SMARCA2 PROTAC | ACBI2

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Summary

ACBI2 is a highly potent, orally bioavailable VHL-recruiting PROTAC preferentially degrading SMARCA2 over SMARCA4. ACBI2 can be used as an *in vitro* and *in vivo* tool compound. Cis-ACBI2 is available as a negative control.

Chemical Structure

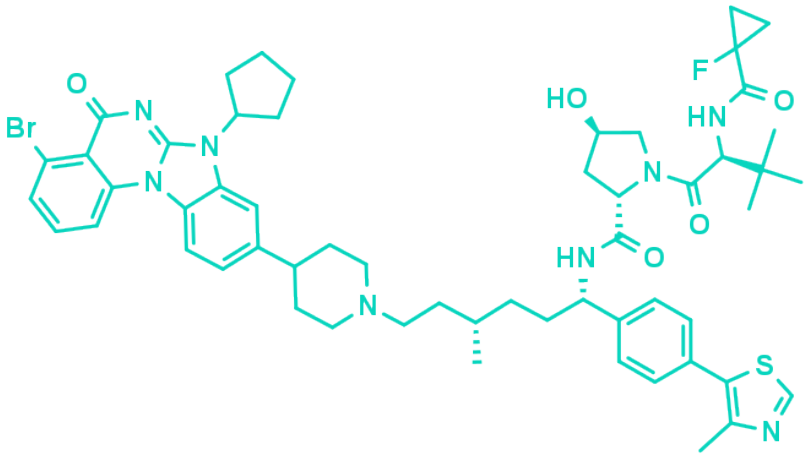

Figure 1: 2-D structure of ACBI2 a highly potent, orally bioavailable VHL recruiting PROTAC preferentially degrading SMARCA2 over SMARCA4.

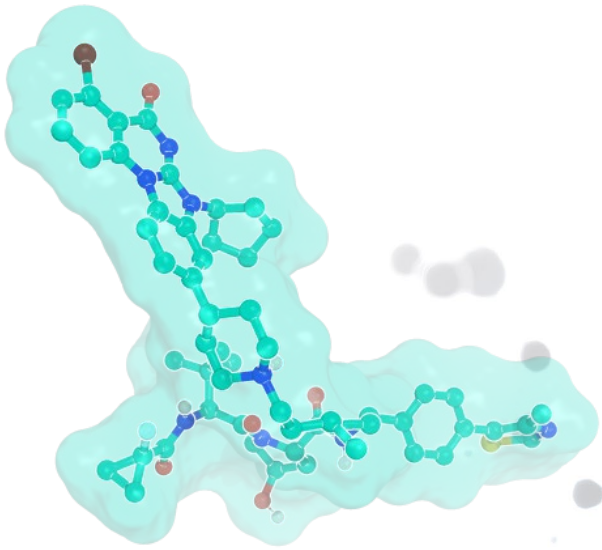

Figure 2: ACBI2, 3D conformation.

Highlights

ACBI2 is a highly potent VHL-recruiting SMARCA2 PROTAC degrader. It shows selective degradation of SMARCA2 over SMARCA4 in ex vivo human whole blood assays. This compound is orally bioavailable and is, thus, suitable not only for *in vitro* but also for *in vivo* studies. *In vivo* efficacy was demonstrated in SMARCA4-deficient cancer models, where significant antiproliferative effects of ACBI2 were observed.

Target information

The ATP-dependent activities of the BAF (SWI/SNF) chromatin remodeling complexes affect the positioning of nucleosomes on DNA and thereby many cellular processes related to chromatin structure, including transcription and DNA repair. The SMARCA2 and SMARCA4 genes encode the two mutually exclusive ATPases of the complex, also known as BRM and BRG1. BAF complex subunits are mutated in approximately 20% of human cancers, several of which are hypothesized to be vulnerable to the loss of SMARCA2 and/or SMARCA4. Hence, selective suppression of SMARCA2 activity has been proposed as a therapeutic concept for SMARCA4-mutated cancers and validated by genetic methods[1-3], but pharmacological validation and exploitation of this synthetic lethal relationship has been hampered by the lack of suitably selective small molecules, in particular for effective *in vivo* use in animal models.

ACBI2 is a PROTAC that causes preferential degradation of SMARCA2 over SMARCA4, as well as the facultative BAF complex subunit PBRM1, whose degradation likely does not contribute to the antiproliferative effects elicited by SMARCA2 degradation in SMARCA4-deficient cancer cells[4]. We qualify ACBI2 as an orally bioavailable SMARCA2 degrader[5] that permits pharmacological evaluation of the SMARCA2-SMARCA4 synthetic lethality concept *in vivo* and *in vitro*[1-3]. We also offer ACBI1 as potent and cooperative PROTAC degrader of SMARCA2, SMARCA4, and PBRM1 on opnMe free of charge.

ACBI2 was jointly developed by scientists from the University of Dundee and Boehringer Ingelheim.

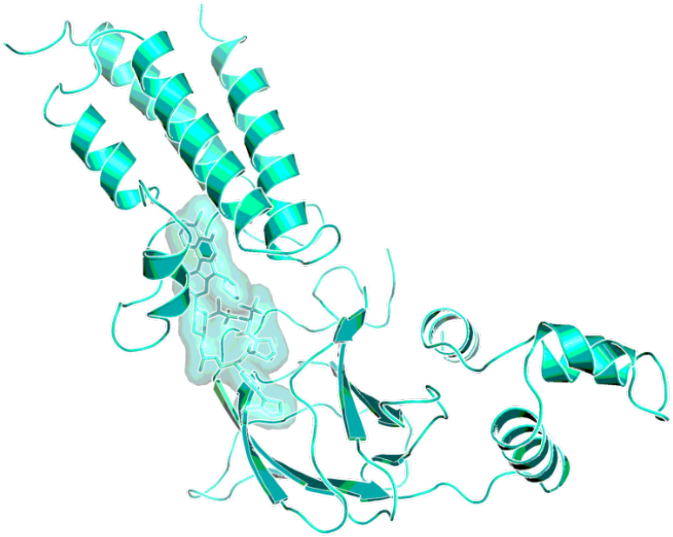

Figure 3: X-ray of close analog BI-2926 (PDB 7Z76) of ACBI2 forming a ternary complex between SMARCA2 bromodomain and VHL.

In vitro activity

ACBI2 displays an EC_{50} < 45 nM in a biochemical ternary complex affinity assay between E3 ligase (VHL), SMARCA2 and ACBI2, and degrades SMARCA2 protein with a $DC_{50,\,4h/18h}$ of 1-13 nM (in RKO and NCI-H1568 cell lines). Furthermore, ACBI2 achieves significant degradation of SMARCA2 with clear selectivity over SMARCA4 in *ex vivo* treatment of human whole blood, obtained from three different healthy donors (Figure **4**).

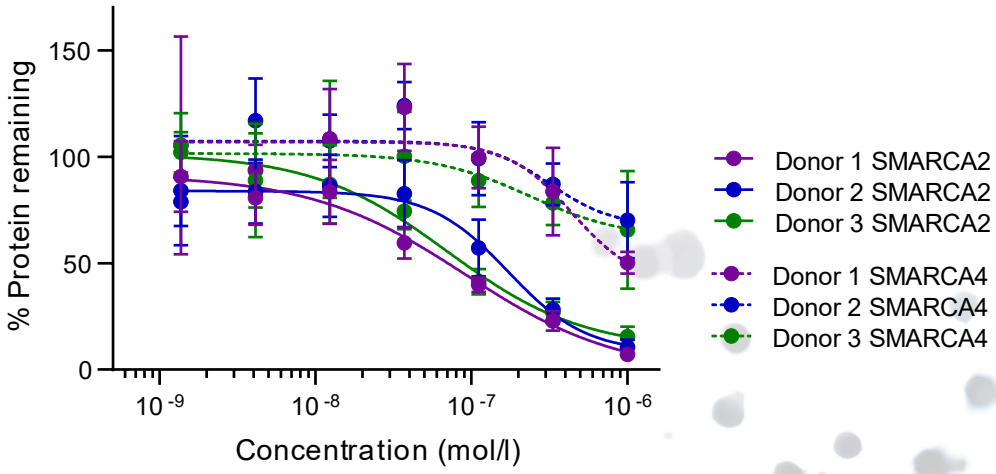

Figure 4: Human whole blood from three healthy donors was treated with ACBI2 for 18h. Protein was extracted from PBMCs and relative SMARCA2 and SMARCA4 levels measured using automated Western blotting (n=3 biological replicates, error bars = SD).

PROBE NAME / NEGATIVE CONTROL		ACBI2	cis-ACBI2
MW [Da]		1,064	1,064
Binary affinity (EC_{50}) [nM][a]	SMARCA2	172 ± 109	203 ± 65
	SMARCA4	314 ± 121	347 ± 41
Ternary affinity + VCB (EC_{50}) [nM][a]	SMARCA2	42 ± 2	181 ± 44
	SMARCA4	86 ± 11	367 ± 38
Cooperativity (binary EC_{50} / ternary EC_{50}) [α][a]	SMARCA2	4.6 ± 2.2	1.1 ± 0.3
	SMARCA4	3.7 ± 0.8	0.9 ± 0.1
Degradation DC_{50}, 18h, RKO cells [nM][b]	SMARCA2	1 ± 1	7,516,423 ± 2,993
	SMARCA4	32 ± 12	5,828 ± 1,535
Degradation D_{max}, 18h, RKO cells [%][b]	SMARCA2	81 ± 7	82 ± 16
	SMARCA4	67 ± 8	94 ± 7
Degradation DC_{50}, 4h, RKO cells [nM][b]	SMARCA2	4 ± 1	>25,000
	SMARCA4	>25,000	>25,000
Degradation, D_{max}, 4h, RKO cells [%][b]	SMARCA2	71 ± 2	NA
	SMARCA4	NA	NA
Degradation DC_{50}, 4h, NCI-H1568 cells [nM][b]	SMARCA2	13 ± 14	NA
Degradation D_{max}, 4h, NCI-H1568 cells [%][b]	SMARCA2	86 ± 6	NA
Proliferation D_{max}, 168h, NCI-H1568 cells [nM][c]		7 ± 4	236 ± 175

[a] TR-FRET competition assay for SMARCA2/4 bromodomains; mean ± SD.

[b] SMARCA2/4 protein degradation after compound treatment for indicated time followed by capillary electrophoresis; mean ± SD. NCI-H1568 cells are SMARCA4-deficient.

[c] Cell viability after compound treatment for indicated time followed by CellTiter Glo assay; mean ± SD. NCI-H1568 cells are SMARCA4-deficient.

In vitro DMPK and CMC parameters

ACBI2 is a large, lipophilic molecule with good absorptive permeability and low efflux ratio in the Caco2-assay. ACBI2 is stable in liver microsomes and hepatocytes and displays a very high plasma protein binding. The aqueous solubility of ACBI2 is low at neutral pH but can be increased using formulations such as Hydroxypropyl-ß-cyclodextrin (HPßCD).

PROBE NAME / NEGATIVE CONTROL	ACBI2	cisACBI2
logP	10.34	10.34
Solubility @ pH 6.8 [µg/ml]	<1	<1
Solubility in HPßCD (10%)/Ringer solution (50%) @ pH 7.2 [µg/ml]	2.0	n.a.
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	1.6	3.4
CACO efflux ratio	4.8	2.5
Microsomal stability (human/mouse/rat) [% Q_H]	<24/<23/<24	31/<23/<24
Hepatocyte stability (human/mouse/rat) [% Q_H]	12/9/15	<4/20/<5
Plasma protein binding (human/mouse/rat) [%]	>99.9	>99.8
hERG (IC_{50} in 30% plasma) [µM]	1.7	n.a.
CYP 3A4 (IC_{50}) [µM]	12.7	7.6
CYP 2C8 (IC_{50}) [µM]	32.8	43
CYP 2C9 (IC_{50}) [µM]	23.2	>50
CYP 2C19 (IC_{50}) [µM]	n.a.	>50
CYP 2D6 (IC_{50}) [µM]	12.1	155.3

In vivo DMPK parameters

ACBI2 displays a low to moderate clearance in mice and rats, leading to a good oral bioavailability 22% and 18% respectively.

ACBI2	MOUSE	RAT
Clearance [% Q_H][b]	2	22
Mean residence time after iv dose [h]	6.2	15.1
t_{max} [h]	1.7	4.0
C_{max} [nM]	5,970	162
F_{oral} [%]	22	18
V_{ss} [l/kg]	0.47	12.8

[b]5 [mg/kg]

No PK data for the negative control cis ACBI2 are available.

In vivo pharmacology

In vivo, ACBI2 displays dose-dependent SMARCA2 degradation in NCI-H1568 and A549 engrafted tumor bearing mice following short-term treatment (Figure **5a and 5b**). Correspondingly, ACBI2 (administered at 80 mg/kg orally once daily) significantly inhibits tumor growth in an A549 xenograft model (**Figure 5c**) and is well tolerated (Figure **5d**). SMARCA2 protein levels in most compound-treated tumors collected at the end of this study were decreased to background levels (Figure **5e**).

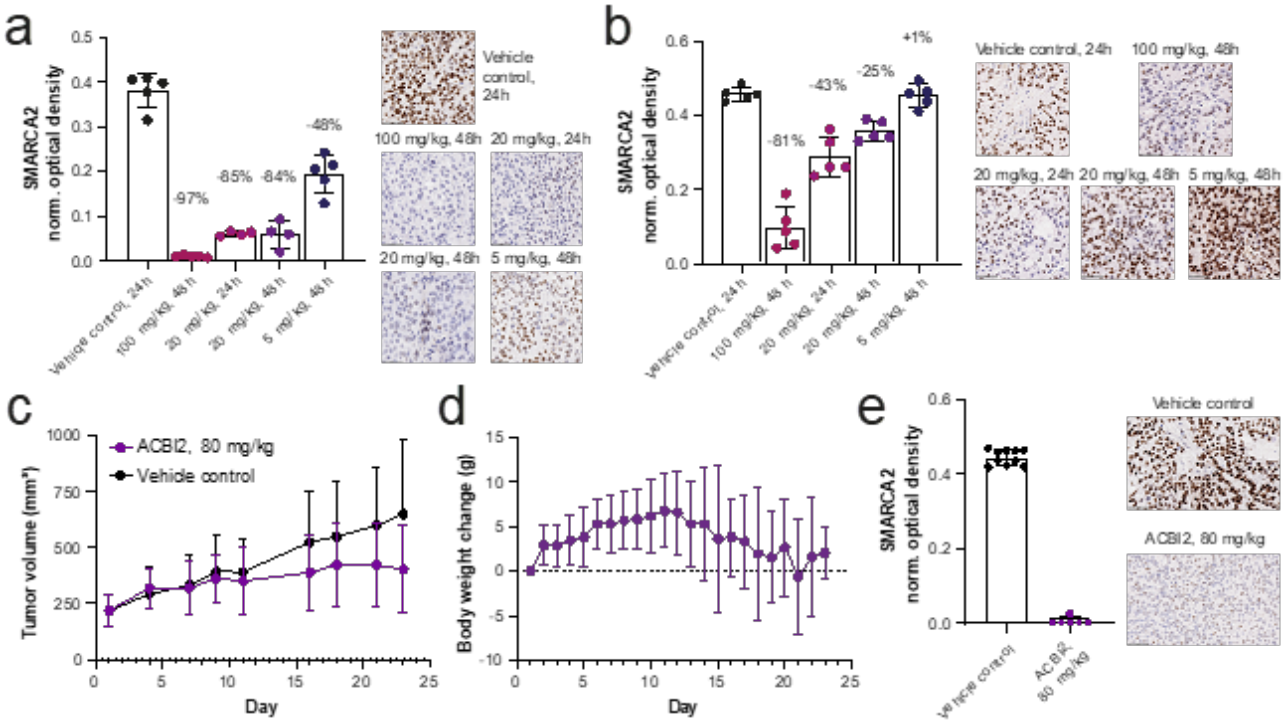

Figure 5: a. NCI-H1568 or **b.** A549 tumor bearing mice were treated orally with ACBI2 (n = 5 animals per group). SMARCA2 levels in viable tumor tissue were determined using IHC staining (mean background-normalized optical density (OD) and SD). **c.** A549 tumor bearing mice were treated orally with ACBI2 once daily (mean of 10 animals per group, error bars = SD). **d.** Body weight of animals in **c.** was measured daily (mean and SD of change per day for n = 10 animals). **e.** SMARCA2 IHC staining at the end of the study shown in **c** and **d** (as for **a** and **b**).

Negative control

Due to the inversion of the stereochemistry of the hydroxy group in the core VHL binding moiety cisACBI2 is no longer able to bind to VHL. Therefore, cisACBI2 can no longer form a functional ternary complex between VHL and SMARCA2, which leads to prohibition of SMARCA2 degradation.

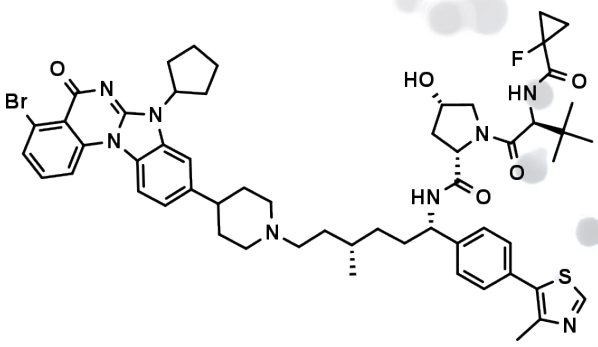

Figure 6: cisACBI2, which serves as a negative control.

Selectivity

ACBI2 hits 6 out of 44 targets (<30% inhibition at 10 µM) in the SafetyScreen44 (NA+/SITE2/R; COX-1@CE; COX-2@CE; M2/H; D2SH_AGON; HERG_DOFETILIDE). Furthermore, in an Invitrogen kinase panel, 6 out of 31 kinases are hit (>70% of control at 10 µM; MAP3K8, CAMK1D, GSK3B, ABL1, MAPKAPK2 and MAPK14). In the DiscoverX Bromoscan 6 out of 31 targets are hit (<30% inhibition at 10 µM; PBRM1, SMARCA4, BRD7, TFQ1, BRDT).

SELECTIVITY DATA AVAILABLE	ACBI2	cisACBI2
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	Yes	No
Dundee	No	No

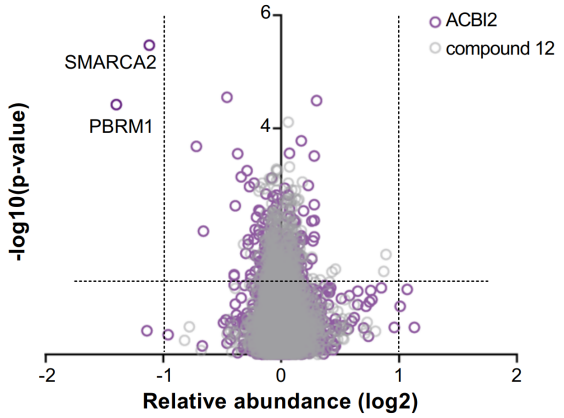

Figure 7: Effects of ACBI2 (purple) and negative control compound 12 (grey, cis-hydroxyproline analogue of ACBI2 which is not capable of binding VHL) on the proteome of the SMARCA4-deficient cell line NCI-H1568, treated with the compounds at 100 nM for 4 h. Data are plotted as the log2 of the normalized fold change in abundance against −log10 of the p value per protein from n = 3 independent experiments (two-tailed t-tests assuming equal variances).

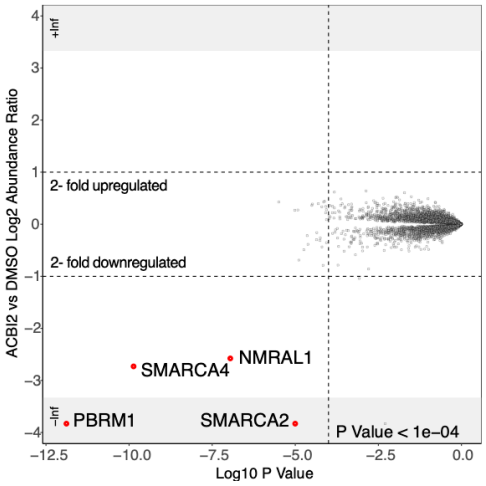

Figure 8: In a separate experiment, the PROTAC ACBI2 was tested by the Eric Fischer Laboratory - Dana-Farber Cancer Institute as part of their Degradation Proteomics Initiative.[6,7] MOLT4 cells were treated with ACBI2 at 100 nM for 5 h. Data are plotted as the log2 of the normalized fold change in protein expression abundance against −log10 p value as calculated by moderated t-test in Bioconductor's limma package. +/- inf box contains proteins that were below detection level in all replicates of a specific treatment group. Here PBRM1 and SMARCA2 were degraded below detection level in ACBI2 treatment and are therefore displayed in the -inf box, indicating complete or near complete degradation of SMARCA2 and PBRM1.

Of the 7,309 proteins quantified in this experiment after in-house filtering for data quality (9,588 prior to filtering), Donovan *et al.* found a high degree proteome-wide selectivity for (P)BAF complex sub-units SMARCA2, SMARCA4 and PBRM1 and also identified regulation of NMRAL1. SMARCA2 and PBRM1 were degraded to a depth below detection level following treatment. It should be noted that quantitative proteomics experiments are designed to provide a global view of targets for a degrader at the specific concentration chosen, and are no substitute for carefully assessing selectivity windows. An in-depth analysis of the quantitation of selectivity windows between SMARCA2 and SMARCA4 supported by dose and time course data across multiple cell lines can be found in Kofink *et al.*, 2022.[5]

Global protein quantification was used to explore the unbiased proteome-wide selectivity of ACBI2 induced degradation. Whole cell protein quantification was performed using label free quantification with the Fischer lab's diaPASEF workflow. Statistical analysis was performed using a moderated t-test in Bioconductor's limma package to generate hit lists containing log2 Fold Change and P-values for each protein.

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

X-ray of close analog BI-2926 (PDB 7Z76) of ACBI2 forming a ternary complex between SMARCA2 bromodomain and VHL available in PDB.

Reference molecule(s)

ACBI1 (ref. 4 and on opnMe)

DT2216 (clinical VHL-based Bcl-xL degrader; ref. 6)

“compounds 21b/c” (orally active VHL-based HMGCR degrader; ref. 7)

PFI-3 (SMARCA2/4 bromodomain binding tool compound; ref. 8)

GEN-1 (PFI-3 derivative; ref. 9)

“compound 26” (PBRM1 bromodomain binder; ref. 10)

Supplementary data

2-D structure files can be downloaded free of charge from opnMe.

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