Science For A Better Life

SMYD2 chemical probe

BAY-598

C. Stresemann, T. Stellfeld, R. Hillig, V. Badock, N. Barak

SGC CPSC, April 9th 2015

SMYD2 Pharmacology

Function as Protein Methyltransferase

Histone H3

Unclear role in gene regulation

H3
K36me2
K4me

Rb
K810me

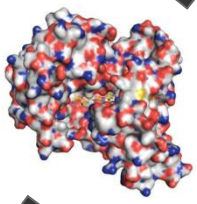
Retinoblastoma protein

Methylation leads to increased cell cycle progression

Estrogen receptor

Attenuation of the chromatin recruitment

ERα
K266me1



p53
K370me1

Tumor suppressor p53

Reduced recruitment of p53 to target genes

(Huang et al., Nature 2006)

Poly (ADP-Ribose) Polymerase

Stimulation of DNA repair activity

PARP
K528me1

Hsp90
K209, K615, K531, K574

Hsp90

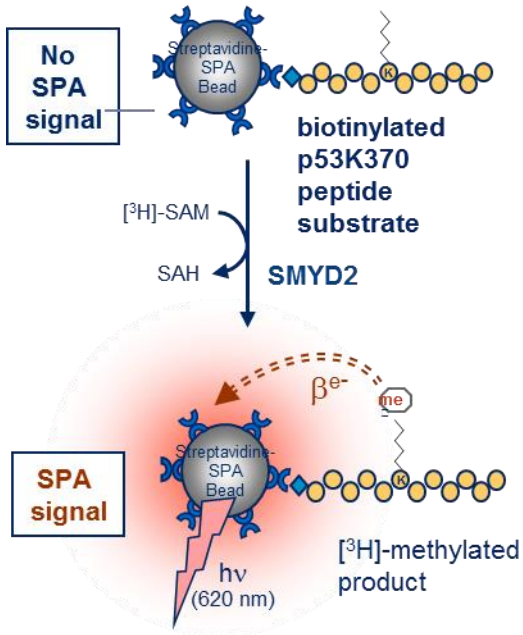
Stabilization of Hsp90 complexes

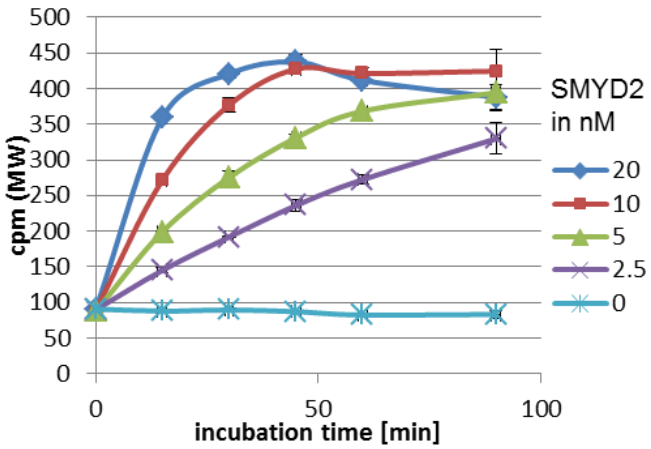
SMYD2 biology is still in an early evolving phase:
Chemical probe suitable for in vivo will foster SMYD2 characterization

SMYD2 biochemical assay

SPA assay with p53 peptide substrate

SMYD2 Scintillation Proximity Assay (SPA)





Protein:
3 nM recombinant His-tagged SMYD2 expressed in Sf9 insect cells

Cofactor:
60 nM 3H-SAM (Km)

Substrate:
1 µM p53K370 peptide

Beads:
25 µg/well streptavidin PS beads

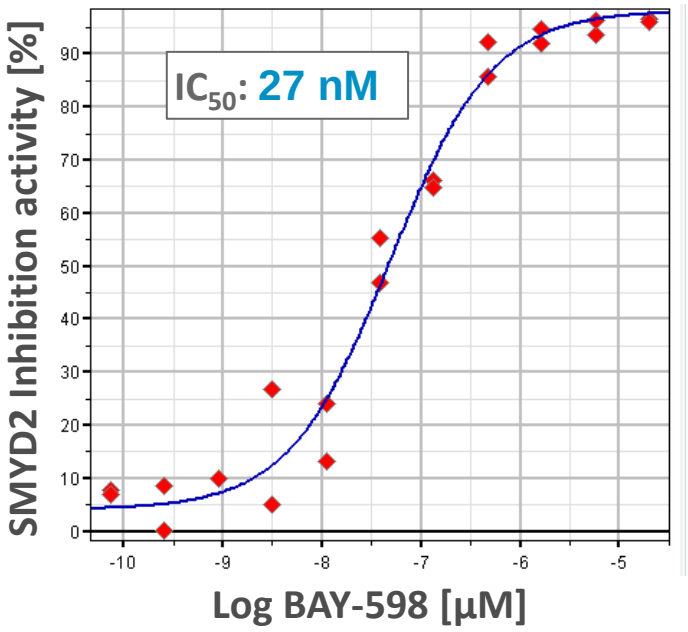
Buffer condition
50mM Tris/ HCl pH 9, 0.0022% Pluronic, 1mM DTT, 0.01% BSA

Other parameters:
- 4 µl assay volume →10 µM compound concentration, 0.1% DMSO
- Assay code: HCS-PYH

Chemical probe BAY-598 resulted from an uHTS campaign

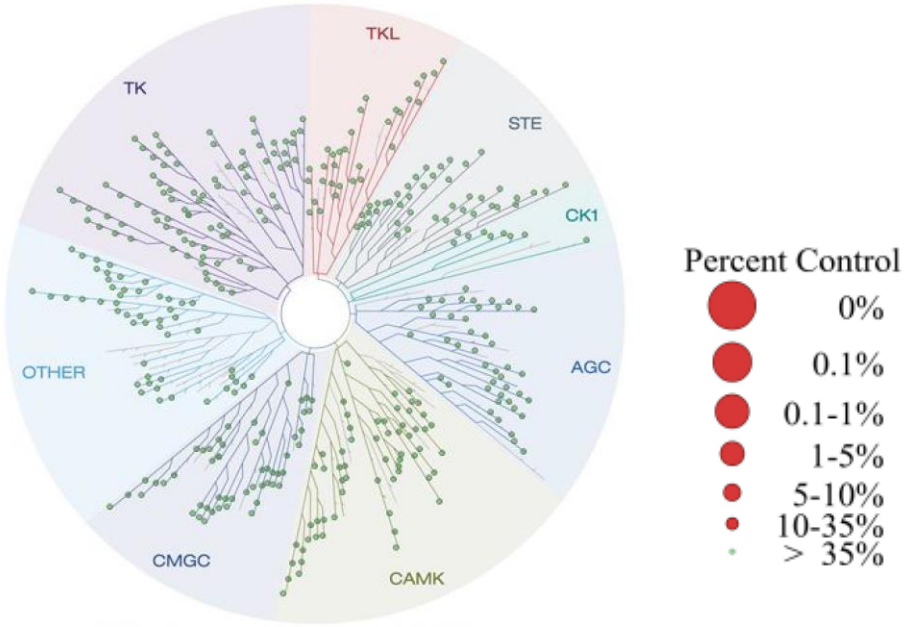
BAY-598
Enzymatic data

SMYD2 enzymatic SPA assay



Kinase selectivity [DiscoveRx-Panel]:

→ No significant activity @1 µM on 395 kinases:



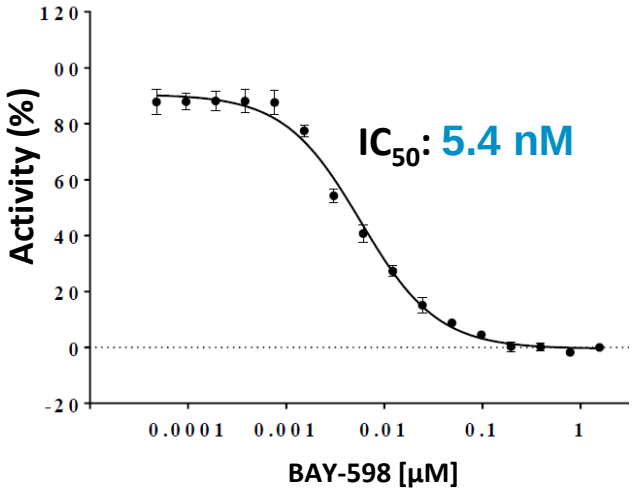
BAY-598 is a potent (IC_{50} < 50 nM) and selective SMYD2 inhibitor

BAY-598

Protein methyltransferase selectivity

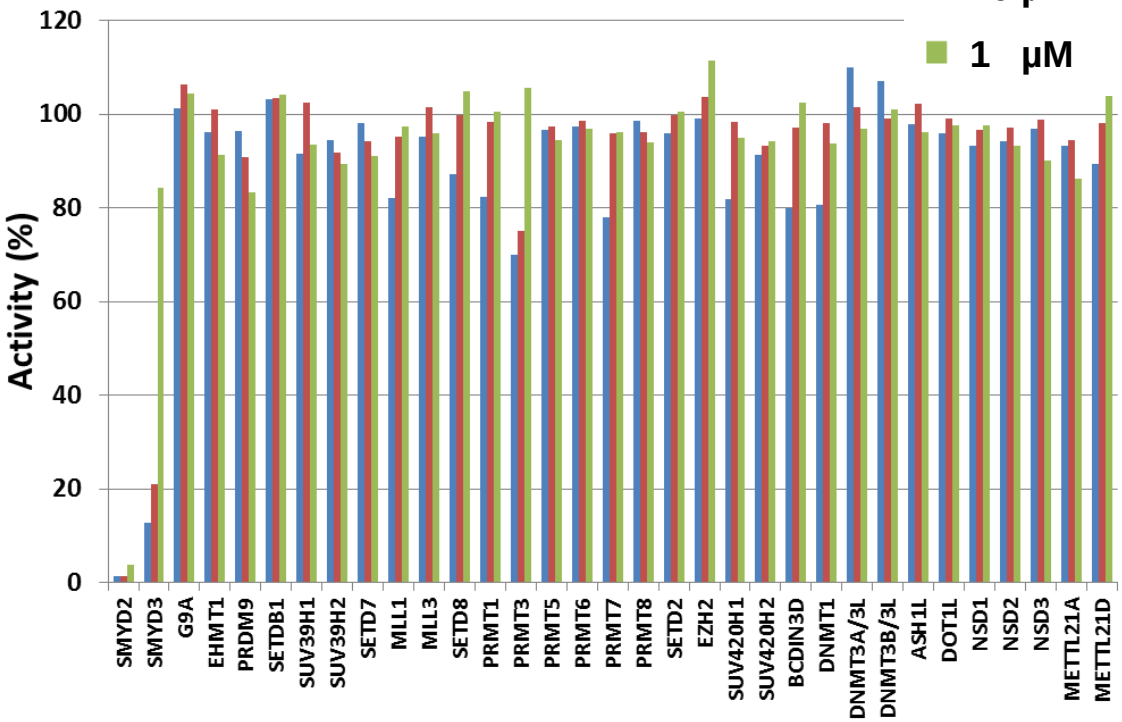
SGC data: Masoud Vedadi, Steven Kennedy, Fengling Li, Taraneh Hajian, Elisa Gibson

SMYD2 (SGC assay):



Conditions:
Buffer: 20 mM Tris pH 9, 2.5 mM DTT, 0.01% TritonX
Enzyme: 5 nM
Peptide 3 µM
SAM 70 nM
23°C for 20 min

Methyltransferase panel (SGC):



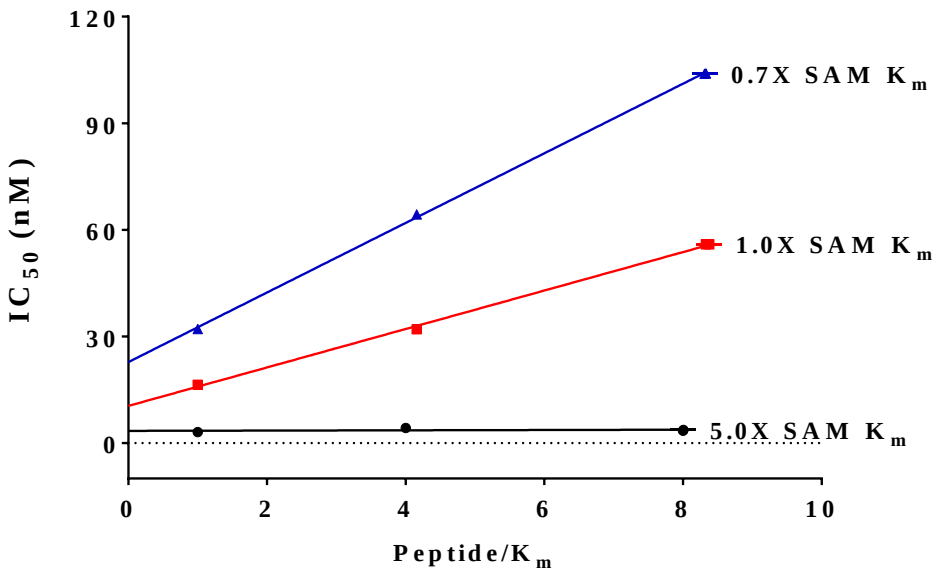
BAY-598 is > 100 fold selective over other protein methyltransferases

Mode of action Assay

SAM/Peptide competition

SGC data: Masoud Vedadi, Steven Kennedy, Taraneh Hajian

MOA of BAY-598:



- At a saturating concentration of SAM BAY-598 has an apparent non-competitive behavior with respect to peptide
- Assay at Km of SAM and 0.7 K_m of SAM resulted in a linear increase in IC_{50} values consistent with a peptide competitive mode of inhibition.

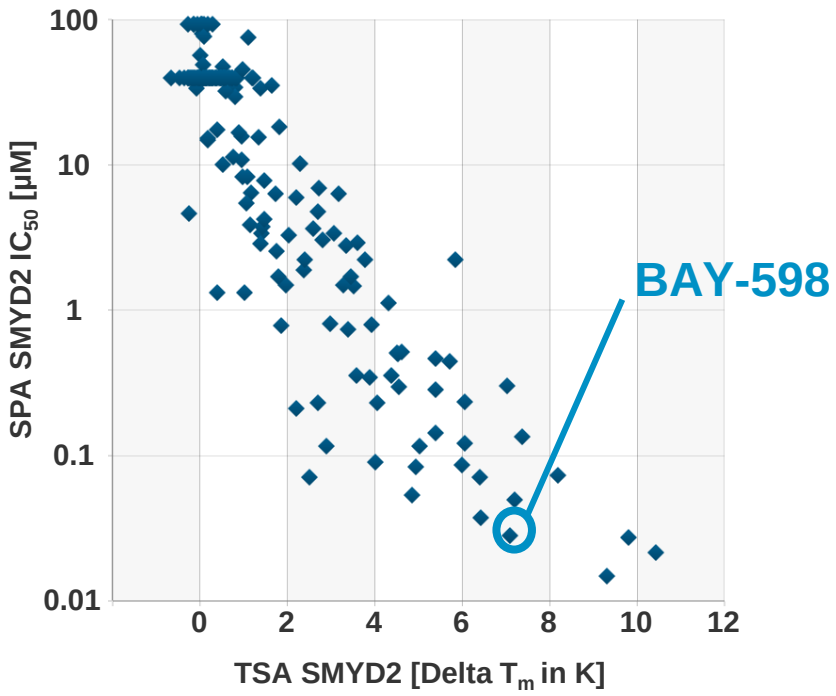
→ These data indicate SAM dependent inhibition of BAY-598

BAY-598 is a peptide competitive inhibitor with SAM contribution

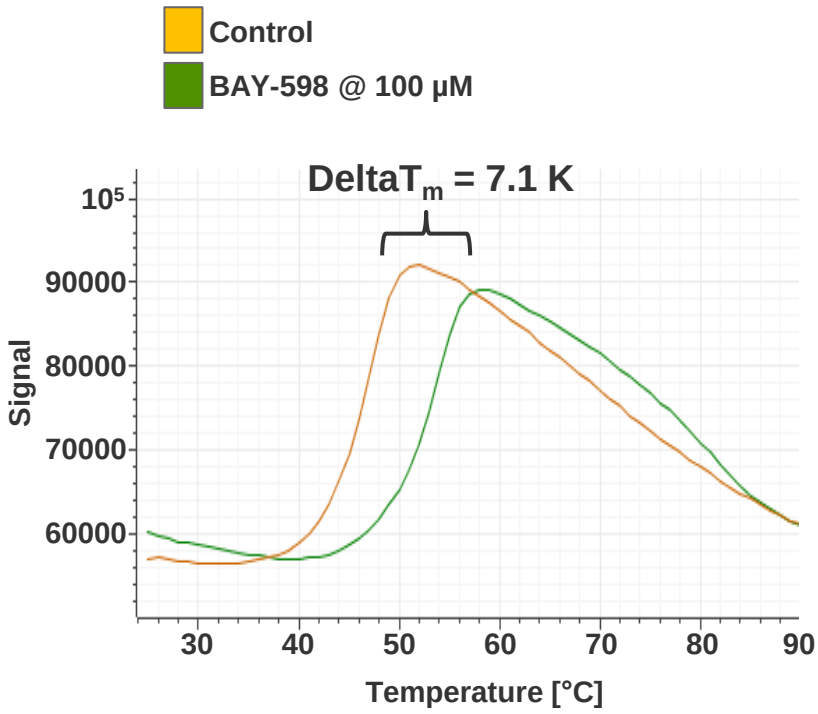
SMYD2 Inhibitor Optimization

Use of TSA-Data for Compound Selection

SMYD2 stabilization vs. potency



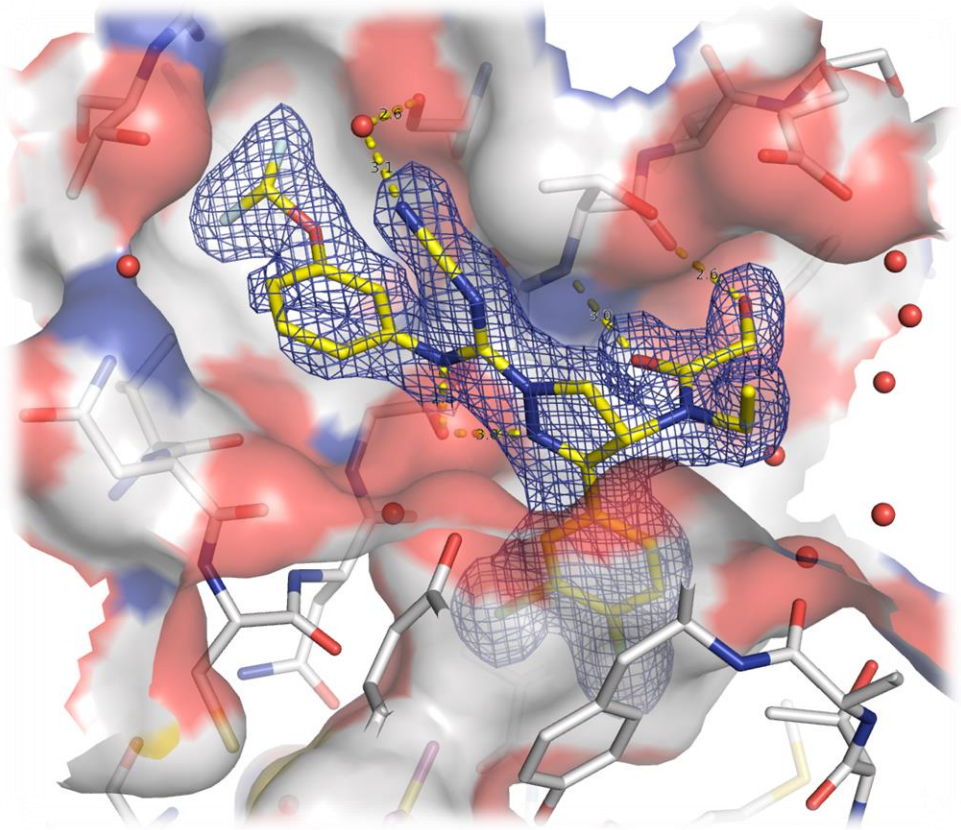
BAY-598 stabilizes SMYD2

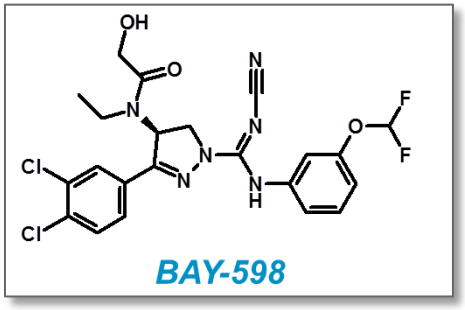


Very good correlation between SMYD2 stabilization and biochemical potency

Binding Mode of BAY-598

X-Ray Structure of SMYD2 with BAY-598





- BAY 598 soaked into preformed SMYD2-SAM crystals
- **2 Å resolution**; clear density for bound ligand in peptide binding pocket
- BAY-598 forms network of hydrogen bonds with SMYD2; **binding is mainly enthalpy-driven** based on ITC studies

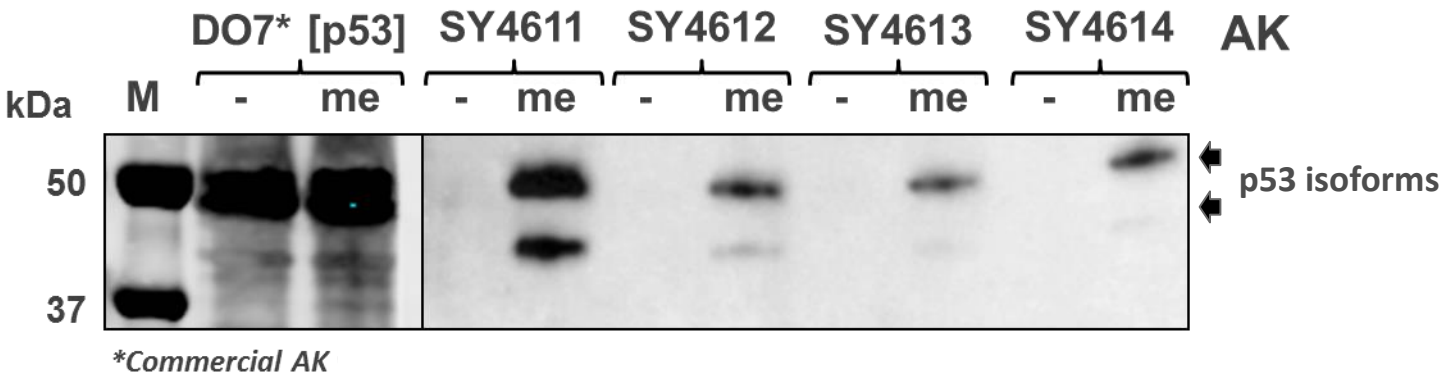
Ladbury *et al. Nat. Rev. Drug Disc.* **2010**, *9*, 23.
Tarcsay et al. *DDT* **2015**, *20*, 86.

BAY-598 binds into the peptide binding site of SMYD2

Cellular Mechanistic Assay

p53: Validated Substrate for SMYD2

Test of customized antibodies on recombinant methylated (me) vs. non-methylated p53:



*Commercial AK

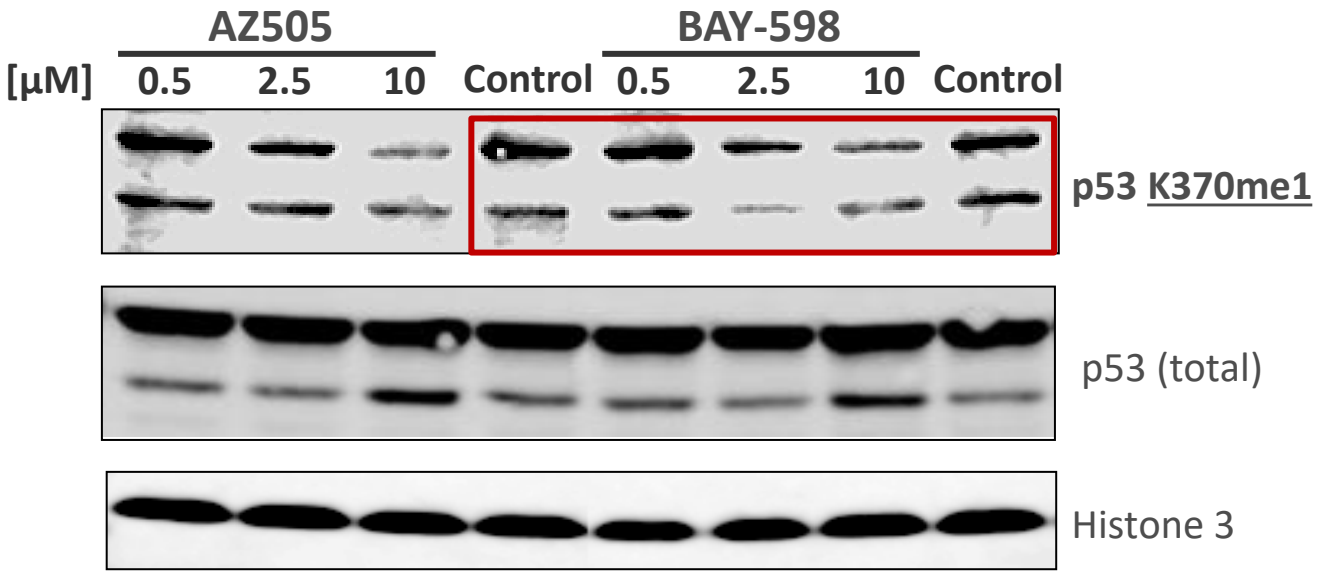
- Recombinant full-length p53 has been methylated in vitro by SMYD2
- Four different polyclonal antibodies have been generated against mono-methylated p53 protein (p53K370me1)
- AKs were purified against p53K370me0 and p53K370me2

p53 was validated in vitro as a substrate of SMYD2

Cellular Mechanistic Assay

Inhibition of endogenous p53 methylation

Analysis of endogenous p53 methylation in KYSE-150 cells:



KYSE-150 (p53 mutated + SMYD2 amplified) cells have been treated for 120h

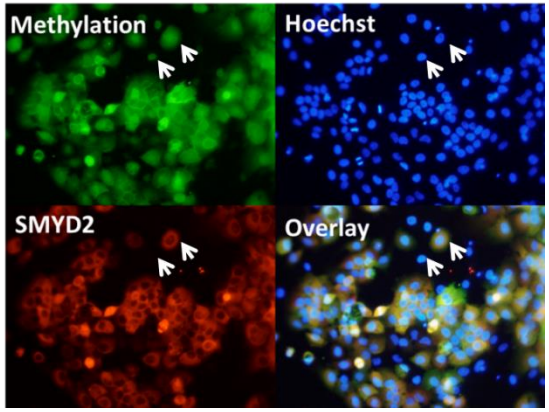
BAY-598 inhibits significantly endogenous methylation of p53

SMYD2 Inhibitor Optimization

Cellular Mechanistic Assay

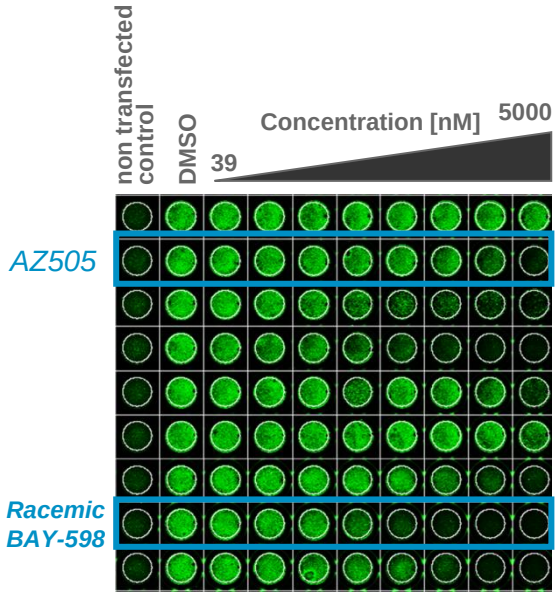
Engineered KYSE-150 cells:

- Cells have been engineered to further overexpress SMYD2 for increased methylation signals
- Methylation was detected with antibody originally directed against methylated p53



In-Cell-Western assay (ICW):

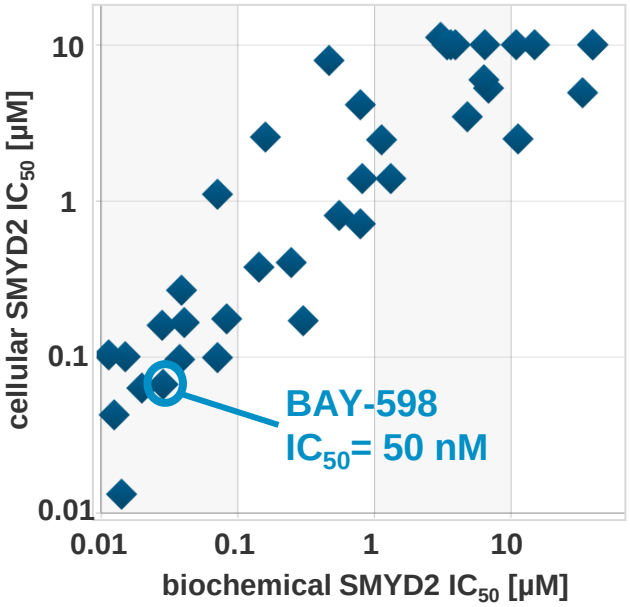
- Used for assessment of cellular potency in lead series



Treatment for 72h

Correlation ICW vs. SPA assay:

- Good correlation between biochemical and cellular data



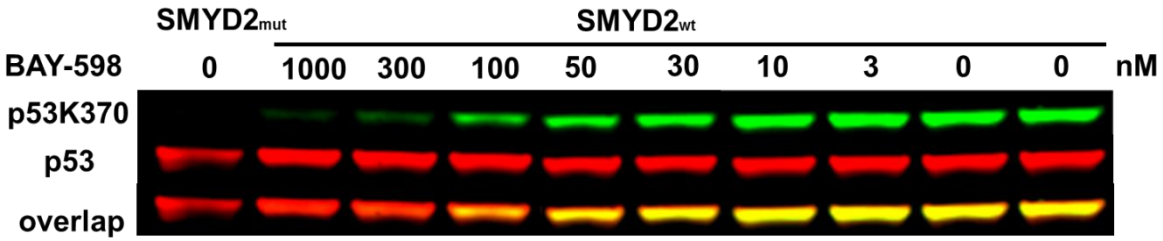
BAY-598 shows potent (IC_{50}= 50 nM) cellular activity

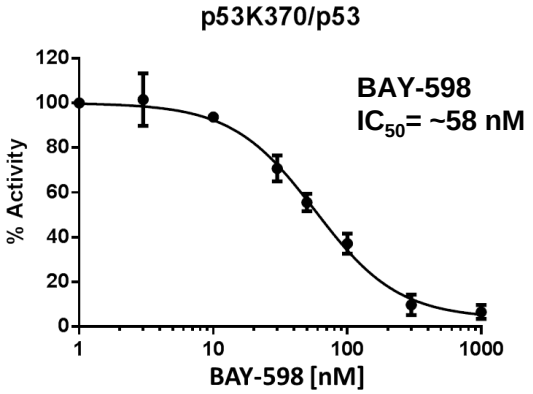
Compound Profile BAY-598

Cellular p53 Methylation Inhibition

Western Blot detection of cellular p53 methylation (2nd cellular mechanistic assay)

- HEK 293 cells co-transfected with p53-FLAG and SMYD2-FLAG or catalytically inactive $SMYD2_{mut}$-FLAG and treated with BAY-598 for 24h (n=3)





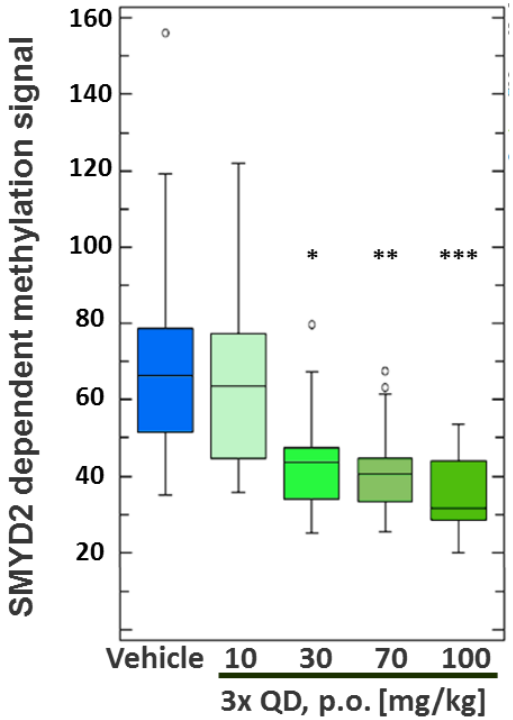
Magdalena Szewczyk & Dalia Barsyte (Structural Genomics Consortium Toronto)

Cellular BAY-598 activity was corroborated in a p53 cellular methylation assay
(IC_{50} ~58 nM)

Compound Profile BAY-598

In vivo Mode-of-Action Study

Xenograft study with KYSE-150 cells engineered to further overexpress SMYD2

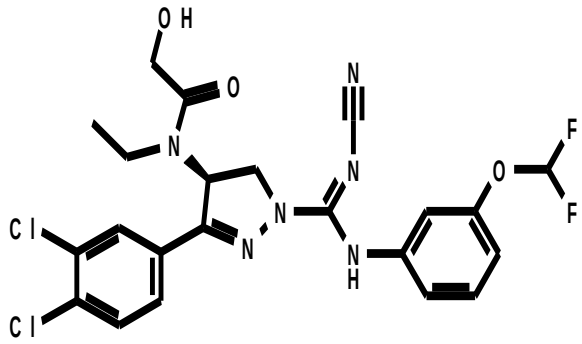


- **Readout:** *ex vivo* analysis of intra-tumoral SMYD2-dependent methylation level
- Significant reduction of SMYD2-mediated methylation was observed after 3 days with **30mg/kg oral daily dosing of BAY-598**
- BAY-598 exposure covered unbound SMYD2 IC_{50} from 1 to more than 7 h depending on dose
- **BAY-598 was well tolerated** up to 500 mg/kg oral daily dosing
- BAY-598 showed **no significant effects in monotherapy** in a xenograft efficacy study with non-engineered KYSE-150 cells up to 500 mg/kg oral daily dosing

BAY-598 can be dosed orally, is well tolerated and shows significant reduction of SMYD2 dependent methylation in vivo

Pyrazoline SMYD2 Inhibitor Probe

Compound Profile BAY-598



BAY-598

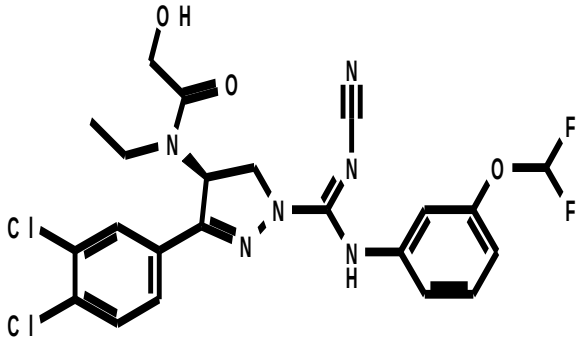
Highly potent, cellularly active and selective	
IC_{50} SMYD2	**28 nM**
IC_{50} Cellular mechanistic assay	**50 nM**
IC_{50} PAR-1	1,700 nM
EC_{50} Platelet aggregation assay	38,000 nM
Selectivity histone methyltransferases (# = 32)	> 50,000 nM SMYD3 = 6,000 nM
Selectivity Kinase (# = 456)	> 20,000 nM
Selectivity Eurofins Panel (# = 68)	>5,000 nM DAT = 4,550 nM, NET = 2,160 nM

Lead-like Properties	
MW	**525 g/mol**
TPSA	**114 Å²**
Measured logD (pH 7)	**3.1**
Calculated logD (pH 7.5)	3.2
Solubility (pH 6.5)	22 mg/L
Stability in human plasma	stable
Stability in rat plasma	stable

Feasible for in vivo studies in rodents				
Cl_{int} Hepatocytes	**Rat**	2.5 L/h/kg	**Rat PK in vivo**	
CL_{int} Microsomes	**Mouse**	3.5 L/h/kg	**Cl_b**	**1.6 L/h/kg**
	Human	0.79 L/h/kg	**V_{ss}**	**2.1 L/kg**
Caco2	**P_{app} AB**	19 nm/sec	**MRT**	**1.6 h**
	Efflux ratio	11fold	**F**	**24 %**

Pyrazoline SMYD2 Inhibitor Probe

Summary



BAY-598

BAY-598 fulfills all SGC chemical probe criteria
We consider BAY-598 as an attractive and novel SGC probe for SMYD2

SMYD2 Inhibitor Program

Acknowledgements

Amaury Fernandez
Andrea Haegebarth
Anke Mueller-Fahrnow
Bernard Haendler
Carlo Stresemann
Christian Stegmann
Christoph Gerdes
Clara Christ
Detlef Stoeckigt
Erik Eggert
H. Ellinger-Ziegelbauer
Hanno Wild
Hilmar Weinmann
Holger Steuber
Ingo Hartung
Joerg Weiske
Karl Ziegelbauer
Luisella Toschi
Manfred Husemann
Marion Hitchcock
Michael Brands
Michael Bruening
Michaela Bairlein
Naomi Barak
Nicole Diedrichs
Olaf Doehr
Oliver Schenk
Peter Spreyer
Ralf Lesche
Roman Hillig
Silke Koehr
Thomas Brumby
Timo Stellfeld
Ursula Egner
Volker Badock
Volker Gekeler

Ian Stefanuti
James Brown
Rosie Crampton
Will Bromley

Al Edwards
Cheryl Arrowsmith
Dalia Barsyte
Elisa Gibson
Fengling Li
Madgalena Szewczyk
Masoud Vedadi
Peter Brown
Santha Santhakumar
Steven Kennedy
Taraneh Hajian