** (**CBPD-268**, trans configuration), and compound **34** (**CBPD-266**, cis configuration). While Compound **34** is not as potent and effective as Compound **32** in reducing CBP and p300 protein levels, Compound **33**(**CBPD-268**) is more potent and effective than compound **32**. Specifically, **CBPD-268** achieves a DC_{50} of 0.5 nM for CBP and 0.8 nM for the DC_{50} of p300. In the HiBit assay, the concentration for p300 is 8 nM, and the D_{max} for both proteins is used is 100%. Therefore, **CBPD-268** is a highly efficient degrader of CBP and p300 proteins.

By extending the linker in **CBPD-268** by one more methylene, compound **35** is obtained, which is CBP and Very weak and ineffective degrader of P300. Moving the methylene group in the **CBPD-268** linker from the right to the left cyclohexyl group yields compound **36**, which reduces CBP and p300In terms of protein, it is much weaker and less effective than **CBPD-268**. Finally, methylene-forming compound **37**, which has a positive effect on CBP and p300, is removed from the **CBPD-268** linkerThe degradation of protein is also weaker and less effective than **CBPD-268**. In summary, the SAR data in Table 4 suggest that **linkers** play a crucial role in the degradation potency and efficiency of the designed **CBP/p300 PROTAC degrader.**

The binding affinity of **CBPD-268** and all other compounds to CBP/p300 and CRBN proteins was tested next, as shown in Table 4. **CBPD-268** exhibits strong binding affinity for both CBP and p300 with IC50 values: 11 and 9.5 nM. Although **CBPD-268** is more potent than compounds **35** and **36** bind to CBP/p300 4−5-fold, showing similarity to compounds **16, 32, 34** and **37**CBP/p300 binding affinity. In addition, **CBPD-268** has a similar CRBN binding affinity (IC50=1.8 μM) with all other compounds (IC_{50} =0.9− 2.8μM）。 **These data suggest that CBPD-268-induced CBP/p300 degradation is superior to other compounds, which may be attributed to the** formation **of** a **more stable and efficient ternary complex than other compounds.**

Further evaluation of **CBPD-268** in AR+ prostate cancer cell lines. **CBPD-268** (compound **33**) is all CBP/p300 evaluated in the HiBit degradation assay the most effective compound in degraders. Next, its ability to reduce CBP/p300 protein levels in AR+ prostate cancer cell lines were evaluated by traditional western blot analysis.

The 22Rv1 cell line is highly expressing the AR-V7 splice variant, which is resistant to current AR-targeted therapies and has been widely used as a CRPC Model. **CCS1477** is a CBP/p300 bromodomain inhibitor that has been shown to be effective in inhibiting tumor growth in vivo. Therefore, the data summarized in Figure 2 and Table 5 were first characterized as **CBPD-268** induced in the 22Rv1 cell line Ability of CBP/p300 protein degradation.

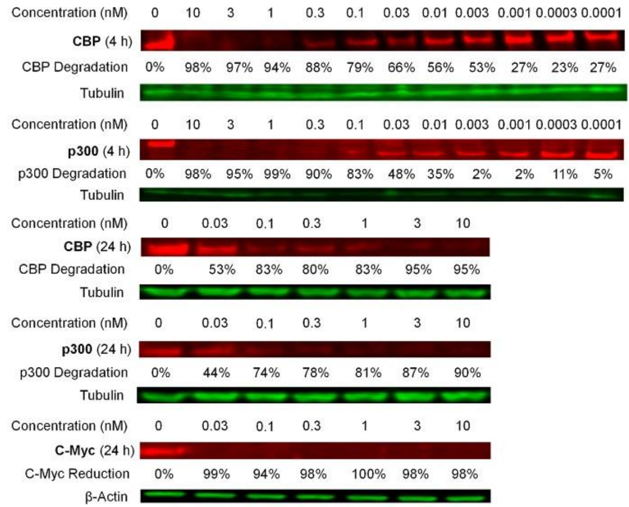


Figure 2. CBPD-268 promotes an exceptional potent and effective CBP/p300 degradation at both 4 and 24 h and induces an efficient reduction of C-Myc protein at 24 h in the 22Rv1 cell line.

CBPD-268

dispose22Rv1cel4

hours, got

targeted CBPD

C_{50} = 0.01 nM

and againstp300 DC_{50} = 0.03 nM

of superior potency, as well as againstCBPp300

high degradation efficiency of protein, D_{max} =98%

treat24 Hours later, **CBPD-268** Against CBP/p300

Protein realized<0.1 nM DC_{50} 90−95% D_{max}**CBPD-268** 24

The processing time of hours can also be efficiently reduced C-Myc protein can be as low as0.03 nM

The concentration of the next will CMyc

Decreased protein>95%。

Next, **CBPD-268** was evaluated to reduce LNCaP and VCaP in AR+ prostate cancer cell lines Ability of CBP/p300 protein levels. As mentioned above, the LNCaP cell line has a T878A mutation in the AR ligand-binding domain, and the VCaP cell line has The AR gene is amplified and therefore has very high levels of the AR protein. Together, these three AR+ prostate cancer cell lines represent three major and distinct mechanisms of resistance to current AR-targeted therapies caused by AR changes. The data of CBP/p300 degradation induced by **CBPD-268** within 4 hours in these three cell lines are shown in the table5.

Table 5. Summary of the Data for CBP/p300 Degradation Induced by CPBD-268 in Three AR+ Prostate Cancer Cell Lines within 4 h Treatment

	CBP Degradation		p300 Degradation	
Cell Line	DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)	D_{max} (%)
22RV1	0.01	98	0.03	98
LNCaP	0.01	98	0.03	98
VCaP	0.02	99	<0.01	99

Similar results in the 22Rv1 cell line, **CBPD-268** is shown in LNCaP and VCaP the depletion of CBP/p300 protein in the cell line is also very efficient. In LNCaP cell lines, DC50 of **CBPD-268** for CBP/p300 protein 0.01−0.03 nM with 98% D_{max}

。 In VCaP cell lines, DC_{50} of CBP and p3000.02 and <0.01 nM, CBP and p300 proteins, respectively D_{max} is 99%.

Next, blocking experiments were performed to investigate **the CBPD-268-induced** mechanism of CBP/p300 degradation, such as: Figure 3. Add the CBP/p300 inhibitor **GNE-049** (10 μM) or CRBN The inhibitor **TX-16** (10 μM) completely abolishes CBP/p300 degradation. Induced by **CBPD-268**, it indicates that CBP/p300 and CRBN protein are required for CBP/p300 degradation.

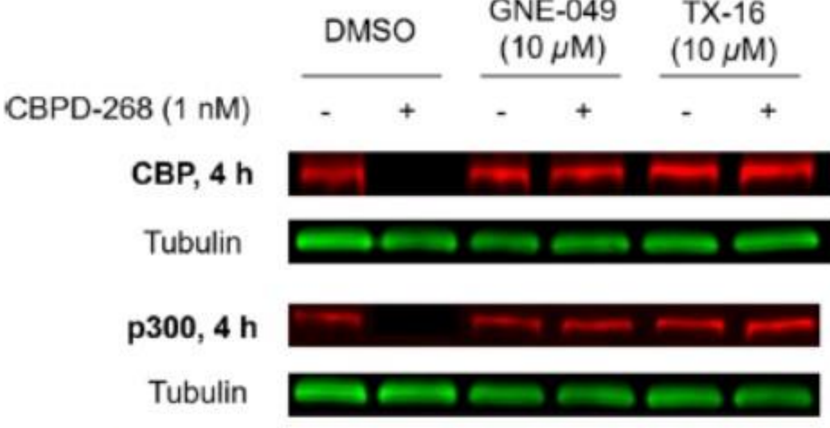
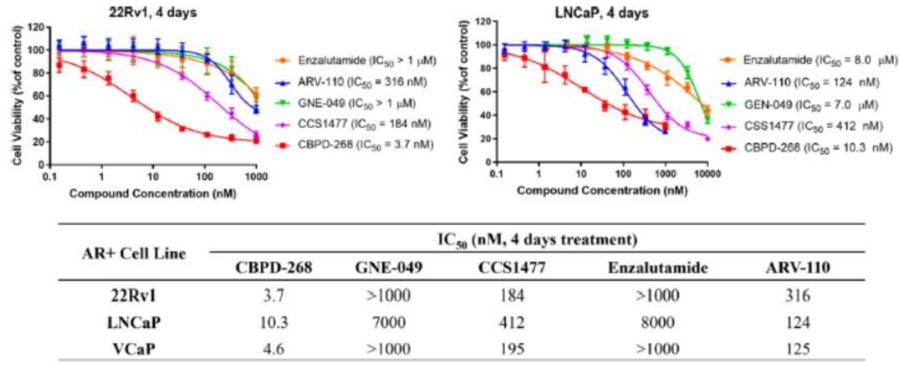


Figure 3. Investigation of the degradation mechanism of CBP/p300 proteins by CBPD-268 in the VCaP cell line.

To evaluate the cell growth inhibition effect of CBPD-268 on three AR+ prostate cancer cell lines

Next, two CBP/p300 bromodomain inhibitors (**GNE-049** and **CCS1477**), an AR PROTAC, are used A degrader (**ARV-110**) and an AR antagonist evaluated **CBPD-268** in AR+22Rv1, LNCaP and VCaP prostate cancer cell lines inhibited cell growth (**enzalutamide**) as controls. The data is shown in Figure 4.



AR+ Cell Line	IC_{50} (nM, 4 days treatment)				
	CBPD-268	GNE-049	CCS1477	Enzalutamide	ARV-110
22Rv1	3.7	>1000	184	>1000	316
LNCaP	10.3	7000	412	8000	124
VCaP	4.6	>1000	195	>1000	125

Figure 4. Summary of cell growth inhibition of **CBPD-268** and control compounds in three AR+ prostate cancer cell lines. Cells were treated for 4 days, and cell viability was determined by CellTiter-Glo luminescent assay.

CBPD-268 potently inhibits cell growth in all three cell lines, at 22Rv1, LNCaP, and VCaP $_{IC50}$ values in the cell line are 3.7 nM and 10.3 nM, respectively and 4.6 nM. In comparison, **GNE-049** is at 22Rv1, LNCaP, and VCaP$_{IC50}$ values in cell lines are >1 µM, 7 µM, and >1 µM, respectively, which are weaker than CBPD-268>250, >650, and >215, respectively Fold. Although **CCS1477** is more effective than **GNE-049**, it is still 40−50 times lower than **CBPD-268**. In addition, **CBPD-268 was more potent than CBPD-268** in the 22Rv1, LNCaP, and VCaP cell lines, respectively**ARV-110** is 85, 12 and 27 times stronger, **bienzalutamide**>250, 750 and >215 times stronger.

In western blot analysis, **CBPD-268** was very effective at inducing almost complete depletion of CBP/p300 protein in the 22Rv1 cell line, with only 4For hours of treatment, the DC_{50} for these two proteins was 0.01−0.03 nMThe DC_{90} value is <1nM (Figure 2). The cell growth inhibition assay showed that **CBPD-268** showed only anIC_{50} of 4 days of treatment3.7nM 。 To elucidate the difference between potency in CBP/p300 degradation and cell growth inhibition assays, the stability of **CBPD-268** in cell culture media used in the 22Rv1 cell growth inhibition assay was evaluated. The data suggest that **CBPD-268** is unstable in cell culture media; Residual <50% **CBPD-268** after 3 hours, % detected after 7 hours 3C10% **CBPD- 268** (Table S1). Perhaps not surprising in the cell culture instability of **CBPD-268**, as the glutarimide moiety in the cerablon ligand moiety has been shown to be unstable in the cell culture medium, which results in degrader inactivation. Thus, in a 4-day cell growth inhibition assay, **CBPD-268** showed a cell growth inhibition potency (IC) in these three AR+ cell lines$_{50}$) and efficiency ($_{Imax}$) are basically achieved within a few hours of their presence in the cell culture medium.

Overall proteome analysis of CBPD-268

Selectivity for degradation of **CBPD-268** at the global level, proteomic analysis of **CBPD-268** was performed in VCap cell lines. As in Western blotting analysis, **CBPD-268** was performed at 1 nM within a 4 inch VCap cell line The concentration is very efficient in almost completely depleting the CBP/p300 protein (Figure S1), so the VCap is treated with 1 nM CBPD-268Cells were subjected to proteome analysis for 4 h, as shown in Figure 5.

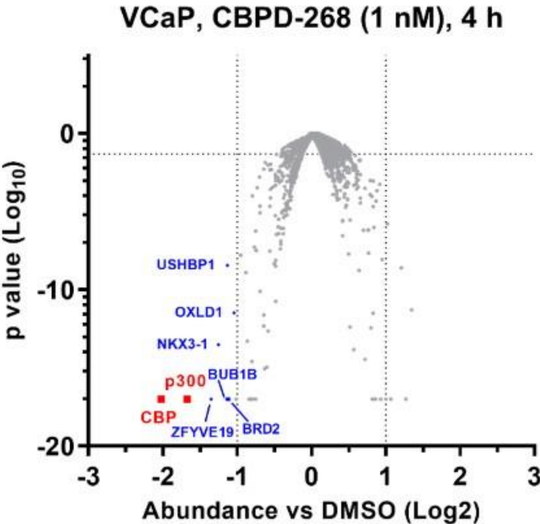


Figure 5. Multiplexed quantitative proteomics analysis (6535 proteins) after the treatment with 1 nM of CBPD-268 in the VCaP cells for 4 h. *P* value: two-sided Student's *t*-test. The data were provided as the representative of three biological replicates. Proteins with *P* values less than 0.05 (*y* axis) and fold decreases greater than 2 (*x* axis) are colored in blue or red.

Overall proteomic analysis showed that CBPD-268 showed depth and significant depletion of CBP/p300 protein, CBPand p300 protein levels were reduced by 75% and 69%, respectively. Several other proteins, including NKX3-1, ZFYVE19, BUB1B, OXLD1, USHBP1 and BRD2, also with modest reductions. NKX3-1 is an AR target gene, and its expression is co-regulated by CBP/p300. The significance of the reduction of other proteins needs to be further studied. In addition, GSPT1, one of the previously reported new substrates for CRBN-based PROTAC degraders, was not treated by **CBPD-268 in proteomics studies** reduction, which was further confirmed by Western blot analysis (Figure S1). As a result, **CBPD-268** reduced the levels of only a few proteins, with CBP/p300 protein being the most reduced of the total 6535 proteins analyzed.

Pharmacokinetic study of CBPD-268

The pharmacokinetics (PK) of **CBPD-268** in rats and mice were evaluated as shown in Table 6。

In rats, **CBPD-268** has half-lives of 1.9 and 1.3 h for intravenous and oral administration, respectively, with a good steady-state distribution volume (Vss = 4.9 L/kg), moderate clearance (Cl = 34.6 mL/min/kg), and at a high dose of 3 mg/kg, oral plasma-exposed C_{max} and AUC were 220.6 ng/mL and 936.9 h*ng/mL, respectively and has excellent overall oral bioavailability (F=67%).

In mice, **CBPD-268** also exhibited desirable PK characteristics when administered intravenously ($T_{1/2}$ = 3.4 h) and oral administration ($T_{1/2}$ =3.1h) both have good half-lives and moderate steady-state volume of distribution (Vss =1.6 L)/kg), low clearance (Cl = 6.0 mL/min/kg), and good oral plasma exposure at a dose of 3 mg/kg (C_{max}=724.7 ng/mL, AUC=4190.4 h*ng/mL), as well as oral bioavailability (F=60%.）。

Table 6. PK Profile of CBPD-268 in Rats and Mice[a]

Species	IV (mg/kg)	$T_{1/2}$[b] (h)	V_{ss}[b] (L/kg)	Cl[b] (mL/min/kg)	PO (mg/kg)	$T_{1/2}$[c] (h)	C_{max}[c] (ng/mL)	AUC[c] (h*ng/mL)	F[c] (%)
Rats	1	1.9	4.9	34.6	3	1.3	220.6	936.9	67
Mice	1	3.4	1.6	6.0	3	3.1	724.7	4190.4	60

[a]The definitions are as follow: IV, intravenous administration; PO, oral administration; $T_{1/2}$, elimination half-life; AUC, area-under-the-curve; V_{ss}, volume of distribution at steady state; Cl, clearance; C_{max}, maximum drug concentration; *F*, oral bioavailability; [b]IV. [c]PO.

In vitro DMPK and safety analysis of CBPD-268

The metabolic stability, plasma stability, plasma protein binding, and sensitivity of **CBPD-268** to human ether-a-go-go-related genes (hERG) in liver microsomes were evaluated Inhibition of ion channels and cytochrome P450 enzymes (CYPs) as shown in Table 7show.

Table 7. Metabolic Stability, Plasma Protein Binding, and Safety Profiling for CBPD-268

Liver Microsomal Stability $T_{1/2}$ (min)					Plasma Stability $T_{1/2}$ (min)				
Human	Monkey	Dog	Rat	Mouse	Human	Monkey	Dog	Rat	Mouse
48	30	49	65	>120	71	>120	78	48	>120

Plasma Protein Binding (%)				hERG Inhibition	CYP Inhibition IC_{50} (μM)		
Human	Dog	Monkey	Mouse	IC50 (mM)	1A2/2B6/2C9/2C19/2D6	3A4 (Midazolam)	3A4 (Testosterone)
99.1	99.4	99.4	98.4	>30	>10	>10	>10

CBPD-268 exhibits excellent metabolic stability in mouse liver microsomes (>120 min) and in humans ($T_{1/2}$ = 48 min), monkey ($T_{1/2}$ = 30 min), dog ($T_{1/2}$ =49 min) and showed reasonable metabolic stability. $T_{1/2}$ = 65 min) liver microsomes. In

humans ($T_{1/2}$ = 71 min), monkeys ($T_{1/2}$% 3E120 min), canines ($T_{1/2}$ = 78 min) and mice ($T_{1/2}$>120min) but moderately stable in rat plasma ($T_{1/2}$ = 48min). **CBPD-268 has high plasma protein binding in human, canine, monkey, and mouse at free drug concentrations of 0.6−1.6%, which is common for orally bioavailable PROTAC molecules. CBPD-268** is effective against hERG at concentrations up to 30 μMIon channels did not exhibit any significant inhibition and, at concentrations up to 10 μM, were not effective against all evaluated CYP isoforms (3A4, 1A2, 2B6, 2C9, 2C19, and 2D6) without significant inhibitory.

Pharmacokinetic/pharmacodynamic evaluation of CBPD-268 in VCaP and 22Rv1 xenograft mouse models

The ability of **CBPD-268** to reduce CBP/p300 protein levels in VCaP xenograft tumors was evaluated as: Figure 6.

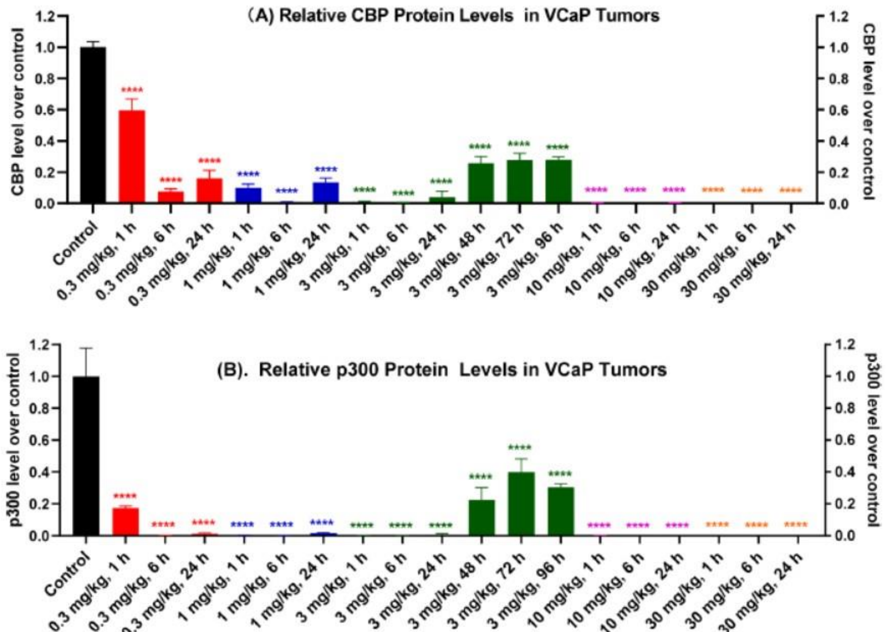


Figure 6. Pharmacodynamic evaluation of **CBPD-268** in the VCaP xenograft mouse model. Tumor-bearing SCID mice were administered with vehicle control or **CBPD-268** (0.3, 1, 3, 10, or 30 mg/kg) via oral gavage using 100% PEG200 as the dosing vehicle. Mice were sacrificed at each time point (1 h, 6 h, 24 h, 48 h, 72 h, or 96 h), and tumor tissues were harvested for analysis. Tumor tissue was analyzed by Western blot for CBP and p300 proteins with tubulin used as the loading control. Each group consisted of 3 mice/tumors. (A) Relative CBP protein levels in VCaP tumors. (B) Relative p300 protein levels in VCaP Tumors. A method of one-way ANOVA was used for calculating *P* value. ****, $P \leq 0.0001$.

CBPD-268 induces the depletion of CBP and p300 proteins in VCaP tumor tissues in a very efficient manner in a dose-dependent manner. A single oral dose of 0.3 mg/kg **CBPD-268** at 1, 6, and 24Hour time points reduced CBP protein levels by 40%, 92%, and 84%, respectively, and resultedP300 protein decreased by 83%, 100%, and 1, 6 The 24-hour time points were 99%. A single oral dose of 1 mg/kg **CBPD-268** is capable of 1, 6 and 24The hourly time points decreased CBP protein water by 90%, 100%, and 87%, respectively, at 1, The 6-hour and 24-hour time points reduced p300 protein levels by ≥98% 。 Further doses of **CBPD-268** can be increased to 3, 10, or 30 mg/kg, as a single oral dose The 1, 6, and 24-hour time points were effective in reducing CBP and p300, respectively Protein level >95%.

As a single oral dose of 3mg/kg **CBPD-268** is highly effective in lowering CBP andp300 protein levels, thus further assessing the effects of **CBPD-268** at extended time points (48, 72, and96 hour time point) on CBP and p300 protein. The data showed that at the 48-96hour time point, a single oral dose of 3 mg/kg of **CBPD- 268**, CBP and: The level of p300 protein was reduced by 60-70%, demonstrating its long-term effect in vivo.

The concentrations of **CBPD-268** in plasma, tumor, liver, and spleen tissues of VCaP tumor-bearing mice at different doses and time points were determined as shown in Table 8show. A single oral administration of **CBPD-268** 0.3-3 mg/kg exhibits a dose- and time-dependent exposure pattern in plasma, tumor, liver, and spleen tissues. Interestingly, while the drug concentration in tumor tissue was lower at the time point of 0.3−3 mg/kg **CBPD-268** administered at 24 hours, CBP and The p300 protein remains effectively depleted for at least 24 hours, suggesting that transient exposure to **CBPD-268** in tumor tissues is sufficient to effectively reduce CBPand p300 protein levels for at least 24 hours.

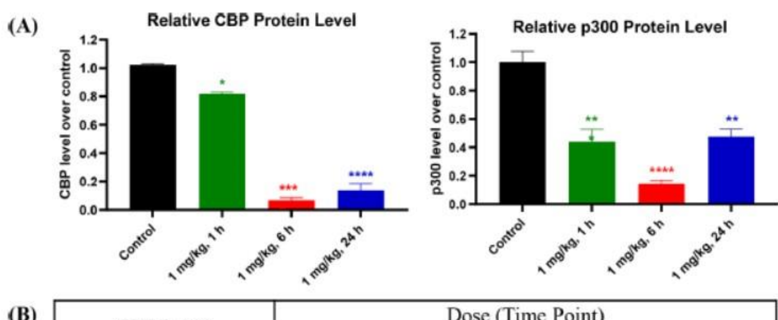
Table 8. Tissue Distribution (Mean ± SD) of CBPD-268 in VCaP Tumor-Bearing Mouse Model at 0.3, 1, and 3 mg/kg PO Doses[a]

PO Dose (mg/kg)	Time Point (h)	Plasma (ng/mL)	Tumor (ng/g)	Liver (ng/mL)	Spleen (ng/mL)
0.3	1	35.2 ± 11.5	27.8 ± 2.7	322.9 ± 33.8	91.0 ± 16.4
	6	12.7 ± 3.1	14.4 ± 3.5	86.8 ± 23.0	27.4 ± 3.3
	24	BLQ	BLQ	5.6 ± 0.4	BLQ
1	1	212.5 ± 55.0	154.9 ± 32.7	1643.4 ± 240.0	446.3 ± 21.9
	6	43.2 ± 20.4	64.3 ± 24.9	301.2 ± 85.7	126.8 ± 70.3
	24	3.3 ± 3.2	BLQ5	13.7 ± 7.3	2.8 ± 0.2
3	1	746.7 ± 826.1	249.0 ± 60.8	3295.0 ± 826.1	936.7 ± 260.8
	6	763.3 ± 384.3	293.5 ± 58.1	1753.3 ± 384.3	738.3 ± 176.1
	24	12.7 ± 69.7	12.0 ± 27.8	61.1 ± 69.7	21.2 ± 22.4
	48	BLQ	BLQ	BLQ	BLQ

[a]BLQ, below the limit of quantification.

Next, **CBPD-268** eliminated CBP/p300 in 22Rv1 xenograft tumor tissue from mice was evaluated using the data summarized in Figure 7The ability of the protein. **Concentrations of CBPD-268** in plasma and tumor tissues were also determined at each time point, which is also summarized in Figure 7.

(A)



(B)

CPBD-268	Dose (Time Point)		
	1 mg/kg (1 h)	1 mg/kg (6 h)	1 mg/kg (24 h)
CBP Protein Reduction over Vehicle Control	18 ± 12%	93 ± 2%	86 ± 5%
p300 Protein Reduction over Vehicle Control	56 ± 12%	86 ± 3%	53 ± 8%

(C)

PO Dose (mg/kg)	Time point (h)	**CPBD-268** Concentration	
		Plasma (ng/ml)	Tumor (ng/g)
		Mean ± SD	Mean ± SD
1	1	140.8 ± 27.5	30.8 ± 6.4
	6	14.1 ± 11.9	12.6 ± 3.2
	24	2.8 ± 2.1	6.3 ± 0.4

Figure 7. PK/PD evaluation of **CBPD-268** in the 22Rv1 xenograft mouse model. Tumor-bearing SCID mice were administered with vehicle control or **CBPD-268** (1 mg/kg) via oral gavage using 100% PEG200 as the dosing vehicle. Mice were sacrificed at each time point (1 h, 6 h, or 24 h), and blood samples and tumor tissues were harvested for analysis. Tumor tissue was analyzed by Western blot for CBP and p300 proteins with tubulin used as the loading control. Each group consisted of 3 mice/tumors. (A) Relative levels of CBP/p300 proteins at 1 h, 6 h, 24 h time-points in 22Rv1 tumors. A method of one-way ANOVA was used for calculating P value. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. (B) Summary of the CBP/p300 degradation data. (C) Concentration of CBPD-268 in plasma and tumors at 1, 6, and 24 h time-points.

CBPD-268 can effectively reduce the levels of CBP and p300 protein in 22Rv1 tumor tissues

A single oral dose of 1mg/kg of **CBPD-268** was administered at 1, 6 and The 24-hour time points reduced CBP protein levels by 18%, 93%, and 86%, respectively and reduced p300 protein levels by 56%, 86% and 53%, respectively1, 6 and 24 hours. Interestingly, a 1 mg/kg dose of **CBPD-268** was used in 22Rv1 tumor tissue1The drug concentrations achieved at the 6 and 24 h time points were much lower than those obtained at the same dose in VCap tumor tissue. In contrast, the PD effect of 1 mg/kg of **CBPD-268** in 22Rv1 tumors (Fig. 7) is weaker than the PD effect in VCaP tumors (Fig5), which is associated with lower drug exposure in 22Rv1 tumor tissue (Figure 7) than VCaP Drug exposure in tumor tissue (Table 8) is consistent. 。

Since **CBPD-268** showed a modest reduction in BRD2 protein levels in in vitro proteomic studies (Figure 5), its effects on mice were evaluated In vivo effects of BRD2−4 protein in 22Rv1 xenograft tumor tissues. The data (Figure 8) showed that CBPD-268 at 1 and 3 mg/kg was administered orally and did not reduce BRD2 in 22Rv1 tumor tissue at the 6 and 24 h time points examined, BRD3 and BRD4 protein levels. Therefore, PD data showed that **CBPD-268** performed better than BRD2/3/4 for CBP/p300 protein Selectivity for in vivo degradation of proteins.

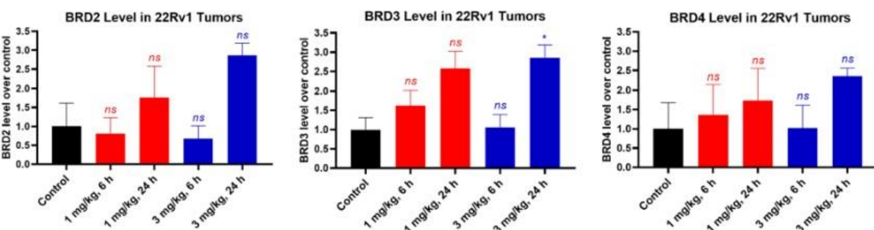


Figure 8. Analysis of BRD2−4 protein levels in the 22Rv1 tumors after the treatment with **CBPD-268**. Tumor-bearing SCID mice were administered with vehicle control or **CBPD-268** (1 or 3 mg/kg) via oral gavage using 100% PEG200 as the dosing vehicle. Mice were sacrificed at each time point (6 or 24 h), and tumor tissues were harvested for Western blot analysis for BRD2−4 proteins with β-actin used as the loading control. Each group consisted of 3 mice/tumors. A method of one-way ANOVA was used for calculating P value. ns, not significant, $P > 0.5$; *, $P \leq 0.5$.

Antitumor efficacy of CBPD-268 in VCap and 22Rv1 xenograft mouse models

Based on PD data, the antitumor activity of **CBPD-268** in a VCaP xenograft tumor model was first evaluated at 1 mg/kg and 3 mg/kg PO doses under different regimens, as shown in Figure 9.

CBPD-268 was administered orally at 1 mg/kg twice weekly or 3 mg/kg weekly at the end of 4 weeks of treatment (p48 days) inhibited tumor growth by 81% or 57% (Figure 9A). **CBPD-268** administered orally at 1 or 3 mg/kg three times a week, At the end of treatment (day 48), tumor regression resulted in 31% and 67%, respectively (Figure 9A). Due to the highly aggressive nature of VCaP tumors, any previously reported agents targeting AR or AR signaling (including AR degraders) are unable to achieve tumor regression. Therefore, **CBPD268** can highly effectively inhibit tumor growth and achieve tumor regression in VCaP tumor models. Importantly, mice tolerated **CBPD-268** treatment well, and less than 7% of body weight was lost in all dosing groups throughout the experimental period (Figure 9B).

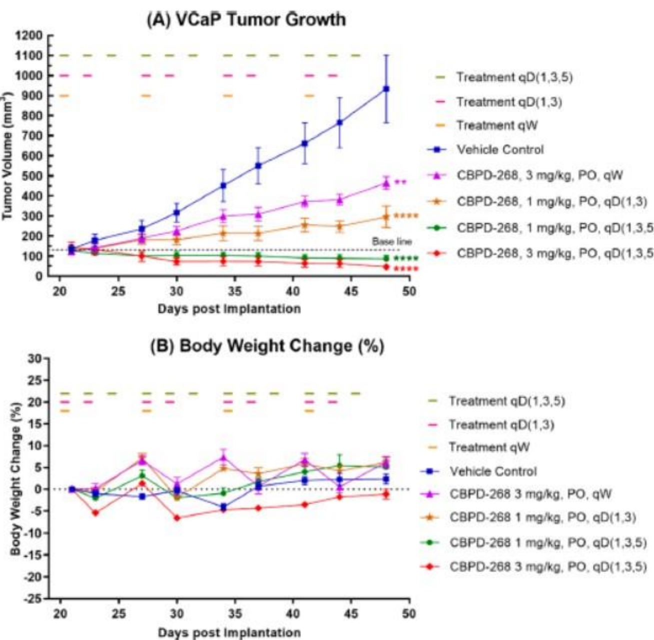


Figure 9. Antitumor efficacy of **CBPD-268** in VCaP xenograft mouse model. **CBPD-268** or vehicle control was administered to mice via oral gavage using 100% PEG200 as the dosing vehicle, with each group consisting of 7−8 mice. (A) Tumor growth for each group. A method of one-way ANOVA was used for calculating the statistical significance of the tumor volumes between groups. **, $P \leq 0.01$; ****, $P \leq 0.0001$. (B) Animal body weight change for each group.

Lower doses (0.3 mg/kg) were further evaluated in VCaP xenograft tumor models, but daily, oneThe antitumor activity of **CBPD-268** five times is shown in Figure 10.
0.3 mg/kg **CBPD-268** orally five times a week for 4 weeks inhibits tumor growth by 84% at the end of treatment (Figure 10A). It is important that **CBPD-268** is well tolerated, with no more than 8% of the maximum body weight loss in mice throughout the experiment (Figure 10B).

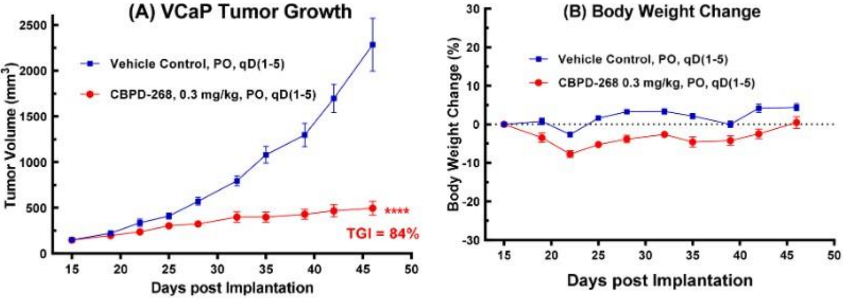


Figure 10. Antitumor efficacy of **CBPD-268** in the VCaP xenograft mouse model. **CBPD-268** (0.3 mg/kg) or vehicle control was administered to mice via oral gavage using 100% PEG200 as the dosing vehicle, with each group consisting of 7−8 mice. (A) Tumor growth for each group. A method of two-tailed student *t* test was used for calculating the statistical significance of the tumor volumes between groups. ****, $P \leq 0.0001$. (B) Animal body weight change for each group.

Inspired by the potent anti-tumor activity of **CBPD26 8** in VCaP tumor models, its in: Antitumor activity in a 22Rv1 xenograft tumor model with very high levels of AR- V7 variant expression and resistance to other AR-targeted agents, as shown in the figure11.

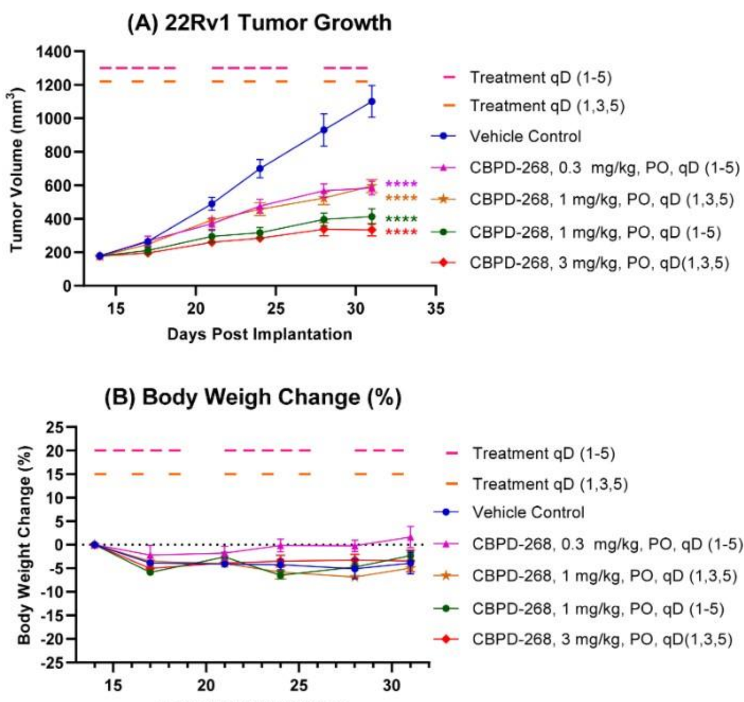


Figure 11. Antitumor efficacy of **CBPD-268** in 22Rv1 xenograft mouse model. **CBPD-268** or vehicle control was administered to mice via oral gavage using 100% PEG200 as the dosing vehicle, with each group consisting of 7−8 mice. (A) Tumor growth for each group. A method of one-way ANOVA was used for calculating the statistical significance of the tumor volumes between groups. ****, $P \leq 0.0001$. (B) Animal body weight change for each group.

At the end of treatment, **CBPD-268** 1mg/kg and 3mg/kg were administered orally 3 times a week for 3weeks, inhibiting 22Rv1 tumor growth by 54% and 83%, respectively. At the end of treatment, **CBPD-268** is administered orally 0.3 mg/kg and 1 mg/kg 5 times a week for 3weeks, inhibiting tumor growth by 56% and 75%, respectively. Importantly, the **CBPD-268-treated** group did not cause any significant weight loss compared to the vehicle control group during the entire experimental period (Figure 11B).

Toxicity study of CBPD-268 in immunocompetent male and female BALB/c mice

The potential toxicity of **CBPD-268** in immunocompetent male and female BALB/c mice was further evaluated as shown in Figure 12show. **CBPD-268** was tested at 3−30 mg/kg twice weekly because it was highly effective in inhibiting tumor growth when administered twice weekly for 5−6 weeks.

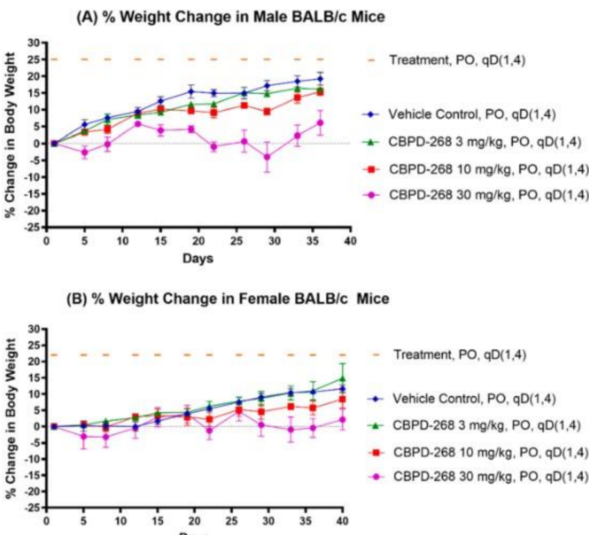


Figure 12. Test the potential toxicity of **CBPD-268** in immune-competent BALB/c male and female mice. Each group of 3 mice was orally administered with **CBPD-268** twice a week for 5−6 weeks at 3−30 mg/kg via oral gavage using 100% PEG200 as the dosing vehicle. (A) % Weight change in male BALB/c mice. (B) % Weight change in female BALB/c mice.

CBDP-268 did not induce weight loss or other signs of toxicity in male and female mice at dose levels of 3 and 10 mg/kg (Figure 12). Male and female mice treated with 30mg/kg **CBPD-268** experienced a modest (<5%) during the experiment weight loss. Thus, **CBPD-268** is well tolerated at doses 10-fold higher than its potency dose in immunocompetent male and female BABL/c mice.

Tissue distribution and toxicity of CBPD-268 in immunocompetent SD rats

PK data showed that **CBPD-268** exhibited excellent PK properties and high oral bioavailability in rats (Table 6). Its tissue distribution in rat plasma, kidney, liver, spleen, and heart was evaluated according to the 3 mg/kg and 10 mg/kg doses, as shown in Table 9show.

Table 9. Tissue Distribution (Mean ± SD) of CBPD-268 in SD Rats at 3 and 10 mg/kg PO Doses[a]

PO Dose(mg/kg)	Time point (h)	Plasma (ng/mL)	Kidney (ng/g)	Liver (ng/g)	Spleen (ng/g)	Heart (ng/g)
3	1	109.3 ± 105.0	513.8 ± 562.3	977.7 ± 645.5	832.5 ± 941.9	245.9 ± 265.1
	6	13.1 ± 9.0	59.2 ± 40.6	147.9 ± 61.7	112.1 ± 59.3	26.4 ± 23.0
	24	1.3 ± 0.2	2.1 ± 3.1	8.4 ± 5.7	8.1 ± 4.7	BLQ
10	1	320.9 ± 150.3	1166.5 ± 320.9	4099.2 ± 1419.0	1993.9 ± 706.0	638.4 ± 249.5
	6	41.4 ± 25.0	224.3 ± 130.2	540.7 ± 210.3	406.4 ± 277.5	83.0 ± 42.2
	24	1.3 ± 0.2	BLQ	20.2 ± 8.9	12.6 ± 8.5	BLQ

[a]BLQ, below the limit of quantification.

CBPD-268 exhibits dose-dependent exposure in plasma and other tissues, and exposure in the kidneys, liver, spleen, and heart is much higher than in plasma, suggesting that **CBPD-268** has a good tissue distribution profile in rats.

To investigate whether **CBPD-268** is effective in reducing CBP and p300 proteins in rat cells. Data suggest that **CBPD-268** is highly effective in inducing protein degradation of CBP and p300, DC_{50}Values are 0.1 nM and 0.3 nM, respectively (Figure S3).

The potential toxicity of **CBPD-268** in immunocompetent SD rats was evaluated at an oral dose of 1−10 mg/kg twice weekly for 5weeks, as shown in Figure 13.

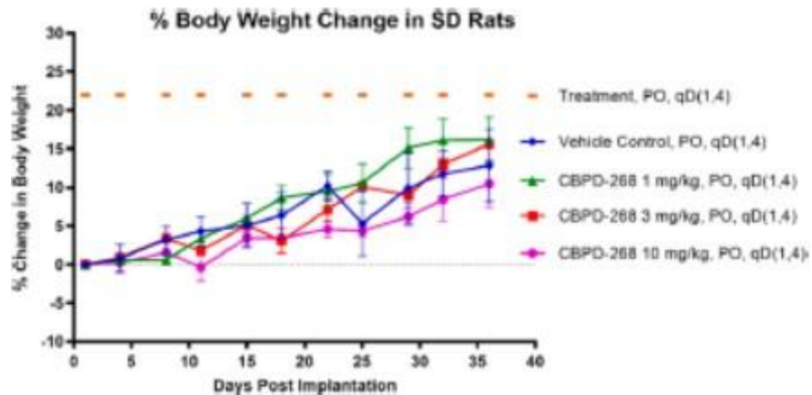


Figure 13. Test the potential toxicity of **CBPD-268** in immune-competent SD rats. Each group of 3 female rats was orally administered with **CBPD-268** twice a week for 5 weeks at 1−10 mg/kg via oral gavage using 100% PEG200 as the dosing vehicle.

CBPD-268 did not cause weight loss in the animals throughout the experiment. In contrast, the body weight of the 1−10 mg/kg treatment group continued to increase in all treatment groups, similar to that of the vehicle control group. At the end of the rat toxicity study treatment, blood samples were collected from each rat for complete blood count (CBC) and blood chemistry analysis, and the resulting data are summarized in Figures S4 and S5.

CBPD-268 was administered orally at a dose of 1−10 mg/kg twice weekly for 5 weeks and did not cause platelets, leukocytes, erythrocytes, neutrophils, basophils, lymphocytes, monocytes, and platelets Any significant change in CBC count. Eosinophils were not present, and the levels of creatinine, total protein, calcium, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, total bilirubin, and albumin were not significantly altered, except that glucose levels were in the 10 mg/kg treatment group Significantly reduced (P = 0.014). In summary, **CBPD-268** is well tolerated in SD rats at an oral dose of 1−10 mg/kg twice weekly for 5weeks, and no signs of toxicity were induced throughout the experiment.

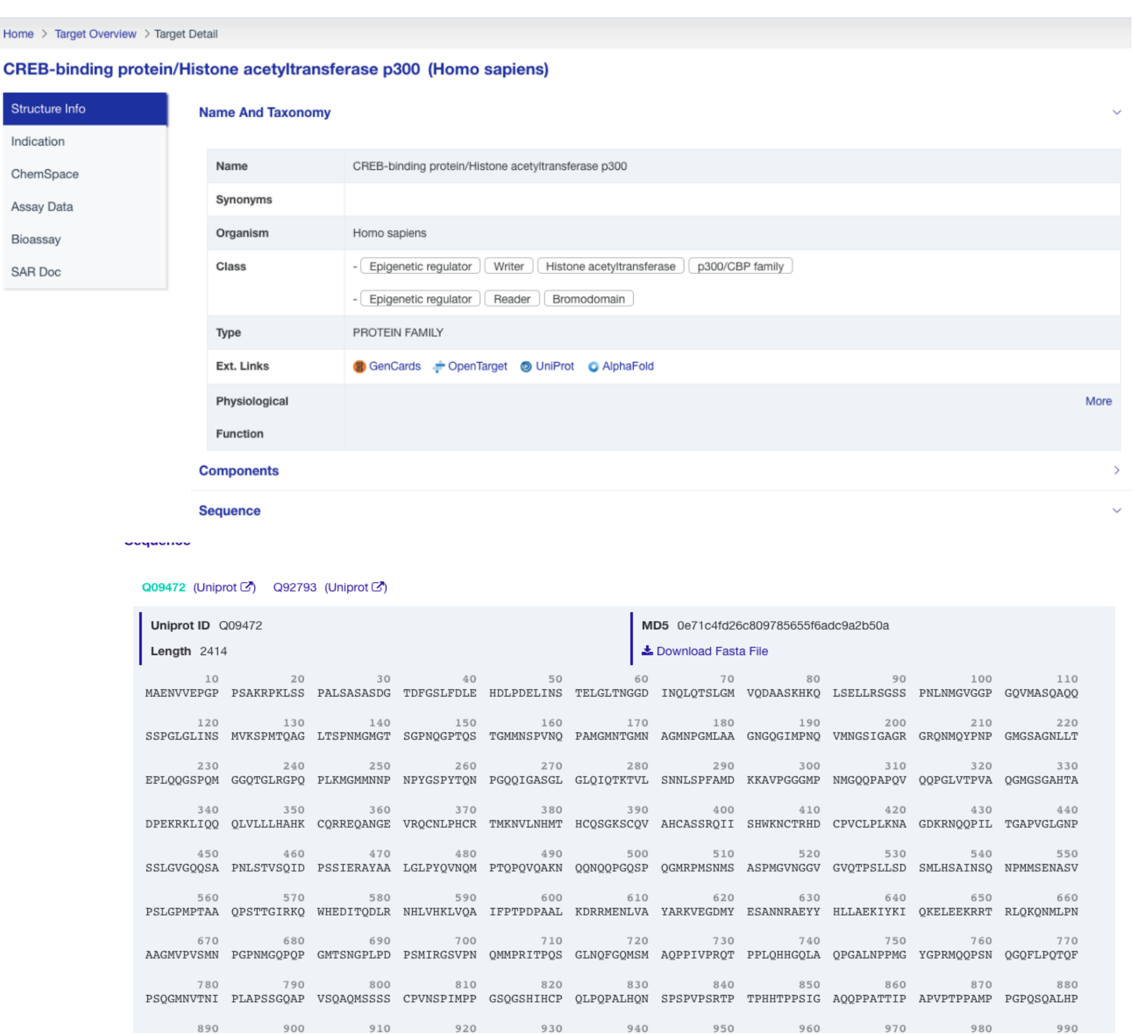
summary

In this study, an attempt was made to develop a particularly effective and orally active CBP/p300 degrader. From the potent and selective CBP/p300 bromodomain inhibitor **GNE-049** and has optimized ADME and PK characteristics A novel cereblon ligand **TX-16** was initiated to design, synthesize, and evaluate a series of potential CBP/p300 PROTAC degraders with different linkers. SAR studies have shown that linkers play a crucial role in degradation potency and oral bioavailability. **CBPD-268** was found to be an abnormally effective, potent, and orally active CBP/p300 degrader. **CPD-268** exhibits excellent oral bioavailability in mice and rats. In addition, **CBPD-268** has good plasma and microsomal stability and tolerance to CYP and hERG inhibition. A single oral administration of 0.3−3 mg/kg of **CBPD-268** is very effective in inducing CBP and p300 in tumor tissues Consumption of proteins. Oral **CBPD-268** has strong antitumor activity in both VCaP and 22Rv1 prostate cancer xenograft models, and is able to be used in VCaP Tumor regression in tumor models. **CBPD-268** was found to be well tolerated in mice and rats and showed a therapeutic index of >10. In summary, **CBPD-268** is a very promising and orally active CBP/p300 degrader that deserves to be used as a treatment for AR Novel therapeutic agents for positive human prostate cancer and other cancers, including hematologic malignancies and other solid tumors, have been studied extensively.

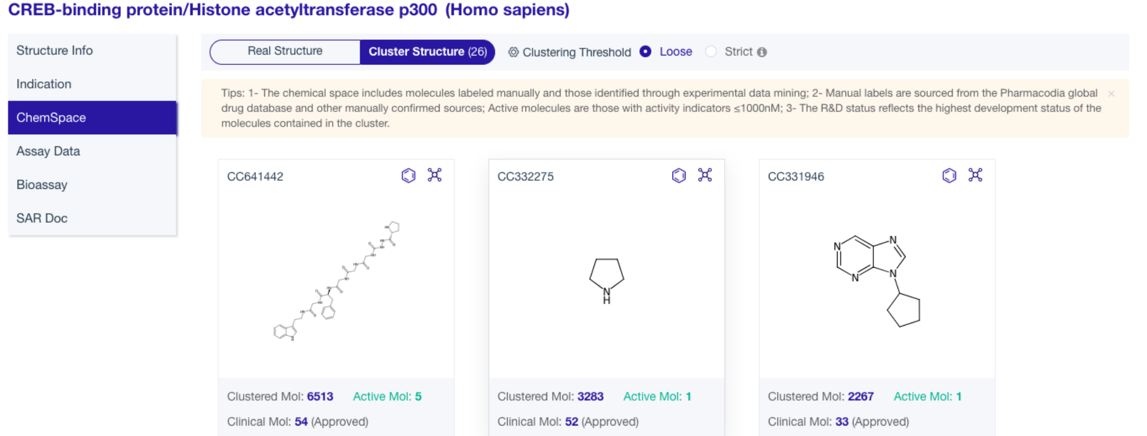
Article source: https://doi.org/10.1021/acs.jmedchem.3c02124

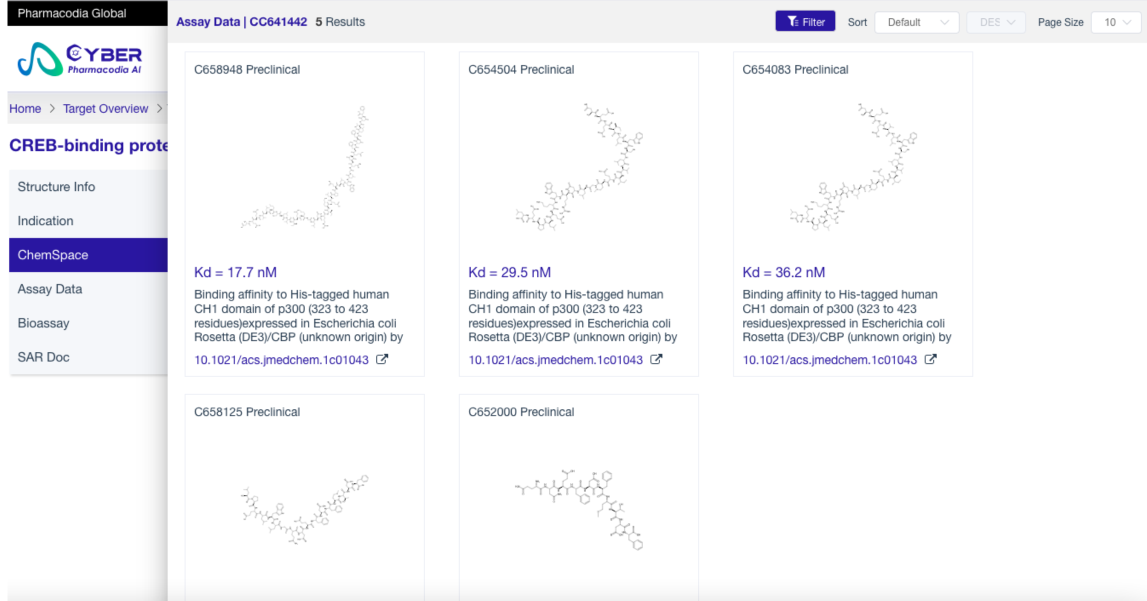
Part III

1. Combined with drug design ideas, the structure of the activity reported in the literature and patents was excavated In order to facilitate the rapid acquisition of targeted structures of interest to developers for the development of ideas, the CREB-binding **protein/Histone acetyltransferase p300 (Homo sapiens)** are examples below:

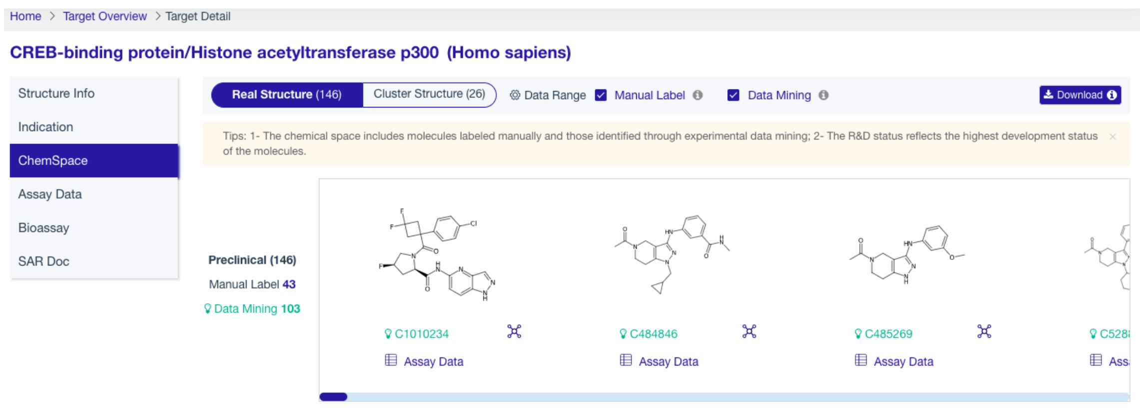


2. In the target interface, select the "Chemical Space" option tab and cascade "Cluster Structure View". tab, you can cluster the literature and patents included in the CyberSAR platform with molecules with activity related to **CBP/p300** related experiments to the "parent nuclear structure." ". The green font highlighted "is the IC_{50}% 3C1000 nM in the in vitro enzyme and cell activity test experiments reported in the literature The structure of the active molecule, the specific experiment, the experimental results and the experimental source.





3. In the target interface, select the "Chemical Space" option tab and cascade "Original Structure View". tab, you can use the literature included in the CyberSAR platform to have molecules with **CBP/p300** related experimental test activity as a "R&D phase timeline." ". Among them, "data mining" highlighted in green font is potential Hit.



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