opnMe by Boehringer Ingelheim

pan-KRAS(on) PROTAC

ACBI4

Boehringer Ingelheim

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Summary

ACBI4 is a first-in-class VHL-recruiting PROTAC molecule, developed through a collaboration between Boehringer Ingelheim and the Centre for Targeted Protein Degradation at the University of Dundee, that potently degrades multiple oncogenic KRAS variants *in vitro*, with a particular focus on KRAS(on) variants. ACBI4 provides a novel chemical tool molecule for studying the impact of degrading KRAS(on) mutants, which is not possible with current pan-KRAS inhibitors or degraders.

Chemical Structure

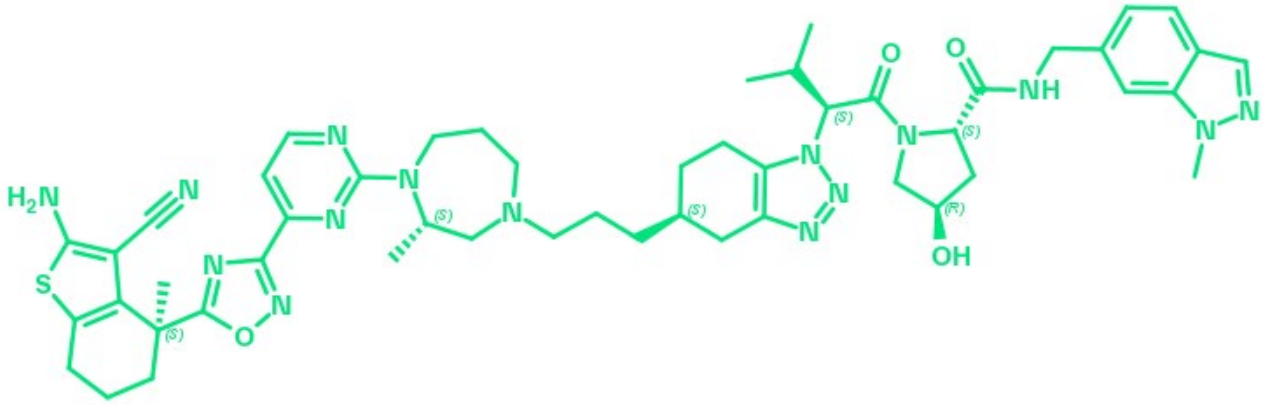

Figure 1: 2D structure of ACBI4, a selective PROTAC that targets KRAS(on) variants

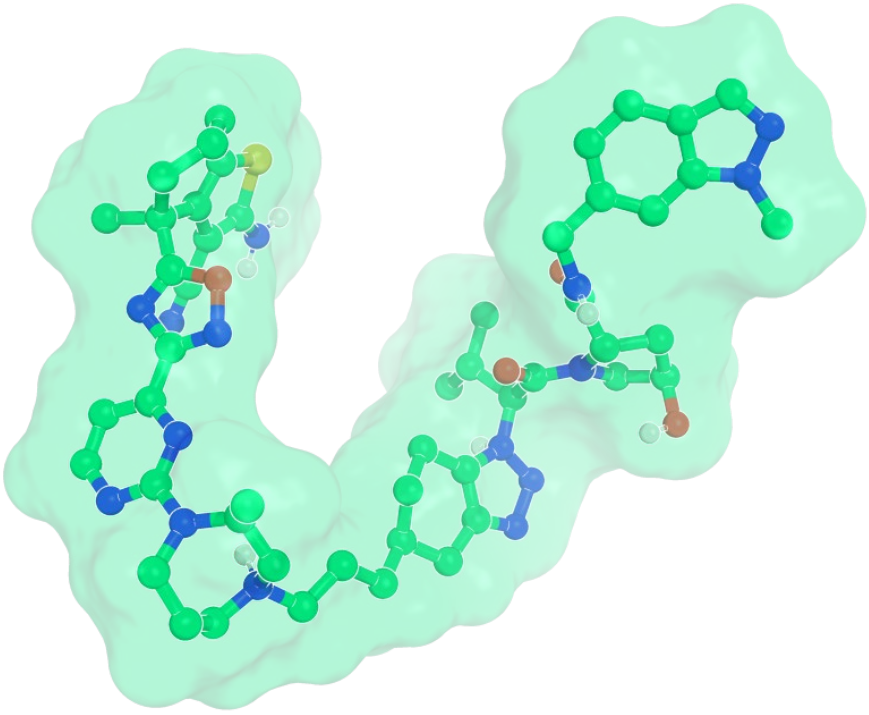

Figure 2: ACBI4 3D conformation, based on the ternary complex X-ray structure of the complex with KRAS and VHL (PDB code 9RKE)[1].

Highlights

ACBI4 is a highly cooperative, potent PROTAC degrader, developed through a collaboration between Boehringer Ingelheim and the Centre for Targeted Protein Degradation at the University of Dundee, that targets GTP-loaded (active) KRAS mutants, including challenging alleles such as G12R. ACBI4 forms exceptionally stable ternary complexes, achieves rapid and profound KRAS degradation, and induces strong antiproliferative effects in KRAS-driven cancer cells *in vitro*.

Target information

Kirsten rat sarcoma viral oncogene homologue (KRAS) is the most commonly mutated oncogene in human cancers. Variants, predominantly mutations at Glycine (G) 12 or Glutamine (Q) 61, increase the proportion of activated, GTP-loaded KRAS, enhancing RAF-MEK-ERK (MAPK) signalling, and drive tumor growth[3]. Developed in collaboration with Centre for Targeted Protein Degradation, University of Dundee, ACBI4 is a first in class proteolysis targeting chimera (PROTAC) potently degrading 14 out of 17 of the most prevalent oncogenic KRAS variants including KRAS(on) variants. ACBI4 is poorly soluble and lacks oral bioavailability. As a negative control, cis-ACBI3, is available that contains the same KRAS binding moiety.

Due to its exceptionally high cooperativity ACBI4 effectively engages the GTP activated state of KRAS, to drive rapid and profound degradation of KRAS(on) mutants, leading to antiproliferative effect in a cell line driven by KRASG12R.[1]

ACBI3, a widely adopted pan-KRAS degrader available via opnMe, is complemented by ACBI4 (pan-KRAS(on) PROTAC). ACBI3 primarily targets the inactive (GDP-bound) form of KRAS and enables broad mutant degradation, whereas ACBI4 selectively engages the active (GTP-bound) state, addressing KRAS(on) variants that are less accessible to off-state degraders. Together, they provide complementary tools to explore KRAS biology across signaling states and degradation mechanisms.

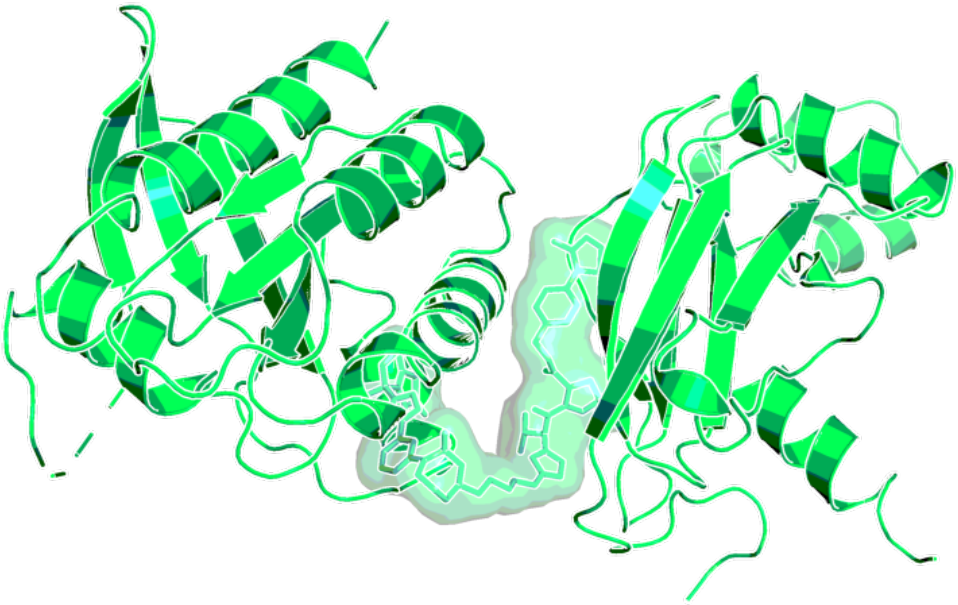

Figure 3: X-ray structure of the complex of ACBI4 with KRAS and VHL (PDB code 9RKE)[1].

In vitro activity

ACBI4 exhibits potent intracellular VHL engagement, ternary complex formation, and ubiquitination translating into efficient E3-ligase dependent cellular degradation and proteome-wide selectivity. ACBI4 displays antiproliferative activity in a cell line panel on *KRAS* mutant but not *KRAS*^{WT} cell lines (geometric mean IC_{50} = 478 nM *vs* 8.3 µM, respectively).

Probe name / Negative control	ACBI4	cis-ACBI3
MW [Da][a]	970.22	1,019.25
Cellular KRAS^{G12D} degradation, 18 h (GP5d cells) (DC_{50}) [nM][b]	7	> 1,000
Cellular KRAS^{G12R} degradation, 18 h (KP-2 cells) (DC_{50}) [nM][b]	151	n.d.
Cellular proliferation, 3 days (KP-2 cells) (IC_{50}) [nM][c]	174	n.d.

[a]Please note that ACBI4 and cis-ACBI3 are supplied in salt form; for the molecular weight of the salt, please refer to the vial label
[b]by capillary electrophoresis using the following antibodies: KRAS^{G12D} (Cell Signaling #14429), KRAS^{G12V} (Cell Signaling #14412), normalized by GAPDH (Abcam #ab9485),
[c]by CellTiterGlo assay (Promega #G7570)

In vitro DMPK and CMC parameters

ACBI4 is a large, lipophilic molecule that exhibits moderate aqueous solubility at physiological pH (6.8), which is improved relative to the negative control cis-ACBI3. It has low stability in liver microsomes while being good to moderately stable in hepatocytes. Despite its size and lipophilicity, ACBI4 demonstrates good apparent permeability in Caco-2 assays, with a measured efflux ratio of 2.6, indicating some transporter-mediated efflux but overall favorable absorptive properties.

Probe name / Negative control	ACBI4	cis-ACBI3
logD @7.4	3.8	4.3
Solubility @ pH 6.8 [µg/ml]	15	<1
Caco-2 permeability @ pH 7.4 [*10^{-6} cm/s]	9.3	n.d.
Caco-2 efflux ratio	2.6	n.d.
Microsomal stability (human/mouse/rat) [% Q_H]	>88/70/>88	>88/>88/75
Hepatocyte stability (human/mouse/rat) [% Q_H]	85/72/34	n.d.

Negative control

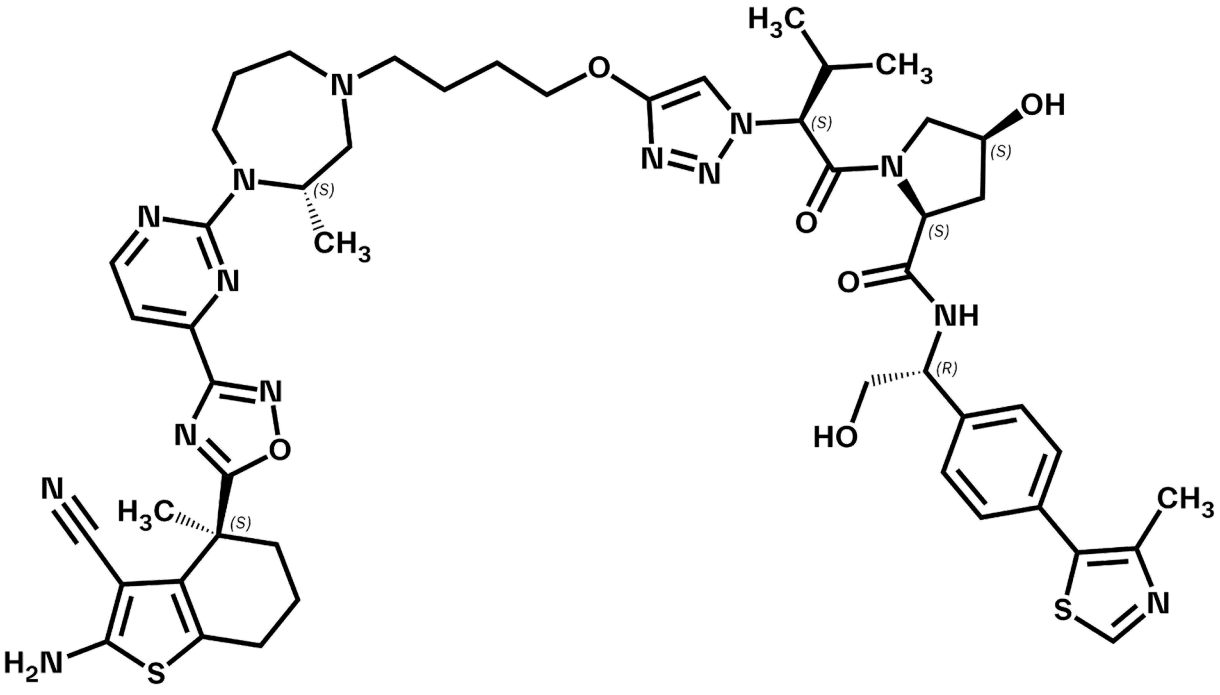

Figure 4: cis-ACBI3, which serves as a negative control.

Selectivity

The degradation selectivity of ACBI4 was assessed by whole cell proteomics MS analysis of Cal-62 cells treated for 6 hours with 1 µM ACBI4 or a negative control. The below figure validates selective degradation of KRAS. Interestingly, NRAS degradation was observed only at very high concentrations of ACBI4, indicating a degree of selectivity for KRAS over NRAS. Additionally, HRAS degradation was noted at higher concentrations of ACBI4, with maximal degradation achieved at 3 µM (DC_{50} = 637 nM, D_{max} = 72%). This represents a roughly 2-fold selectivity window over KRAS degradation (DC_{50} = 382 nM, D_{max} = 88%). These findings suggest that ACBI4 exhibits preferential degradation of KRAS while sparing NRAS and HRAS at lower concentrations, which could be advantageous in maintaining a therapeutic window.

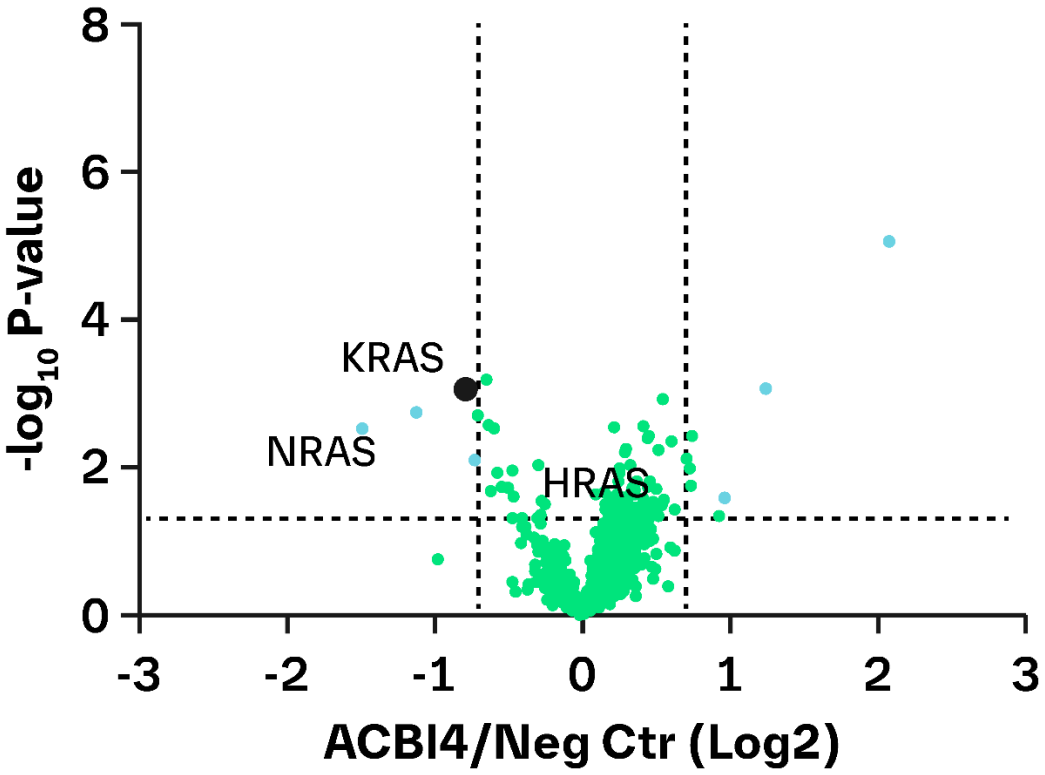

Figure 6: Volcano plot of whole cell proteomics MS analysis of Cal-62 cells treated with 1 µM ACBI4 or negative control (6 hours).

Co-crystal structure

The X-ray crystal structure of target in complex with ACBI4 is available (PDB code: 9RKC and 9RKN)[1].

Reference molecule(s)

Other available tool compounds: ACBI3[2] which is also available to order via opnMe.

Supplementary data

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

References

1. Vetma V., Puoti I., Karolak N. K., Chakraborti S., Diers E., Girardi E., Khan S., Kidd G., Kropatsch K. G., McLennan R., O'Connor S., Samwer M., Trainor N., Whitworth C., Wijaya A. J., Wong J. Y. F., Zollman D., Farnaby W., Popow J., Ciulli A., Ettmayer P., McAulay K. Identification of a Highly Cooperative PROTAC Degrader Targeting GTP-Loaded KRAS(On) Alleles. *Journal of the American Chemical Society* **2025**, 147(45), 41367–41378. https://doi.org/10.1021/jacs.5c10354, PubMed.
2. Popow J., Farnaby W., Gollner A., Kofink C., Fischer G., Wurm M., Zollman D., Wijaya A., Mischerikow N., Hasenoehrl C., Prokofeva P., Arnhof H., Arce-Solano S., Bell S., Boeck G., Diers E., Frost A.B., Goodwin-Tindall J., Karolyi-Oezguer J., Khan S., Klawatsch T., Koegl M., Kousek R., Kratochvil B., Kropatsch K., Lauber A.A., McLennan R., Olt S., Peter D., Petermann O., Roessler V., Stolt-Bergner P., Strack P., Strauss E., Trainor N., Vetma V., Whitworth C., Zhong S., Quant J., Weinstabl H., Kuster B., Ettmayer P., Ciulli A. Targeting cancer with small molecule pan-KRAS degraders *Science* **2024**, 385(6715), 1338-1347. DOI: 10.1126/science.adm8684, PubMed.
3. Huang L., Guo Z., Wang F., Fu L. KRAS mutation: from undruggable to druggable in cancer *Sig Transduct Target Ther* **2021**, 6, 386. DOI: 10.1038/s41392-021-00780-4, PubMed.
4. Simanshu, D. K., Nissley, D. V., & McCormick, F. RAS Proteins and Their Regulators in Human Disease *Cell*, **2018**, 170(1), 17–33. https://doi.org/10.1016/j.cell.2017.06.009, PubMed.