Science For A Better Life

BAY-6035
SMYD3 protein methyltransferase inhibitor

probe presentation 2017 & post-meeting info

Carlo Stresemann, Holger Steuber, and Stefan Gradl

SMYD3 protein methyltransferase

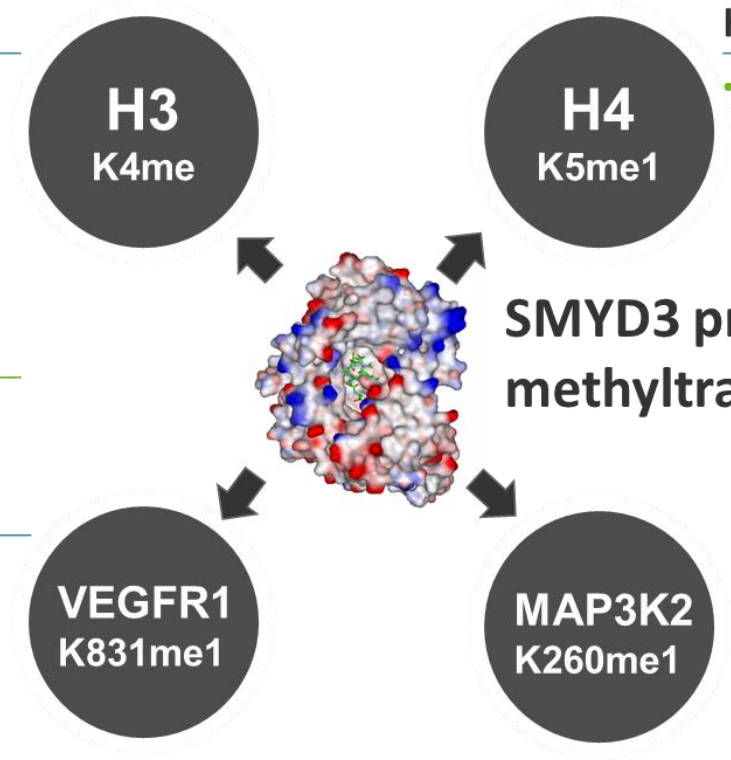
Described roles

Histone H3 (2004)

- H3K4me3 is a hallmark for transcriptional activation
- Transcriptional **up-regulation of oncogenes** e.g. cMET, MMP9, AR
- ER co-factor activity

Histone H4 (2012)

- Function unknown



VEGFR1 (2007)

- Methylated VEGFR1 has increased kinase activity and leads to **angiogenesis stimulation** and **cancers progression**

MAP3K2 (2014)

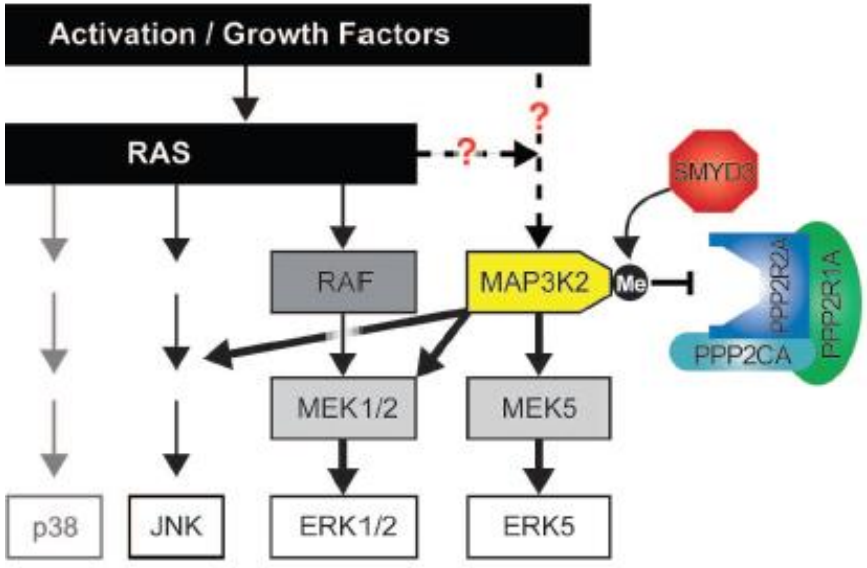
- SMYD3 and MAP3K2 are necessary for full activation of ERK1/2 and MEK1/2 **survival signaling in RAS mutated adenocarcinomas**

SMYD3 is described as a protein methyltransferase regulating transcription of oncogenes and signaling pathways frequently miss-regulated in cancer

SMYD3

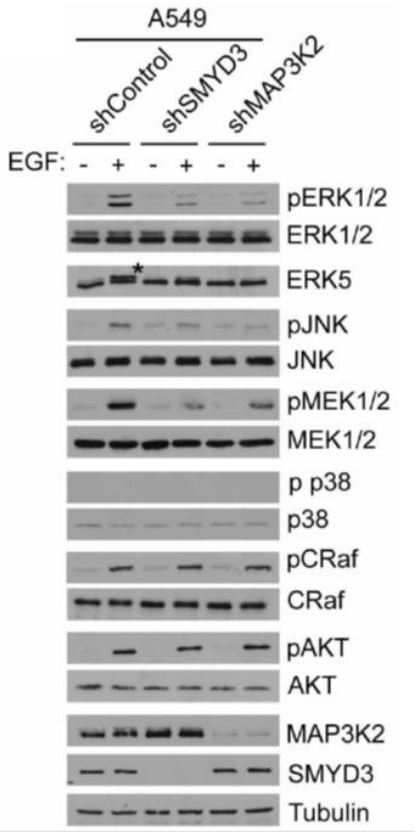
Role in pancreatic cancer (*Mazur et al. 2014*)

SMYD3 links lysine methylation of MEKK2 (MAP3K2) to Ras-driven cancer:

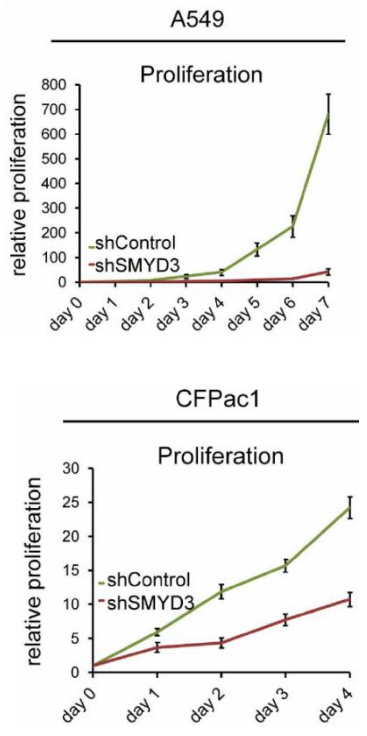


Mazur et al. 2014

MEK/ERK pathway



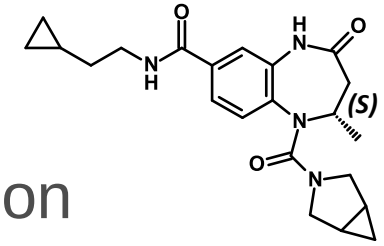
Proliferation



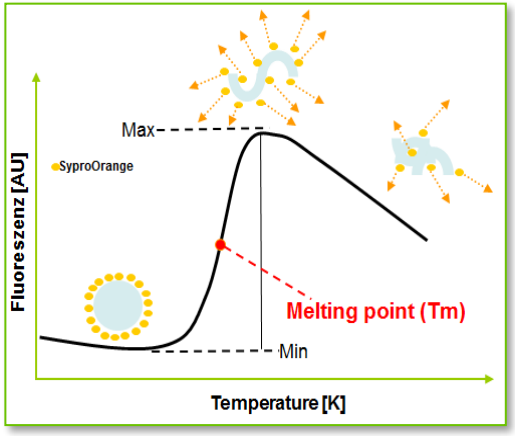
Recent literature has highlighted the role of SMYD3 in MAPK signaling pathways

BAY-6035

Primary screen and optimization

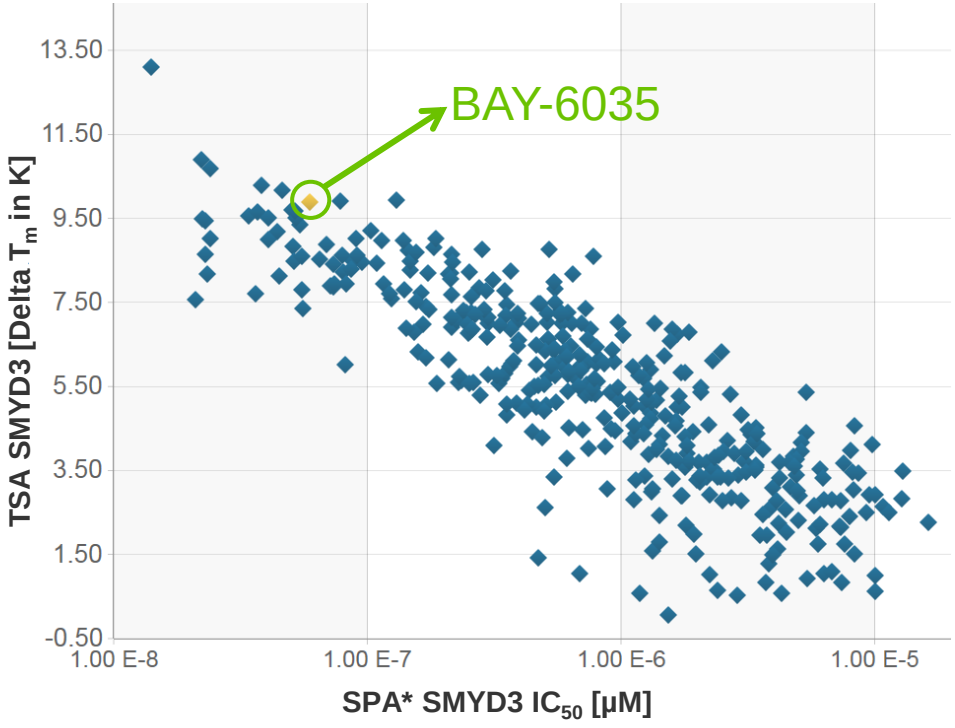


Initial Screening: TSA assay



Principle:	Thermal shift assay (Delta T_m)
Tested cmpds:	410,000
Concentration:	120 µM
Hits:	**1,239** (used for IC_{50} determ.)
Final hit rate:	0.3%

SPA vs. TSA stabilization of BAY-6035 lead series

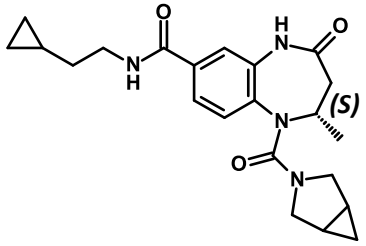


*Scintillation proximity assay with MEKK2 substrate peptide

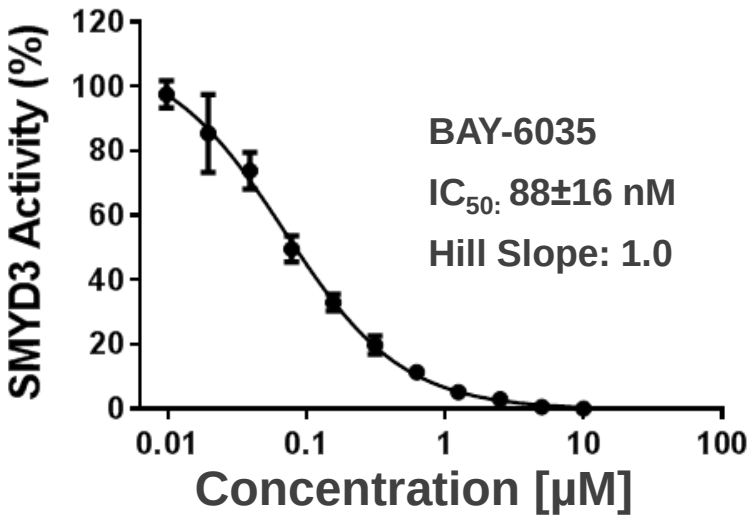
For optimization of compounds SPA assay and TSA was routinely used

BAY-6035

Potency



Scintillation proximity assay



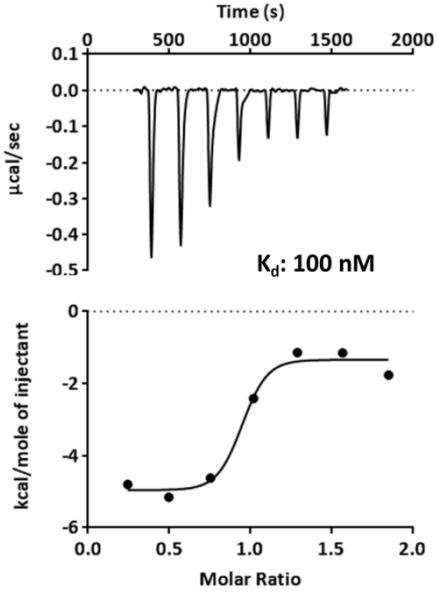
BAY-6035

$IC_{50:}$ 88±16 nM

Hill Slope: 1.0

Assay Conditions:
10 nM SMYD3, SAM: 335 nM, Peptide [MEKK2 derived]: 15 µM. Incubation for 40 min at 23°C. Buffer: 50 mM Tris pH 9, 2.5 mM DTT, 0.02% Tween 20
Experiments were performed in in quadruplicate.

Confirmation by ITC



BAY-6035

Kd: 100 nM
N: 0.8
∆H: -3.6 kcal/mol
∆S: 19.8 cal/ mol.K

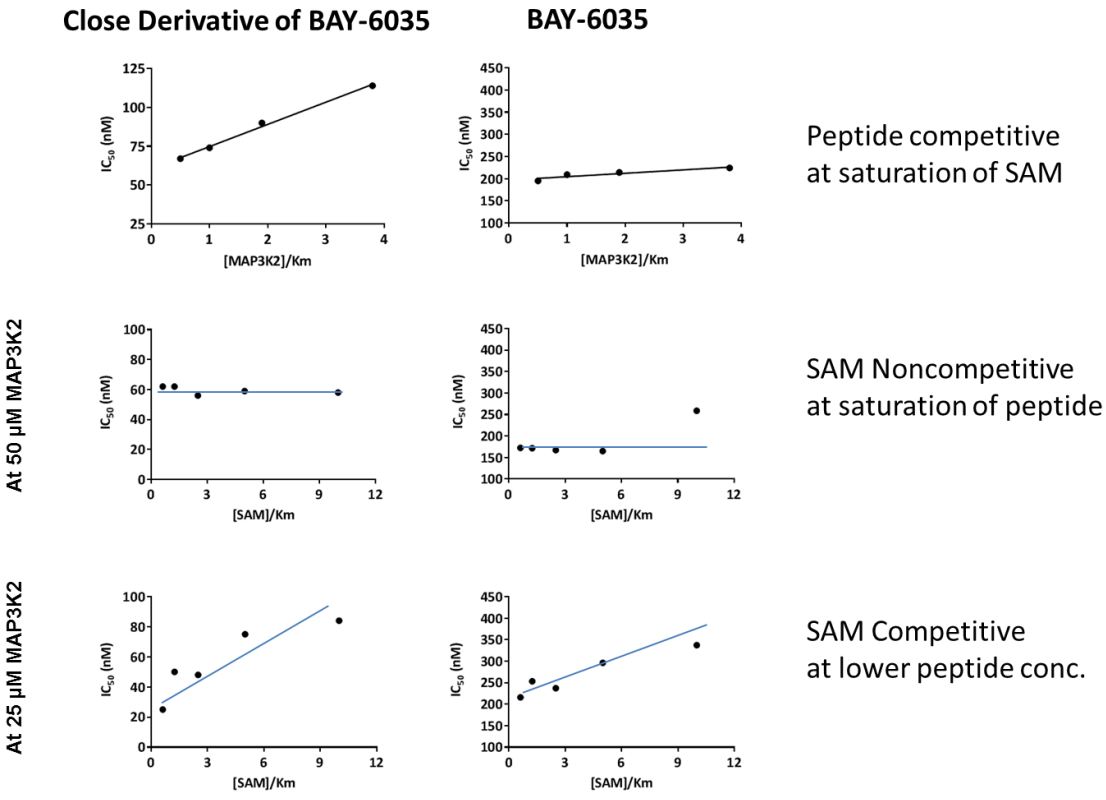
Assay Conditions:
8 injections of 4 µL each in 180 s intervals were performed. The heat production appears during the first few injections. In Syringe: 20 mM HEPES pH 7.4, 150 mM NaCl, 0.005% Tween 20, compound concentration of 0.3 mM and DMSO Concentration 2.5%.

BAY-6035 is a sub 100 nM inhibitor and binding was confirmed by ITC

BAY-6035

MoA study

MOA: Mixed Inhibition Pattern



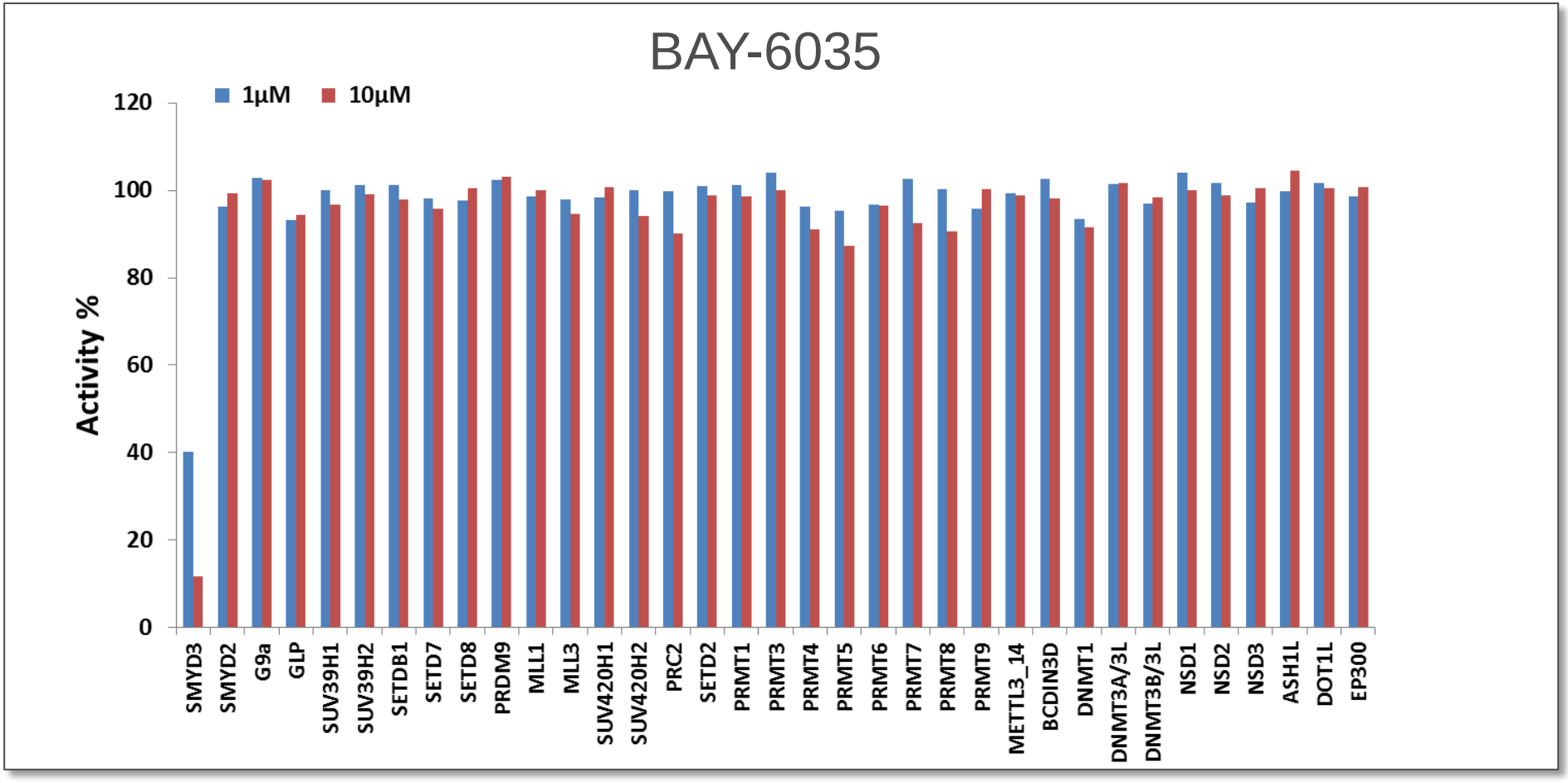
Peptide competitive
at saturation of SAM

SAM Noncompetitive
at saturation of peptide

SAM Competitive
at lower peptide conc.

BAY-6035

Selectivity against PMTs and DNMTs

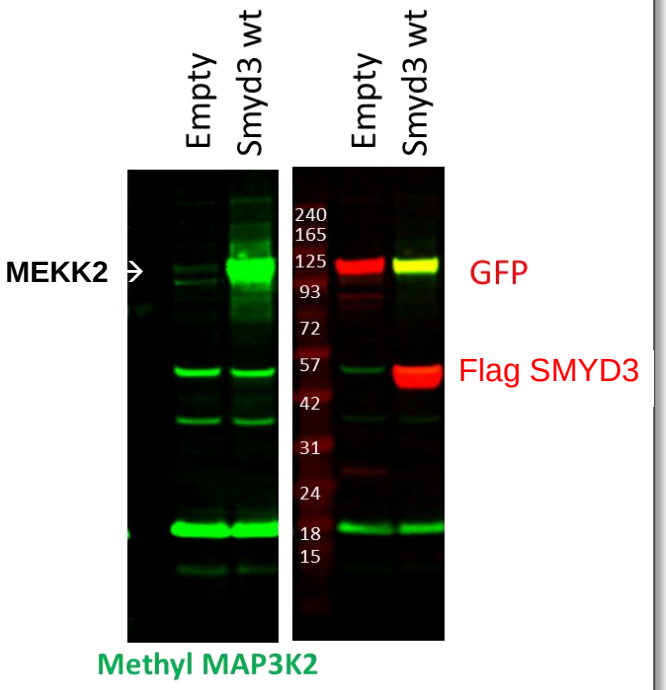


BAY-6035 showed strong selective inhibition of SMYD3

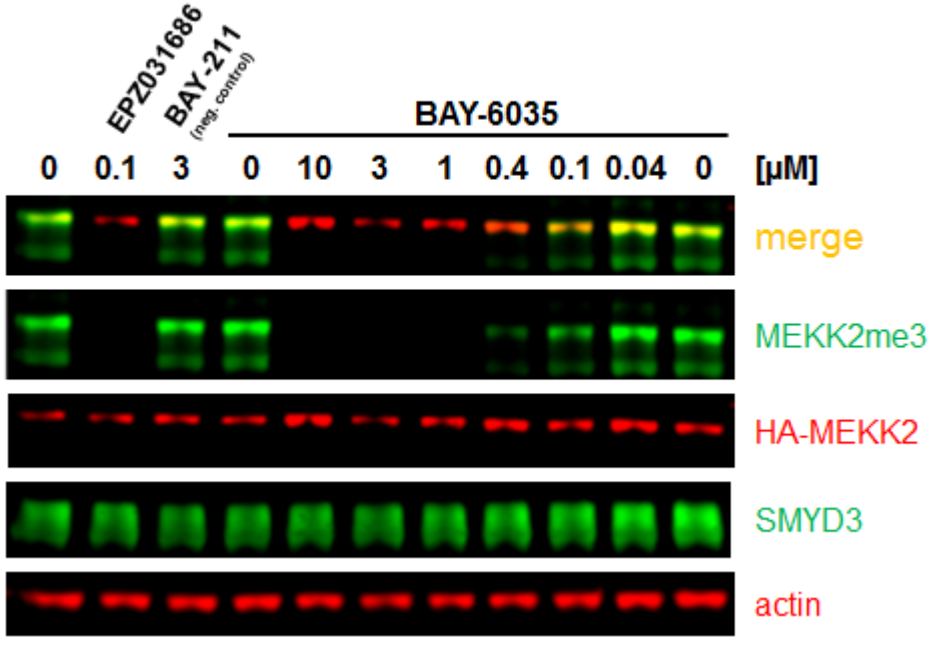
BAY-6035

Cellular mechanistic assay

Methylation of MEKK2-GFP detected with a K260me3 specific antibody:



Cellular assay: Dose response in HELA cells



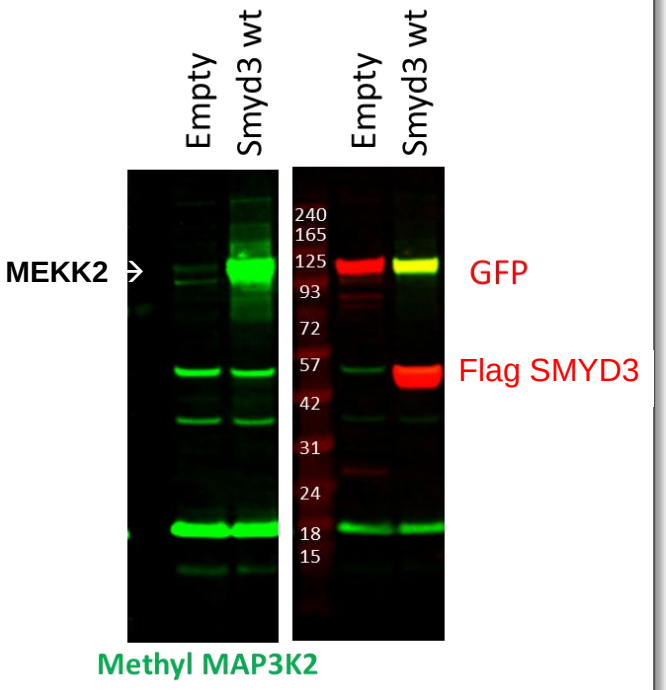
Assay: Hela cells were transfected with Smyd3 and HA-MEKK2, treated with compounds and MEKK2 methylation assessed using specific antibody in western blots. The methyl MEKK2 signal was normalized to total MEKK2.

Cellular methylation of MEKK2 by SMYD3 is inhibited by BAY-6035

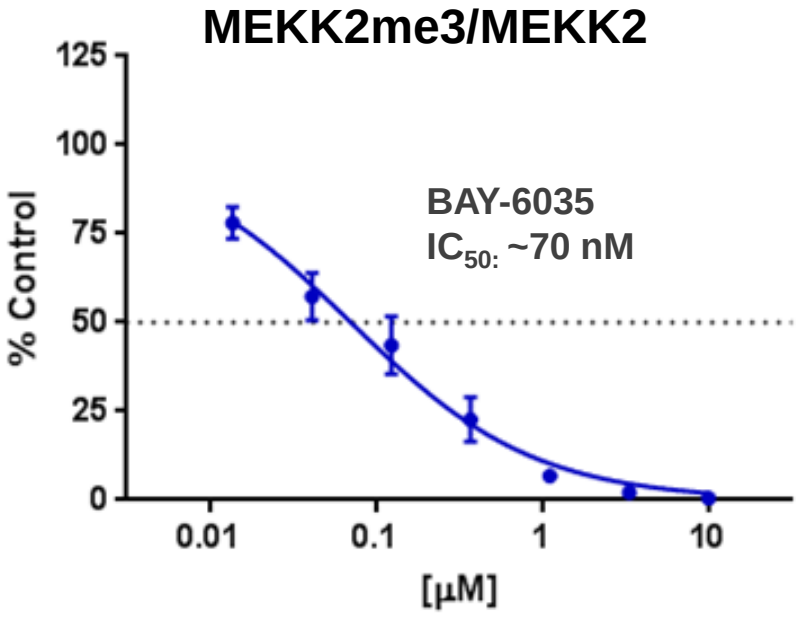
BAY-6035

Cellular mechanistic assay

Methylation of MEKK2-GFP detected with a K260me3 specific antibody:



Cellular assay: IC_{50} determination



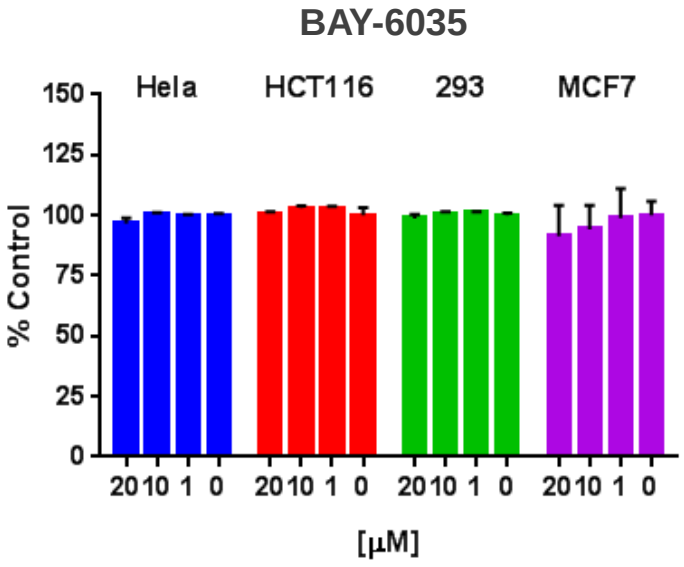
Error bars represent SD from triplicate wells of 1 biological replicate.
Previous biological replicate with duplicates showed same IC50.

BAY-6035 has a cellular mechanistic IC_{50} of 70 nM

BAY-6035

Proliferation effects

Cell lines were treated with compounds for 72h monitoring cell confluence as toxicity readout

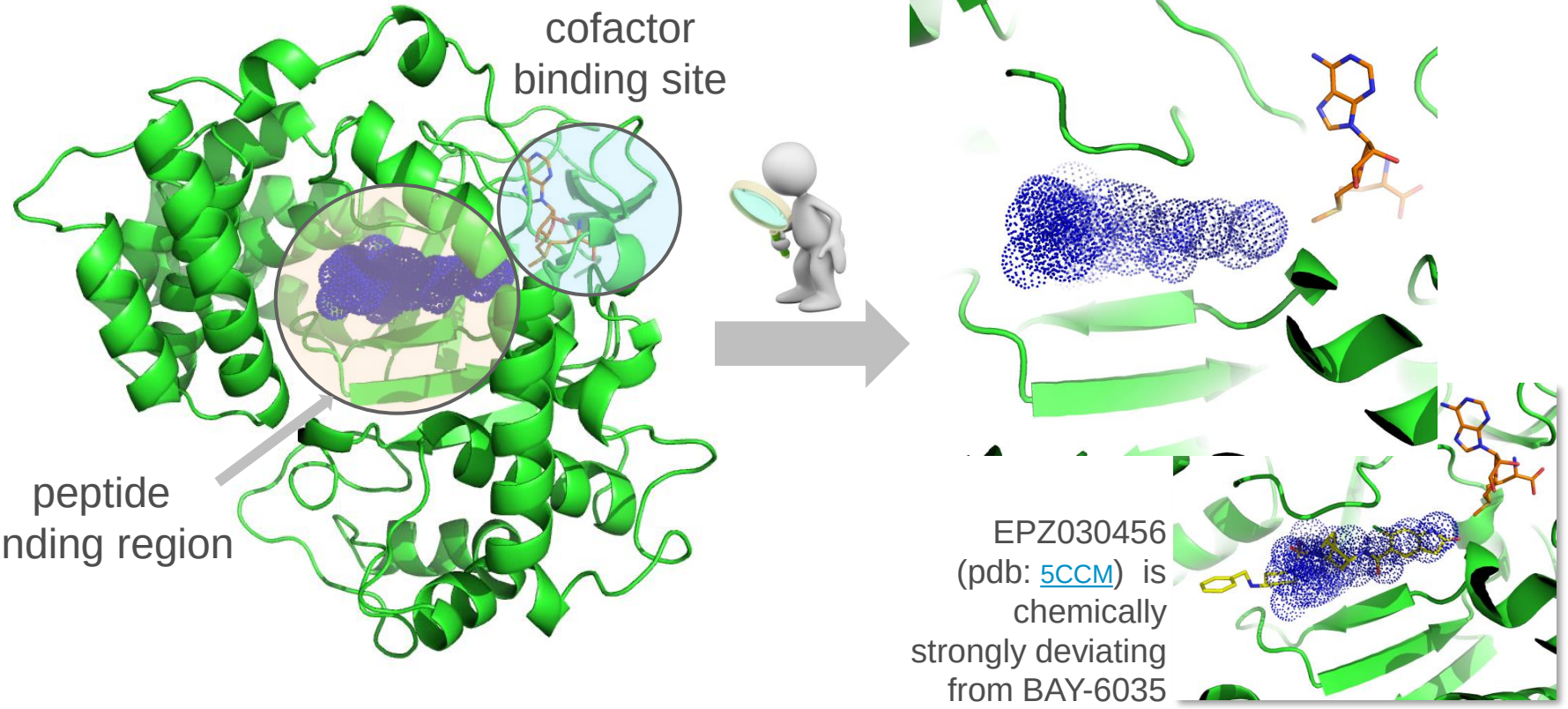


No cell toxicity observed for BAY-6035 up to 20µM at 72h, Compounds should be used at 1uM or below.

Binding mode

X-ray structure of close analogue 7O2A (7O2B)

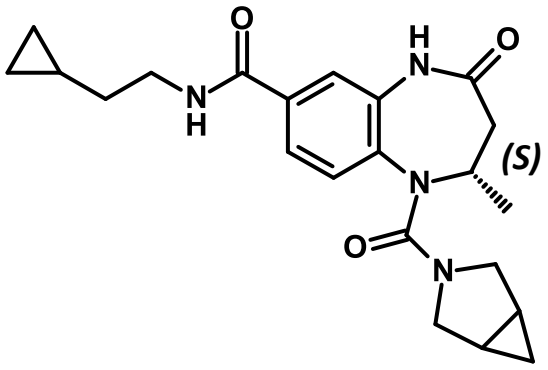
1.6 Å resolution



BAY-6035 occupies peptide binding region in a structurally conserved manner

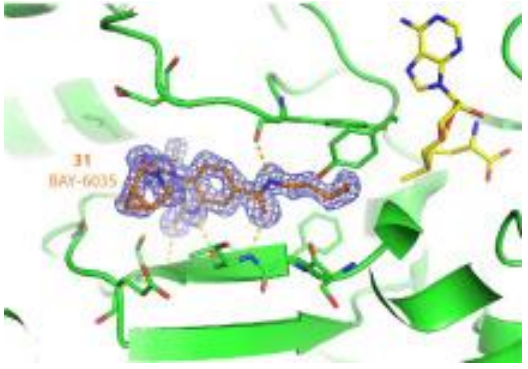
SMYD3 Probe

BAY-6035



Potency	
SMYD3 SPA IC_{50}	88 nM
Cellular mechanistic IC_{50} (MEKK2me3)	~70 nM
TSA Delta Tm [K]	9.9
ITC Kd	100 nM

Selectivity
No activity in 34 other MT's (incl. SMYD2)



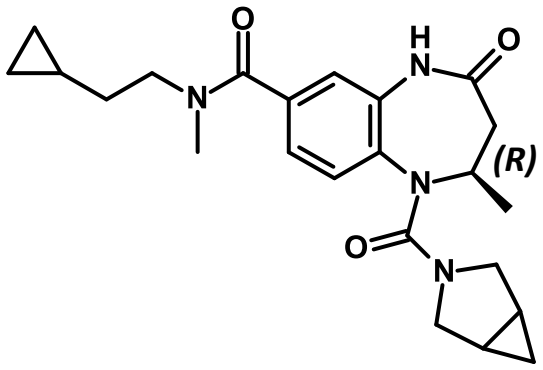
pdb 7O2C

Properties & Physchem	
LogD @pH 7.5	1.9
BEI / LLE (calc)	18 / 5.5
Sw pH 6.5 [mg/L]	363
MW / TPSA [g/mol; Å²]	396 / 82
Stability (plasma)	tbd
Stability (pH 1, 7, 10)	tbd

in vitro PK			
Caco2	A-B [nm/s]	B-A [nm/s]	Efflux Ratio
	4.4	213	48
Metabolic Stability		CL [L/h/kg]	F_{max} [%]
	Human Mics	0.3	74
	Rat Heps	0.83	80

Negative Control

BAY-444



Potency	
SMYD3 SPA IC_{50}	>10 µM
Cellular mechanistic IC_{50} (MEKK2me3)	>10 µM
TSA Delta Tm [K]	n.d.
ITC Kd	n.d.

BAY-444 is a close analogue of BAY-6035

BAY-6035

Summary

- SMYD3 inhibitor BAY-6035 exhibits excellent potency (IC_{50} 88 nM) and selectivity (greater than 50-fold) over other protein and DNA methyltransferases
- BAY-6035 inhibits SMYD3-dependent cellular methylation with an IC_{50} of 70 nM
- BAY-444 is an excellent negative control for BAY-6035 (IC_{50} >10 µM)
- Crystal structure has been solved and BAY-6035 is structurally distinct from other SMYD3 inhibitors (e.g. EPZ031686)
- **S. Gradl et *al*. Discovery of the SMYD3 Inhibitor BAY-6035 Using Thermal Shift Assay (TSA)-Based High-Throughput Screening. *SLAS Discov.* 2021, *26*, 947–960.**

We are offering BAY-6035 as a novel chemical probe for SMYD3 along with BAY-444 as a negative control.

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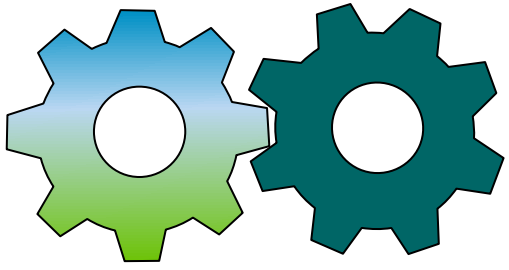

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