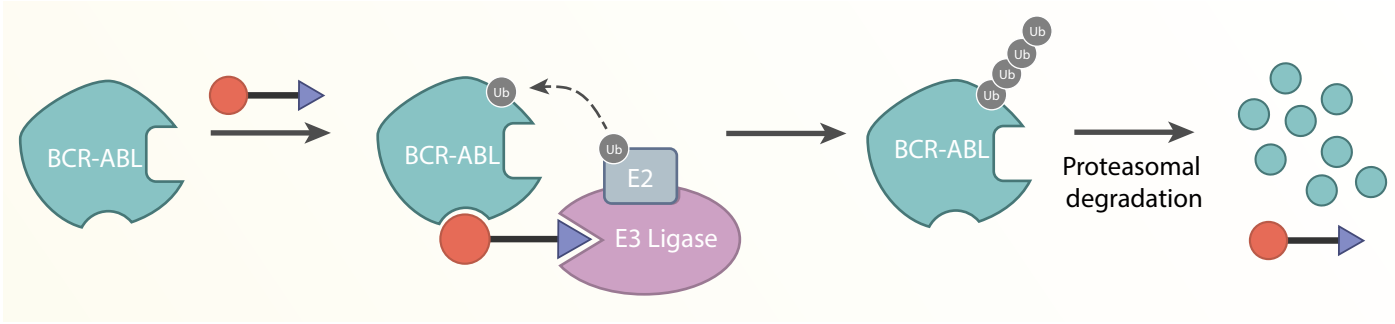
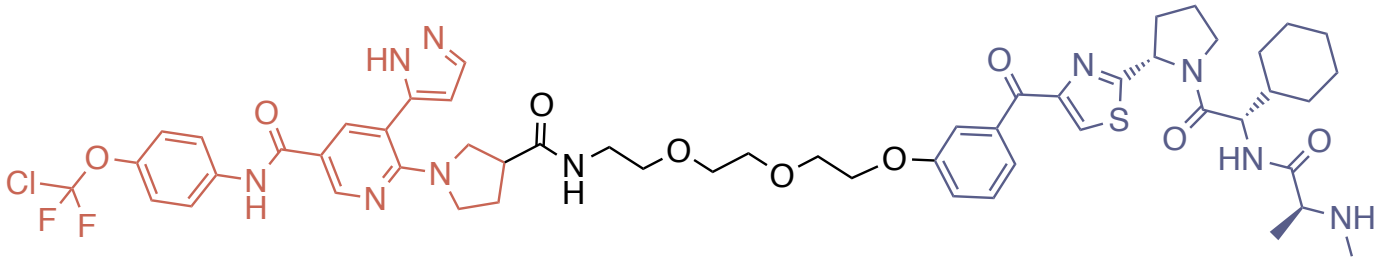
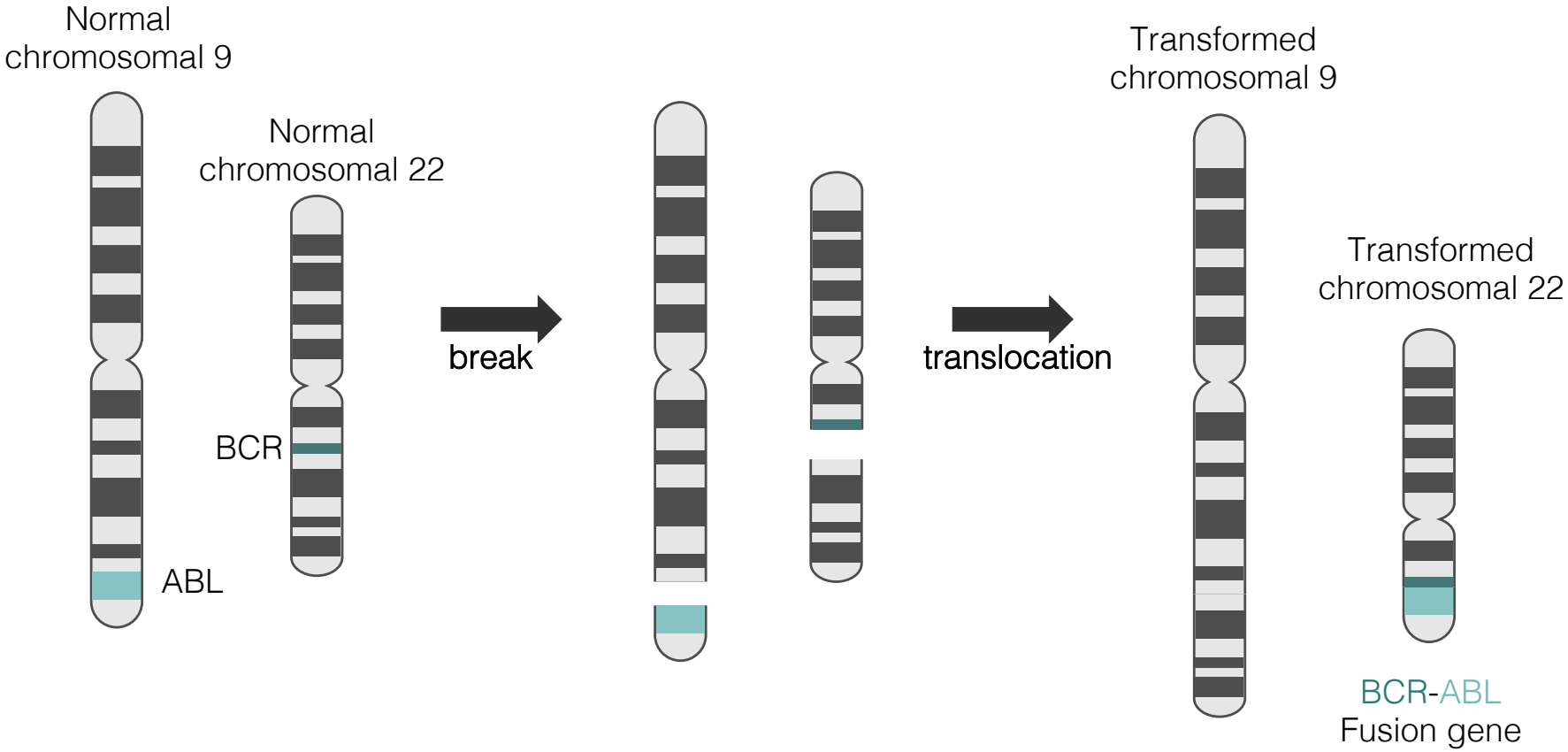
Targeting the Allosteric Site of Oncoprotein BCR-ABL as an Alternative Strategy for Effective Target Protein Degradation

ACS Med. Chem. Lett. **2017**, 139, 6046.





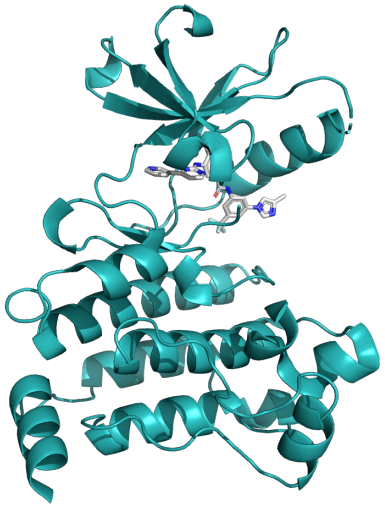
Evan Carder
Wipf Group Current Literature
November 18, 2017



JCI **2007**, 117, 2036.
Nat. Rev. Cancer **2007**, 7, 345.

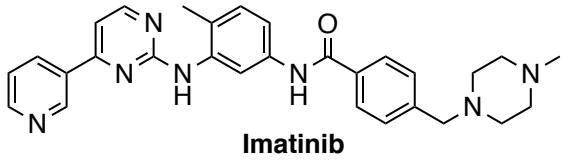
Transformed chromosomal 22

BCR-ABL
Fusion gene

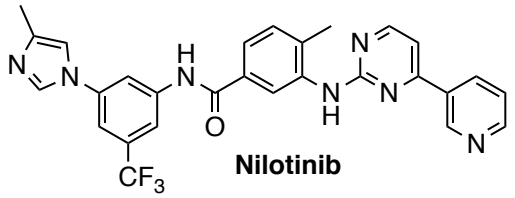


Constitutively-active
ABL tyrosine kinase

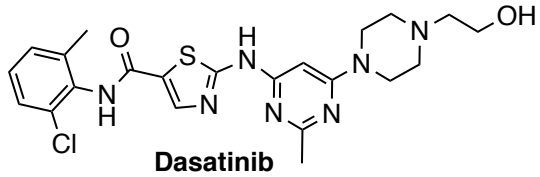
ABL Tyrosine Inhibitors



Imatinib



Nilotinib



Dasatinib

Nat. Rev. Cancer **2007**, 7, 345.

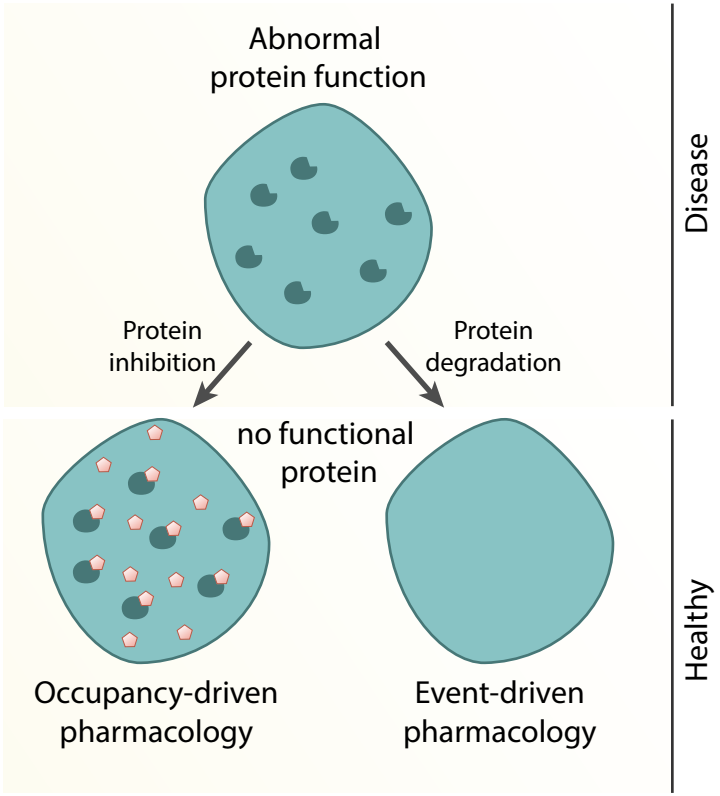
Pharmacology Models: Inhibition vs Degradation

- Occupancy-driven pharmacology

  Stoichiometric activity
  Mostly target active-site binding
  Site occupancy to block function
  Specificity defined by binding

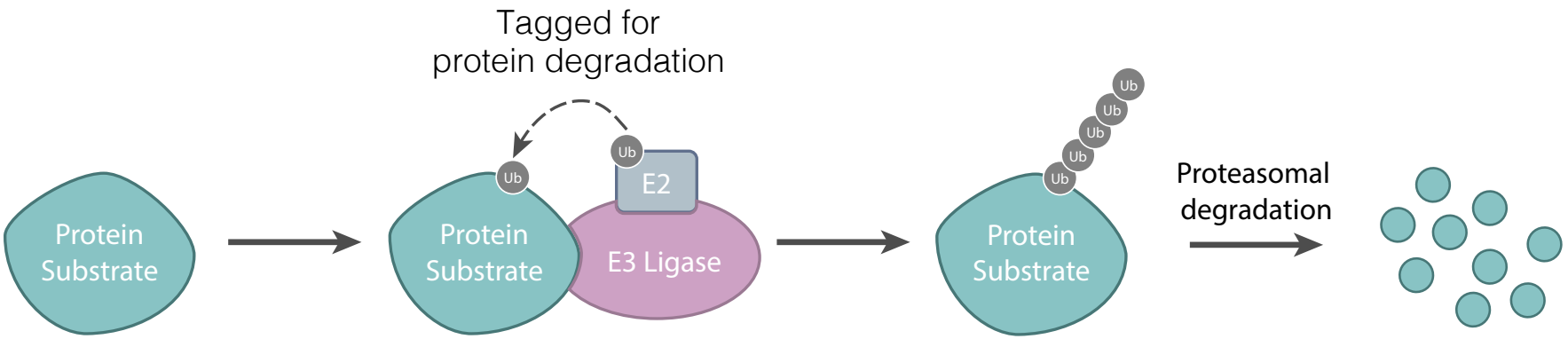
- Event-driven pharmacology

  Sub-stoichiometric activity
  Target active and allosteric sites
  Additional layer of specificity
  Restoration of function requires re-synthesis



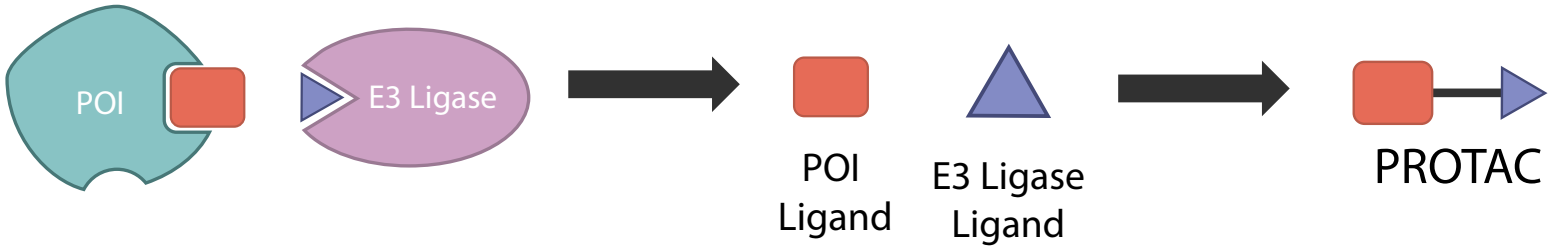
Nat. Drug. Disc. **2017**, 16, 101.
Cell Chemical Biology **2017**, 24, 1181.

Protein Degradation - Ubiquitin-Proteasome System (UPS)



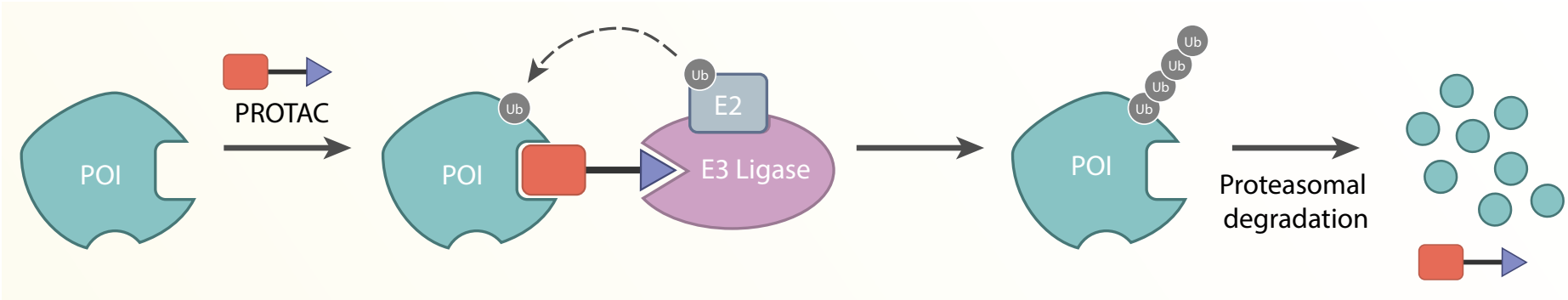
Nat. Rev. Mol. Cell Biol. **2008**, 9, 679.

Proteolysis Targeting Chimeras - PROTACs



-

Small-molecule-mediated Protein degradation:



Natl. Acad, Sci. USA **2001**, 98, 8554.

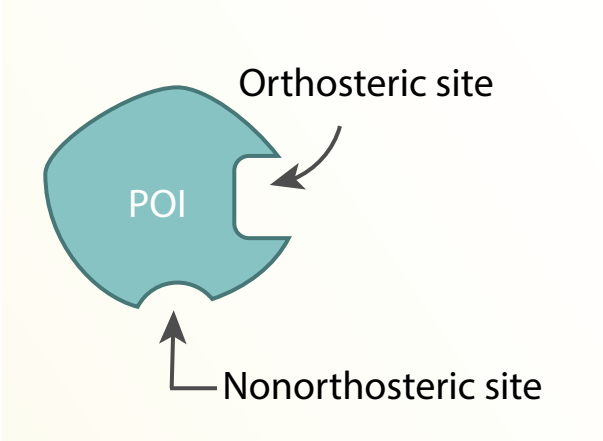
Targeting Protien of Interest

- Orthosteric/Active Site Modulators

  Structurally similar to endogenous ligand
  Employ rational drug design
  Varying degrees of specificity
  Highly susceptible to drug resistance

- Nonothosteric/Allosteric Modulators

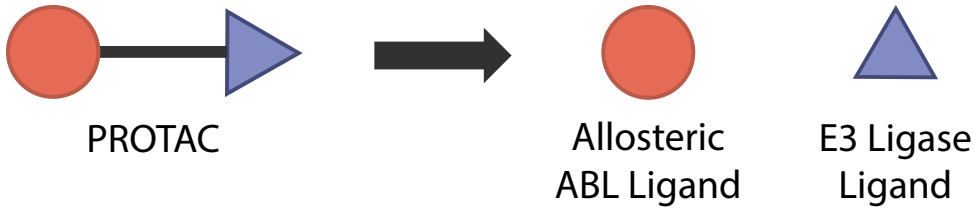
  Lack an endogenous ligand
  Unique chemical structures
  Elevated target selectivity
  Reduced off-targets
  Spatio-temporal specificity
  Reduced susceptibility to drug resistance



ACS Cent. Sci. **2017**, 3, 925.
Med. Princ. Pract. **2013**, 22, 418.
Rev. Pharmacol. Toxicol. **2014**, 54, 165.

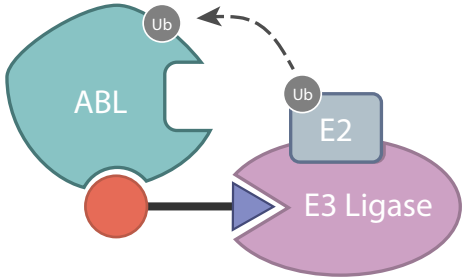
Hypothesis – Application of an allosteric BCR-ABL PROTAC induces BCR-ABL protein degradation

Phase I



Development of an allosteric BCR-ABL PROTAC

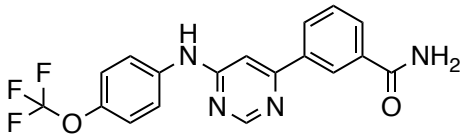
Phase II



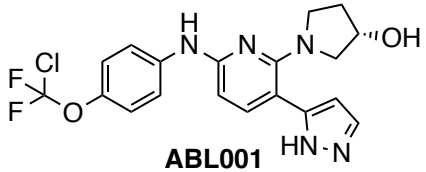
BCR-ABL protein degradation

ABL Allosteric-site Inhibitors

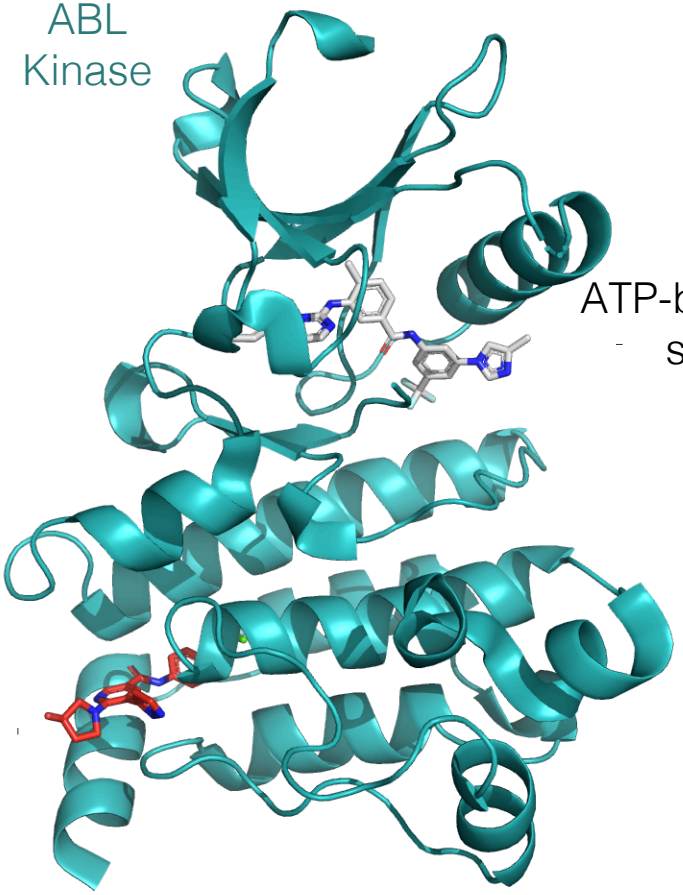
Target Myristate-binding site



GNF-2



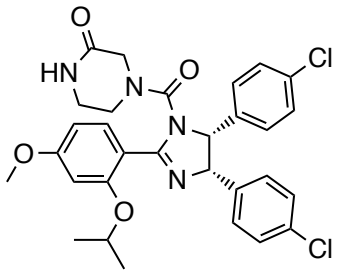
ABL001



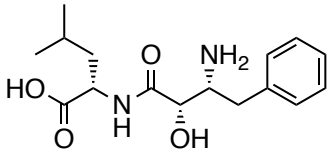
PDB: 5MO4

Nature **2010**, 463, 28.
Nature **2017**, 543, 733.

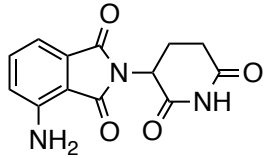
E3-Ubiquitin Ligands



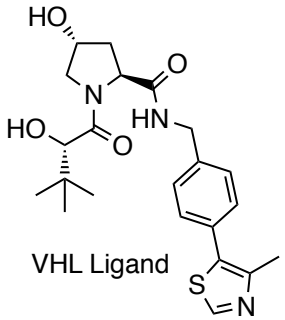
Nutlin-3a
MDM2 Ligand



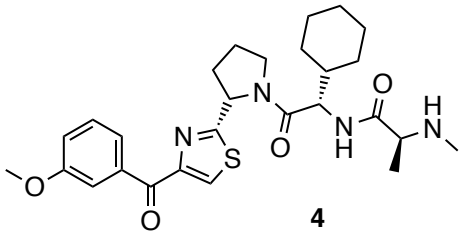
Bestatin
cIAP Ligand



Pomalidomide
CRBN Ligand



VHL Ligand



4
cIAP Ligand

E3-Ubiquitin Ligase

IAPs

MDM2

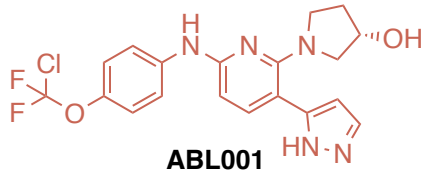
Von Hippel-Lindau

Cereblon

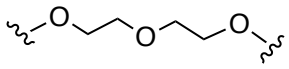
Nat. Drug. Disc. **2017**, 16, 101.

Design of PROTAC

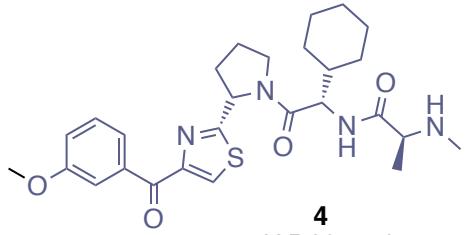
-



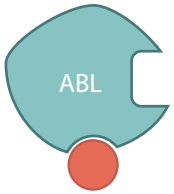
ABL001



Ethylene glycol linker

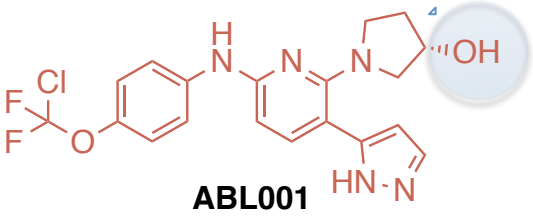


4
cIAP Ligand



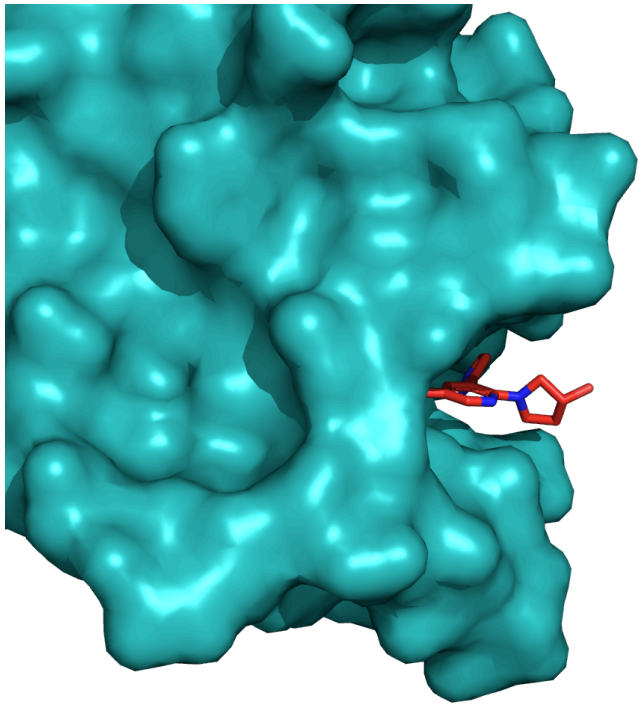
Structural Analysis of ABL001 bound ABL Kinase

Positioning of the linker



ABL001

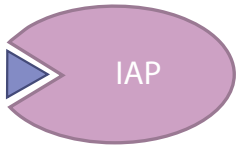
Surface view of ABL Kinase



solvent exposed region

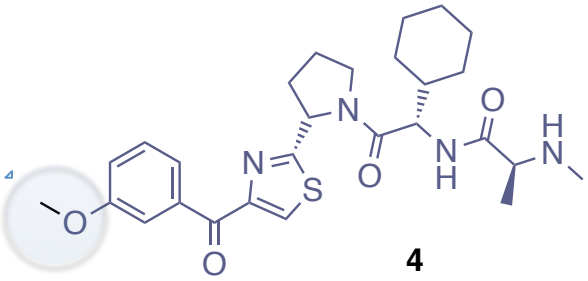
PDB: 5MO4

Nature **2017**, 543, 733.



Structural Analysis of IAP Ligand

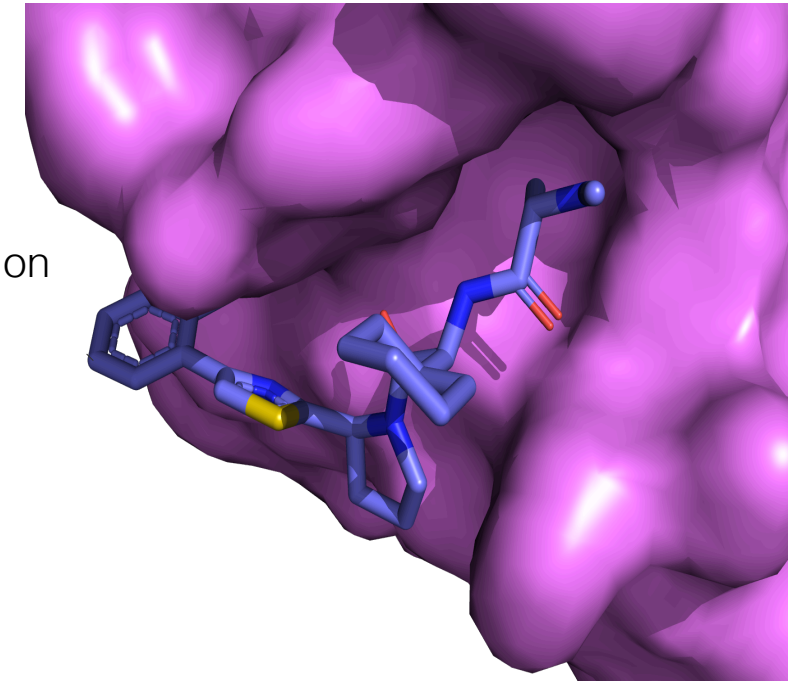
Positioning of the linker



4
cIAP Ligand

Surface view of IAP

solvent
exposed region

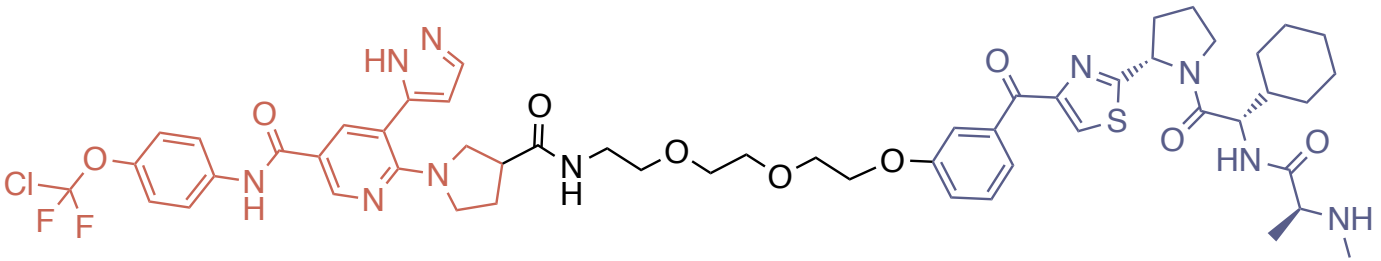


PDB: 3GT9

J. Biological Chemistry **2017**, 292, 4556.
J. Med. Chem. Article ASAP
Bioorg. & Medi.Chem. Lett. **2010**, 20, 2229.

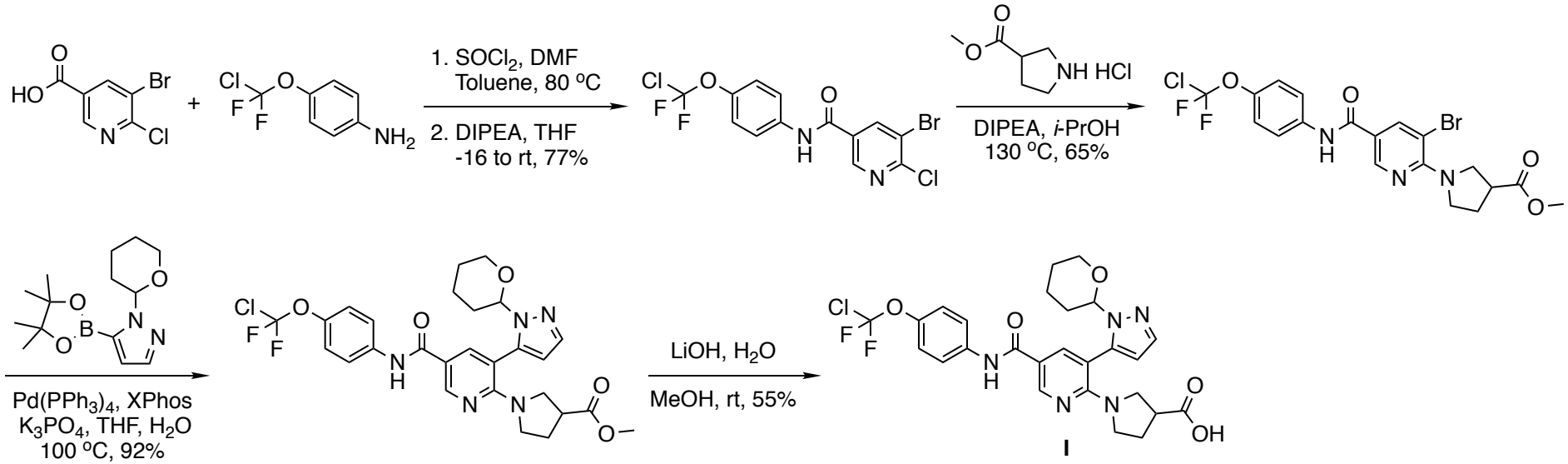
PROTAC Development

ABL Allosteric Ligand | Linker | cIAP Ligand



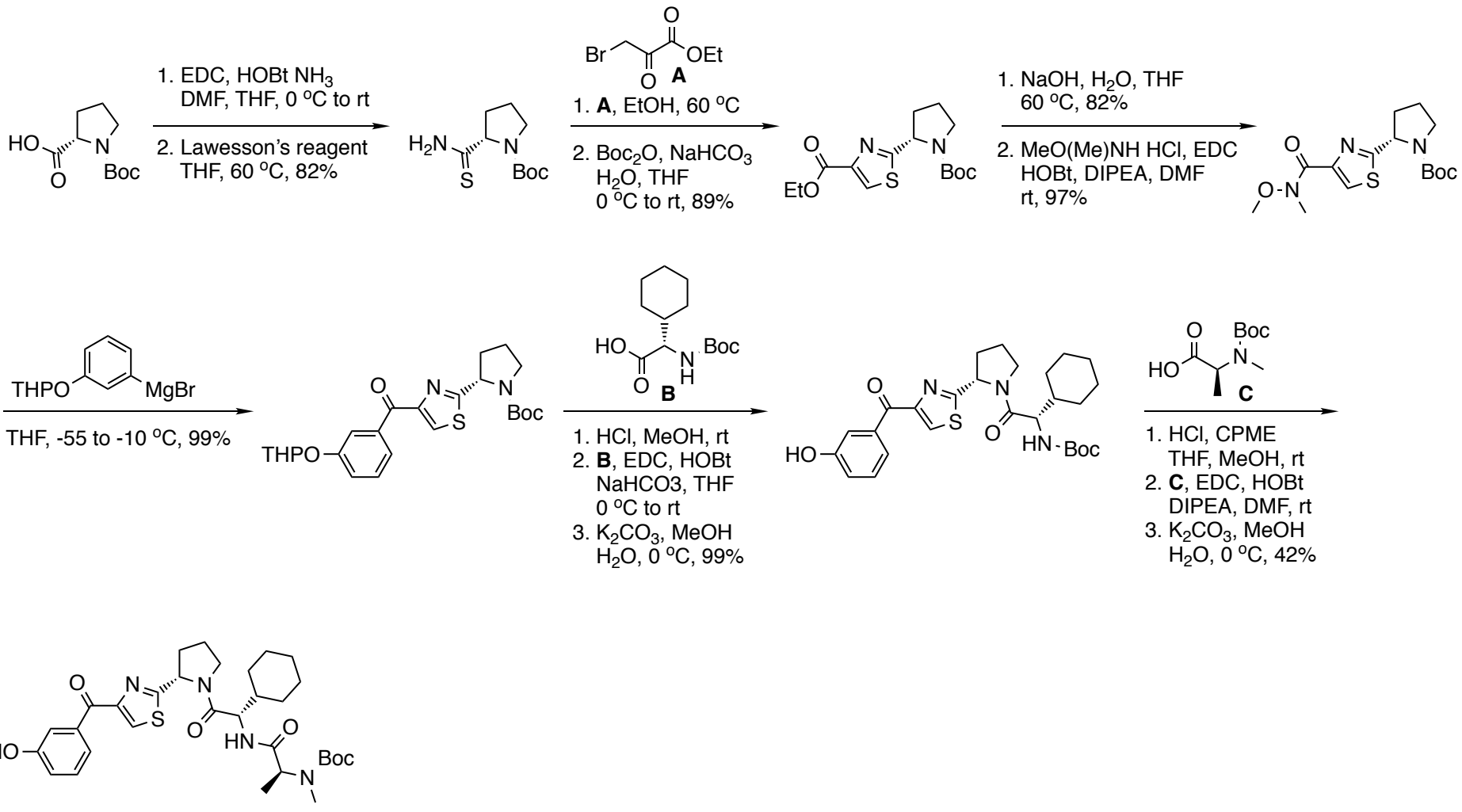
6

Synthesis of the ABL Allosteric Ligand



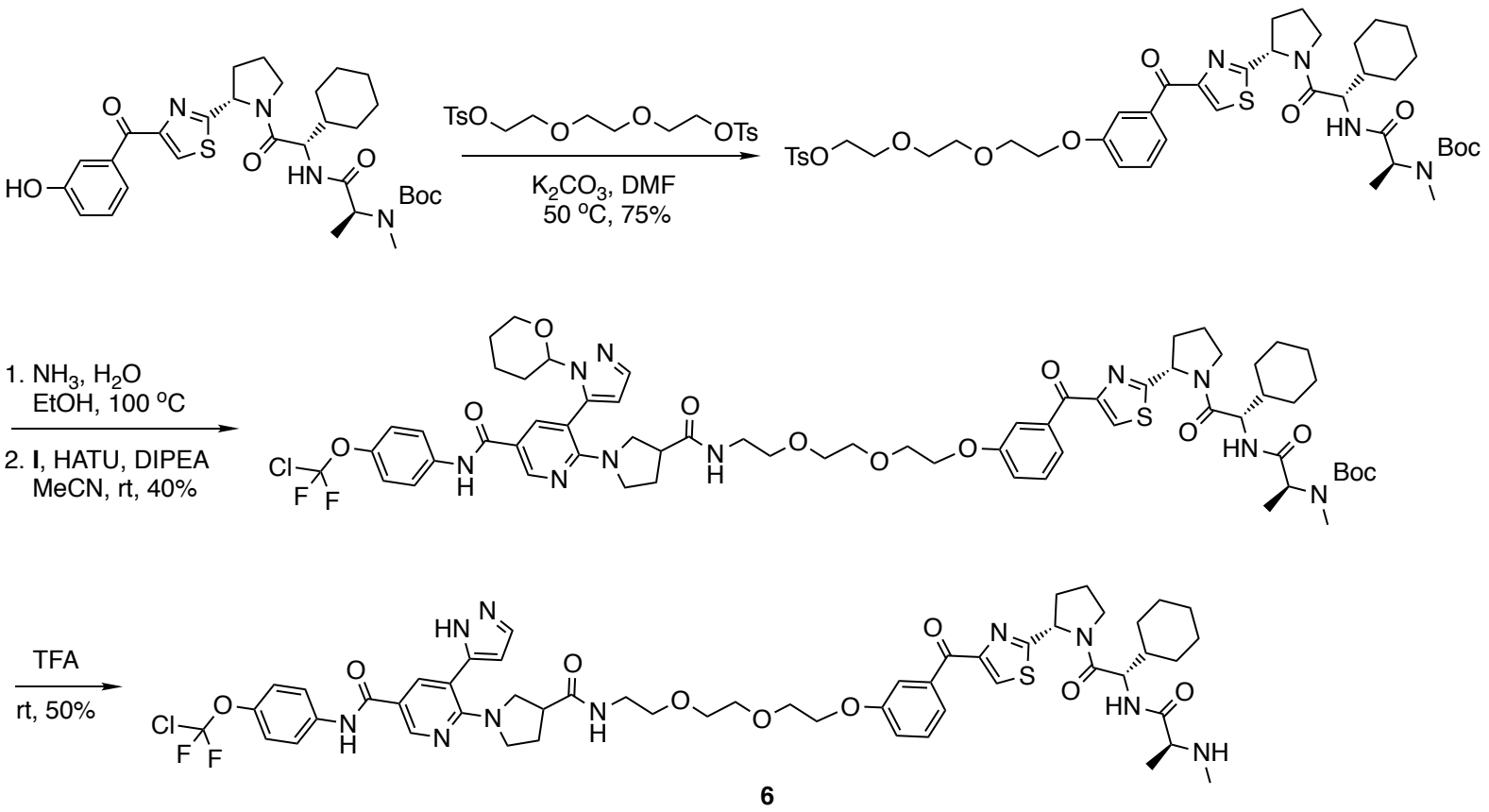
Nature **2010**, 463, 28.
Nature **2017**, 543, 733.

Synthesis of the cIAP Ligand

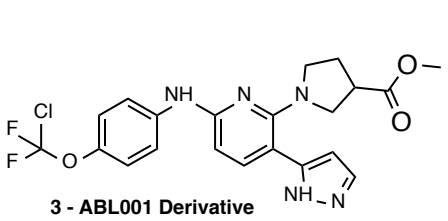


J. Biological Chemistry **2017**, 292, 4556.

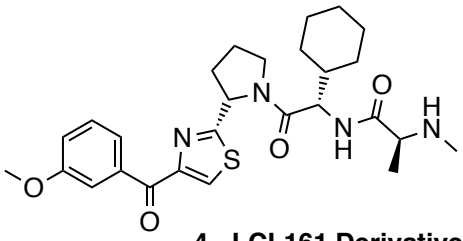
Synthesis of PROTAC – Compound 6



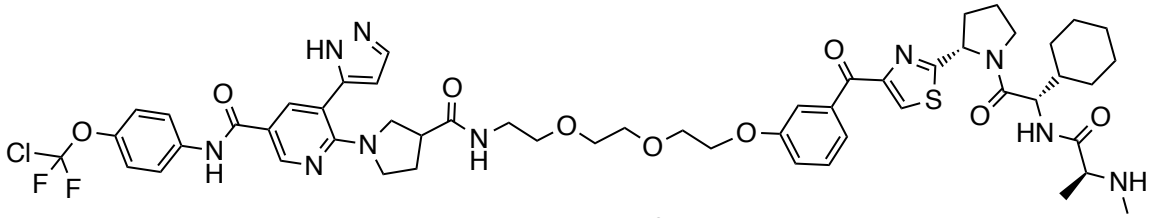
ACS Med. Chem. Lett. **2017**, 8, 1042.



3 - ABL001 Derivative
ABL Ligand



4 - LCL161 Derivative
E3 Ligand



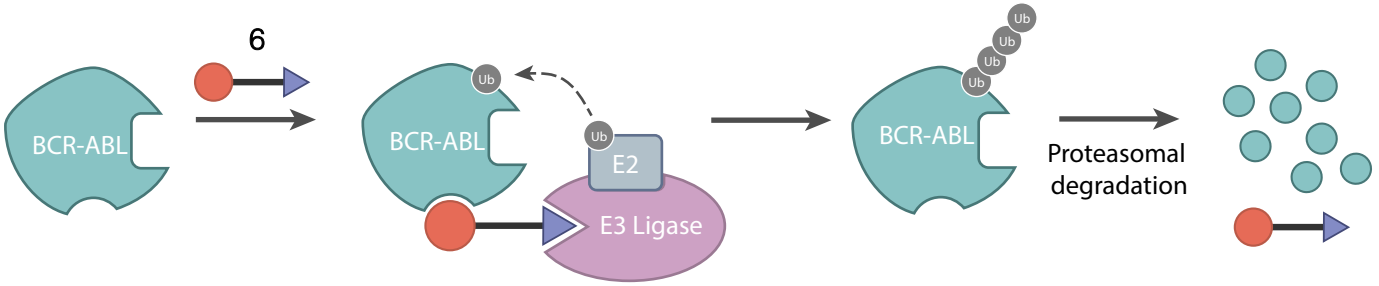
6

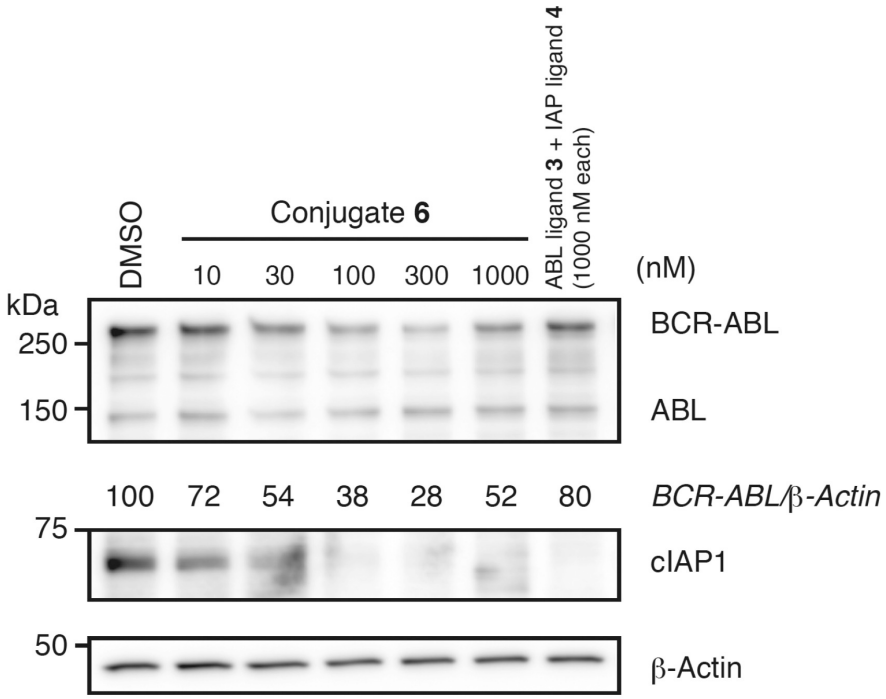
Table 1. Results of TR-FRET Based Binding Assay for ABL1 (Allosteric Site), cIAP1/2, and XIAP[a]

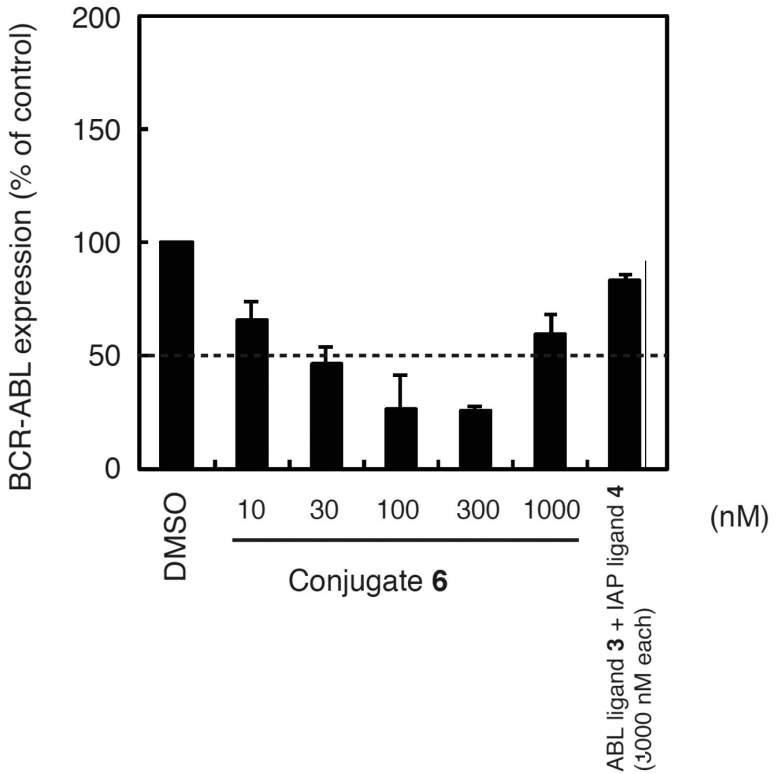
compd	ABL1 IC_{50} (nM)	cIAP1 IC_{50} (nM)	cIAP2 IC_{50} (nM)	XIAP IC_{50} (nM)
1 (GNF-2)	2800 (2000–4000)	n.d.	n.d.	n.d.
2 (GNF-5)	2000 (1400–2900)	n.d.	n.d.	n.d.
3 (ABL001 derivative)	35 (27–45)	n.d.	n.d.	n.d.
4 (LCL-161 derivative)	n.d.	4.8 (4.3–5.4)	5.7 (4.3–7.5)	67 (49–96)
5 (SNIPER(ABL)-21)	2000 (1200–3300)	220 (180–280)	330 (220–500)	94 (70–130)
6 (SNIPER(ABL)-62)	360 (220–590)	86 (78–94)	140 (120–160)	120 (99–140)
7 (SNIPER(ABL)-64)	340 (210–570)	>1000	>1000	>1000

[a]The inhibitory activity of the test compounds at several concentrations were examined as described in Supporting Information. IC_{50} values and 95% confidence intervals were calculated by nonlinear regression analysis of percent inhibition data ($n \geq 3$). n.d.: not determined.

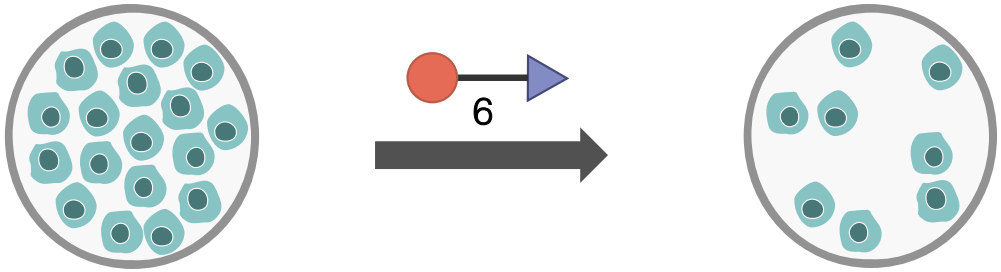
Compound 6-induced BCR-ABL Protein Degradation

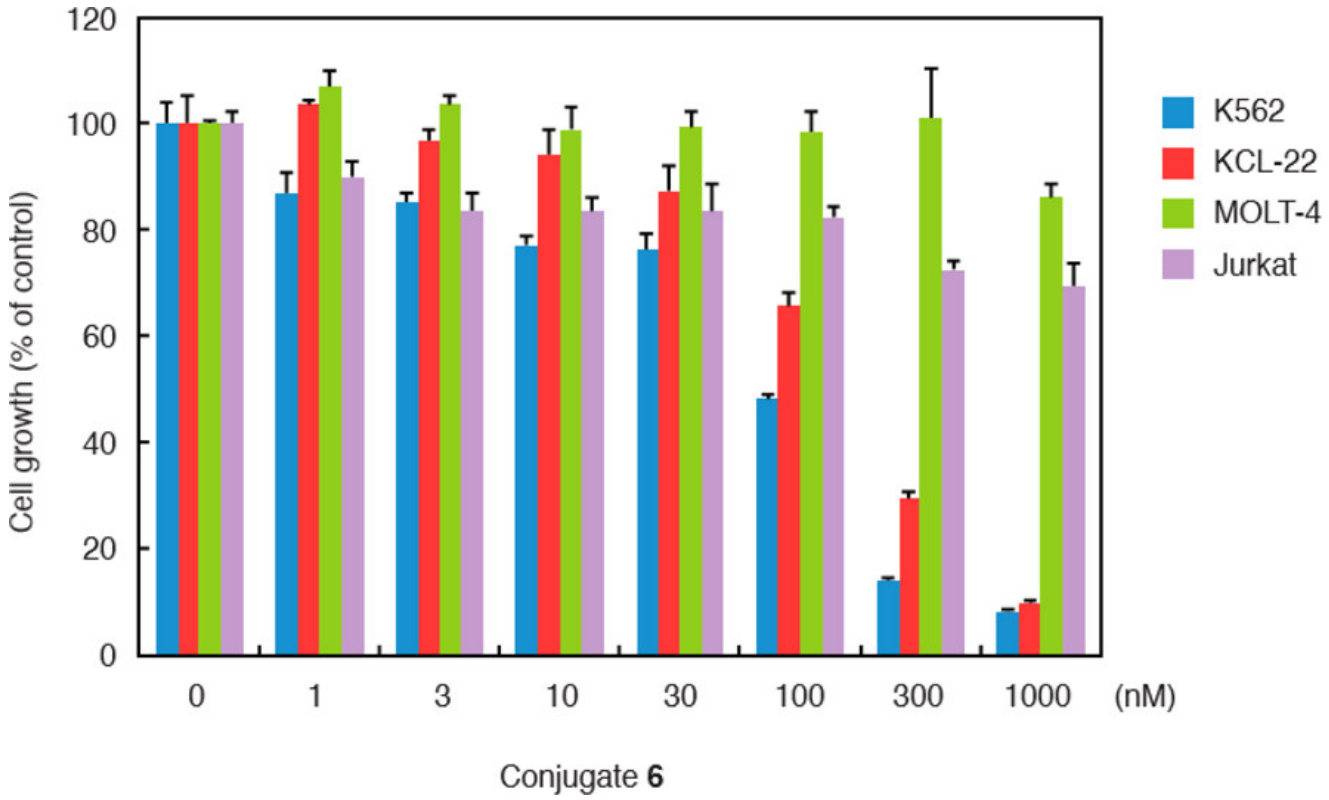






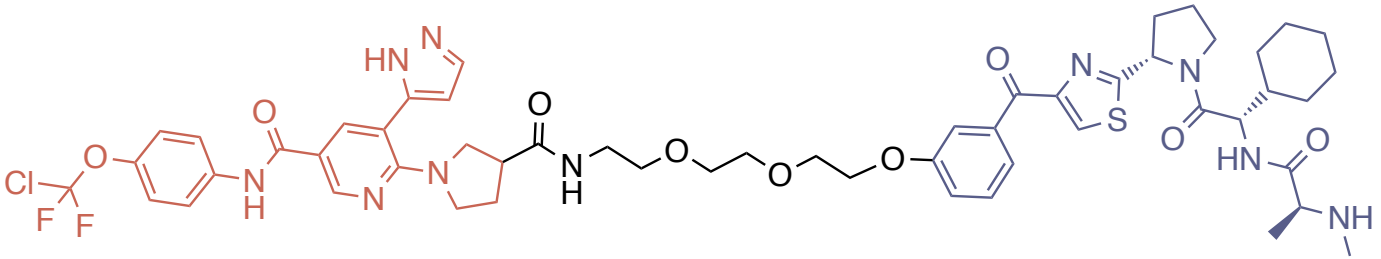
Compound 6 Effect on Cell Proliferation





Summary

ABL Allosteric Ligand Linker cIAP Ligand



6

- Designed and developed an *allosteric* BCR-ABL PROTAC that potently induces BCR-ABL protein degradation in cell assays.

  Half-maximal degradation concentration (DC_{50}): ~30 nM
  Maximum degradation efficacy (D_{max}): ~70% between 100-300 nM

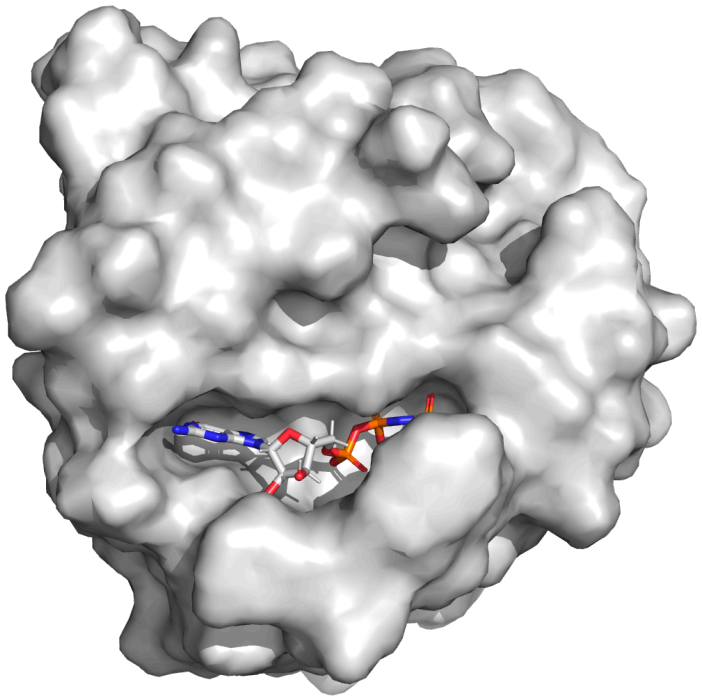
Further developments:

- LCL161 derivative **4** induces auto-ubiquitination leading to IAP protein degradation
- Evaluation of compound **6** in *in-vivo* models

Drugging the "Undruggable"

- The RAS genes constitute the most frequently mutated oncogene family in cancer.
- Approximately ~25% of human tumors have a RAS mutation.
- Despite more than 30 years of effort to develop a pharmacologic inhibitor of RAS, a clinically effective anti-RAS therapy does not exist.
- What approach will prove to be the most effective RAS modulator? Which approach will produce the first clinical anti-RAS agent?

  Perhaps an allosteric RAS PROTAC to induce RAS protein degradation?



Science **2017**, 355, 1158.