Donated Chemical Probe SOS1 inhibitor **BAY-293**

JMC
Structural Genomics Consortium

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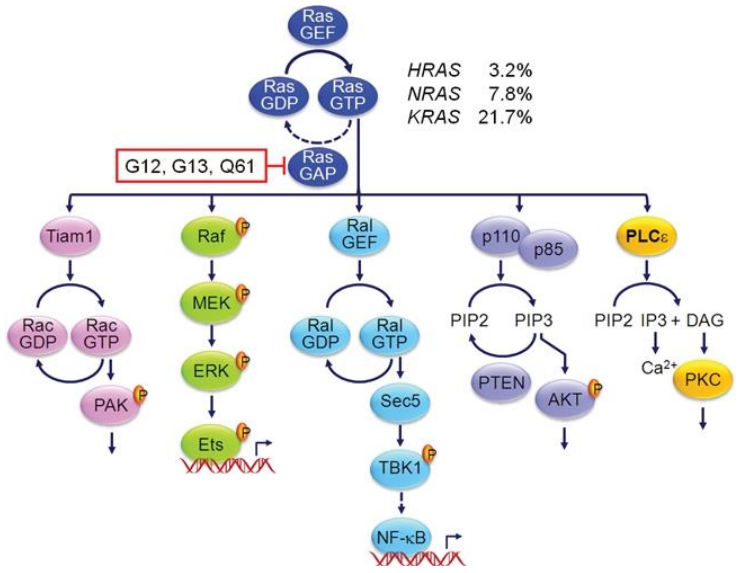
SOS1 & the Ras signalling pathway

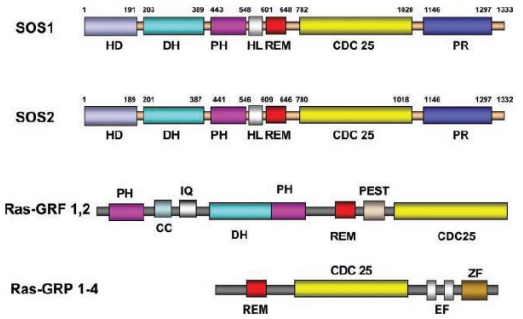
Ras-signalling



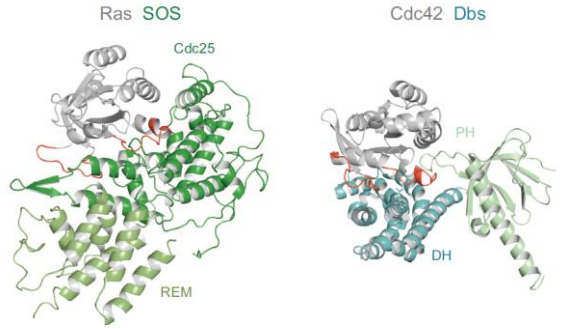
(Neel 2011)

Ras-GEFs

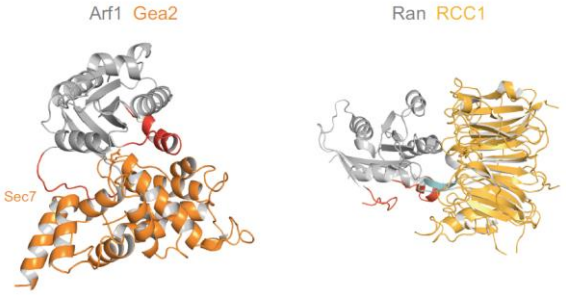
Expression:
- SOS1&2: ubiquitous
- GRPs: brain, immune cells
- GRFs: brain, lung, pancreas



(Rojas 2011)



Closest relatives of SOS1:
- SOS2 is well conserved (80% identity in REM/CDC25 domain)
- All other REM/CDC25 domains show low seq. identity (20-30%)
- GEFs for other GTPase-families show very different protein folds



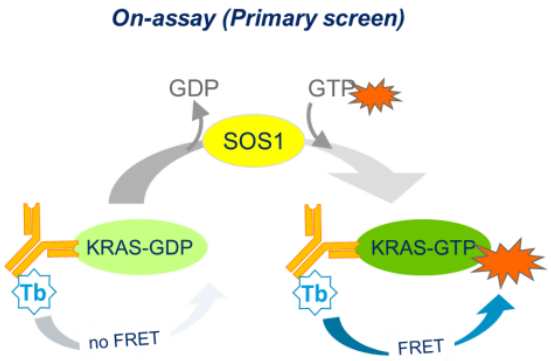
(Cherfils 2013)

SOS1&2 KO-mice
- SOS1: embryonically lethal (trophoblastic defects, Qian 2000)
- Conditional SOS1 KO: viable (Baltranas 2013)
- SOS2: viable & fertile (Esteban 2000)
- SOS1(conditional) + SOS2: severe phenotype with body size reduction, leucocyte reduction, liver failure → lethal 15d post induction (Baltanas 2013)

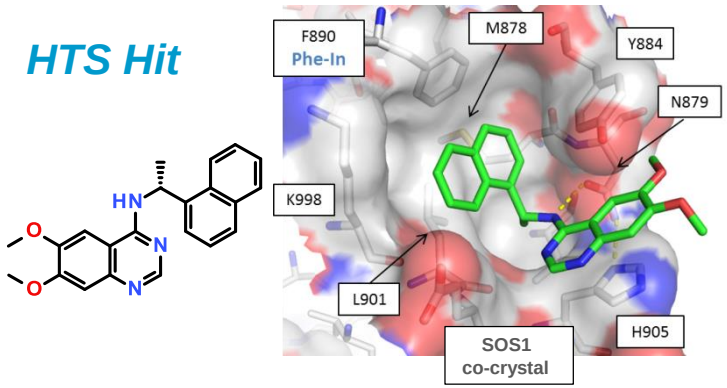
- RAS proteins are molecular switches regulating several tumorigenic pathways
- RAS-Guanine Nucleotide Exchange Factors (GEFs) activate RAS proteins by exchanging GDP for GTP
- SOS1 is main GEF for RAS, SOS2 represents the closest neighbour (80% identity in catalytic domain)
- Recent publications suggest SOS1 as potential tumor target (Wang 2013, You 2018, Cai 2019, see also backup)

Identification of inhibitors of SOS1-KRAS activation

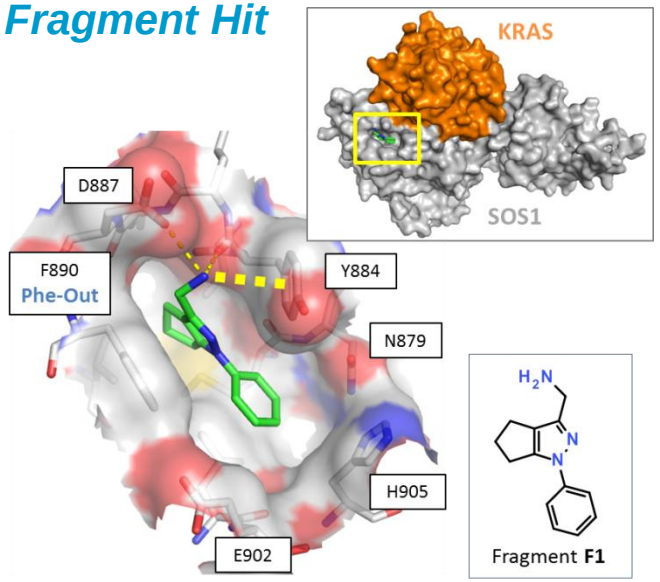
Probe Discovery: Combination of HTS & Fragment Screen



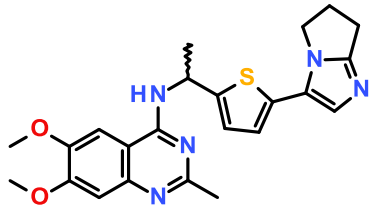
HTS Hit



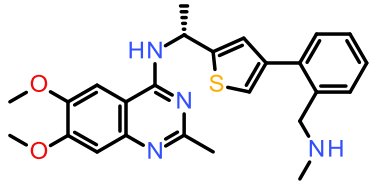
Fragment Hit

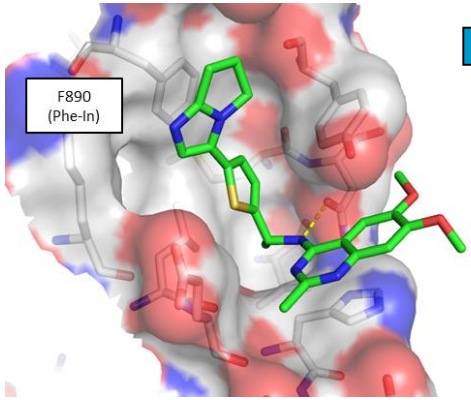


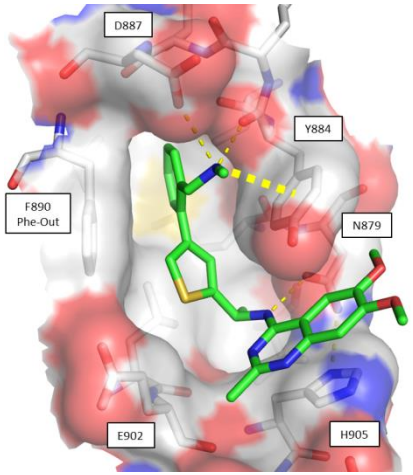
Merged (Phe-in)

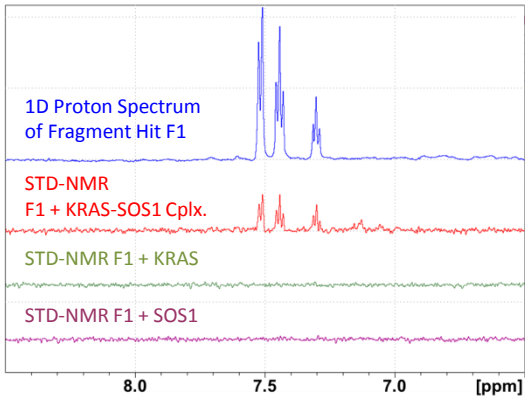


Probe BAY-293 (Phe-Out)









- Hit-to-Lead optimization started from combined HTS & fragment screen efforts
- Opening of back-pocket in SOS1 achieved with probe **BAY-293**

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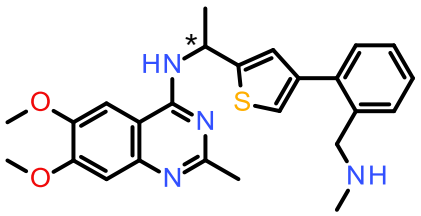
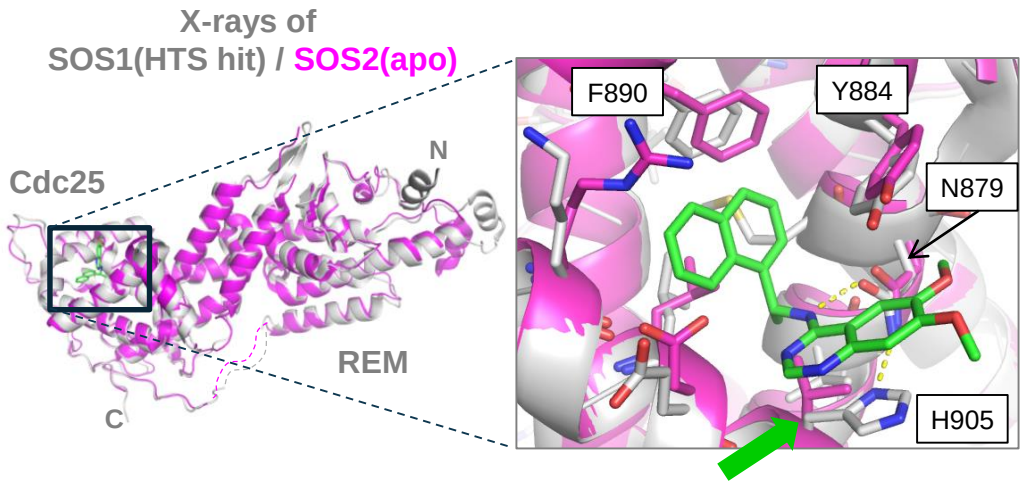
Biochemical potency and selectivity



CPD #	Abs. configuration
BAY-293	*(R)*-enantiomer
BAY-294	*(S)*-enantiomer

IC50 / nM	**SOS1** $KRAS^{G12C}$ interaction	**SOS2** $KRAS^{G12C}$ activation	**DBS** Cdc42 activation	**EGFR** kinase
Racemate	**50**	**> 20000**	**> 20000**	**> 20000**
BAY-293	**21**	**> 20000**	**> 20000**	**> 20000**
BAY-294	**2340**	**> 20000**	**> 20000**	**> 20000**



SOS2 selectivity can be explained by H905V exchange:
loss of stacking interaction of central scaffold (HTS hit in green)

Clean profile in external kinase panel:

- Racemate tested at Millipore against 358 kinases at 1 µM compound concentration: all kinases retain activity > 67%

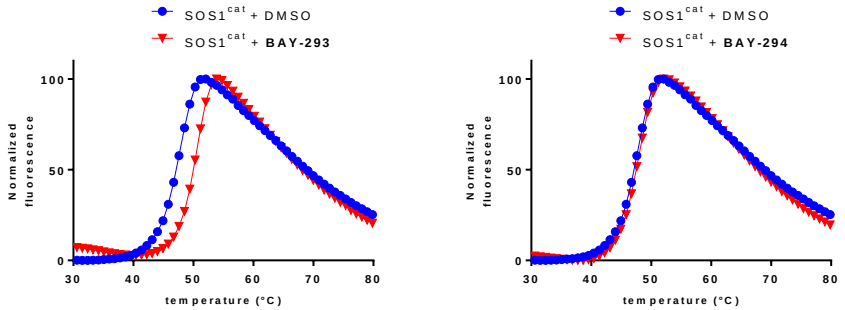
Lead Profiler data:

- Racemate tested against 77 targets: inhibition of 16 GPCRs (mainly aminergic) and 4 transporters (>50% @ 10 µM compound concentration)
- Results not considered causative for on-target and downstream cellular effects

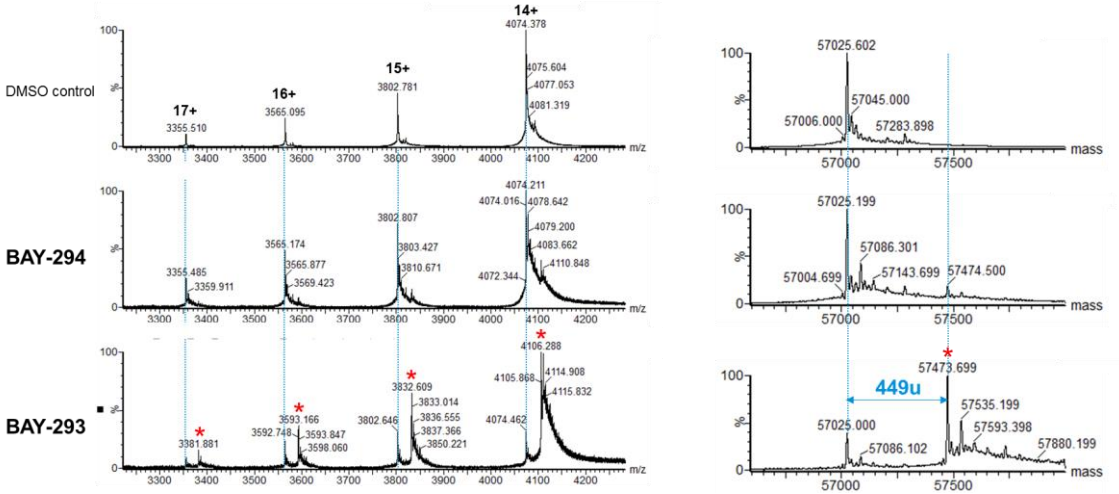
- **BAY-293** potently inhibits SOS1 mediated KRAS activation
- **BAY-293** is inactive against nearest neighbor SOS2 and structurally unrelated GEF (DBS)
- Excellent selectivity against kinases, off-target activity against several GPCRs and transporters
- Enantiomer **BAY-294** as inactive control (> 100-fold difference with batch 99% ee)

Biophysical validation of binding to SOS1

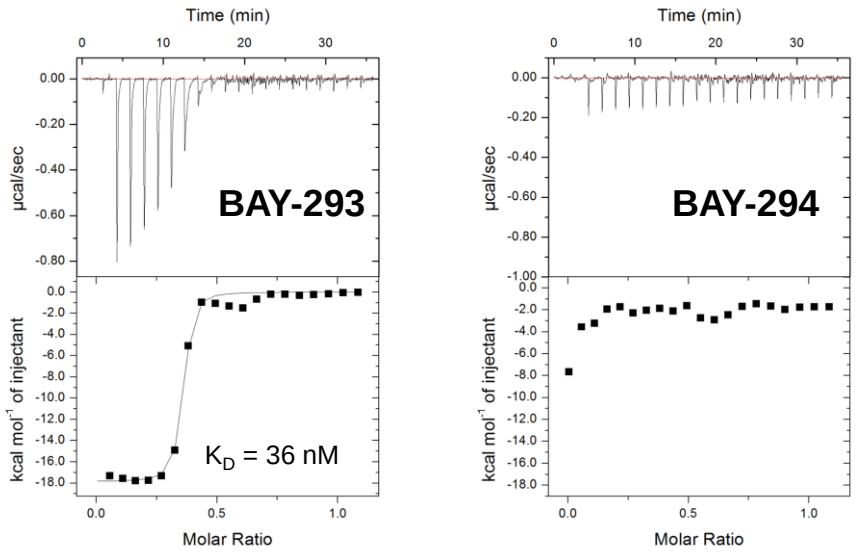
Thermal Shift Assay



Native Mass spectrometry SOS1cat



Isothermal Titration Calorimetry SOS1cat



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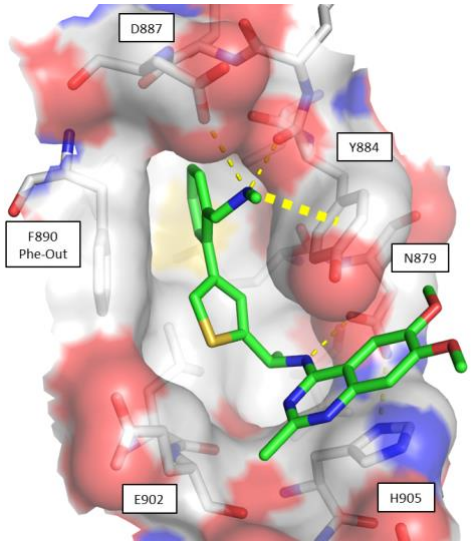
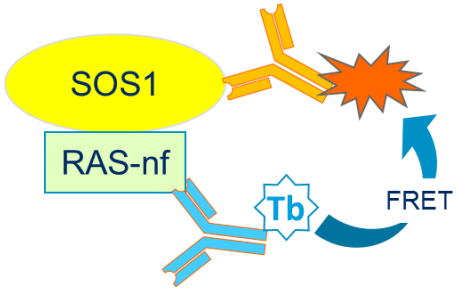
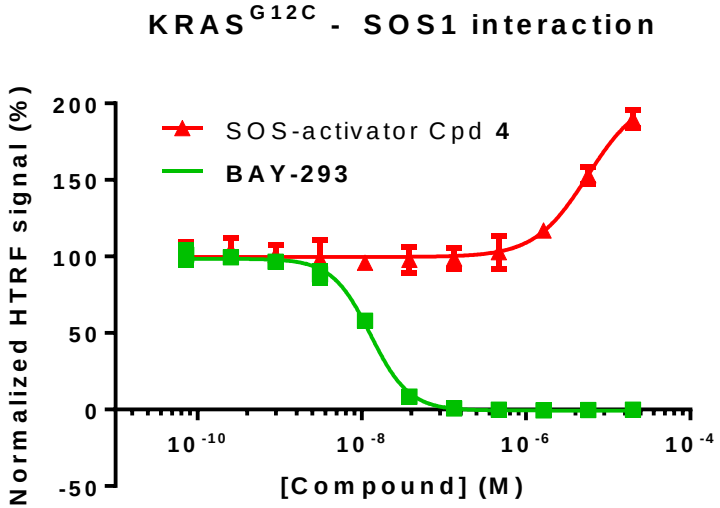


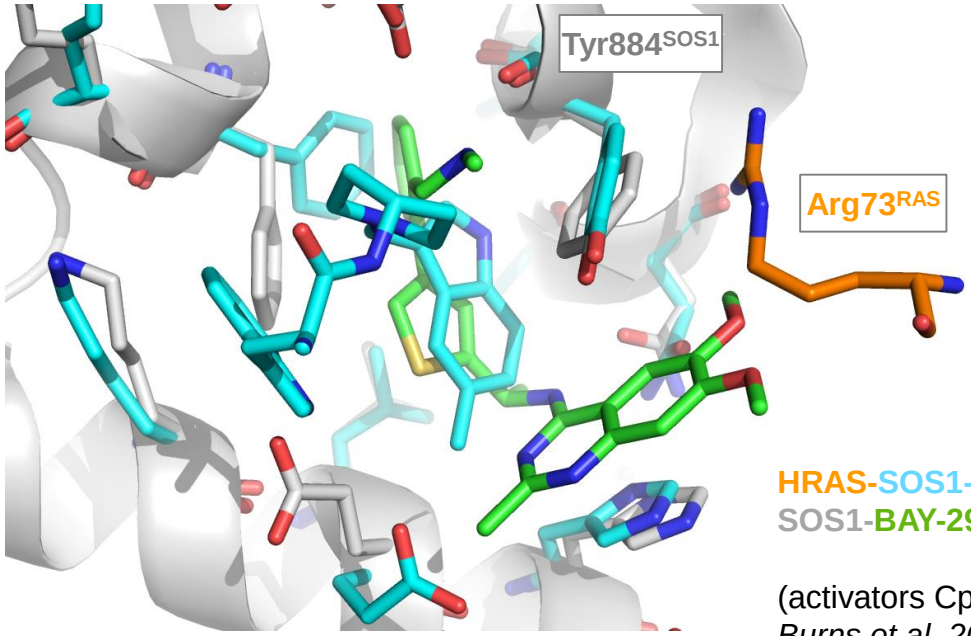
- **BAY-293**, but not inactive **BAY-294**, interacts with SOS1 in TSA, ITC and native MS assays
- X-Ray of **BAY-293** confirms binding mode within SOS1 pocket

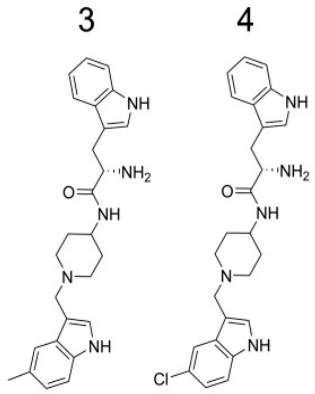
Mode-of-action

Disruption of SOS1-RAS interaction









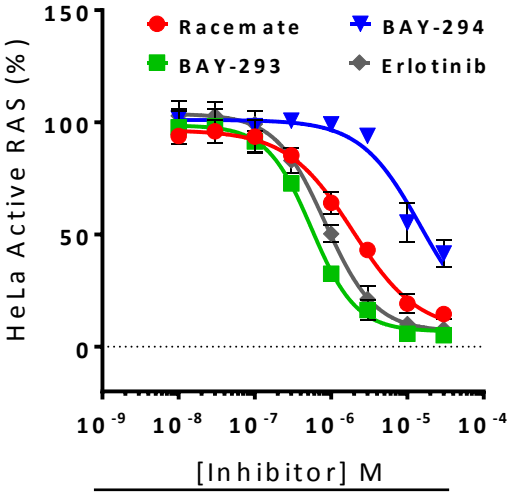
HRAS-SOS1-cpd3 (4NYI.pdb)
SOS1-BAY-293 (5OVI.pdb)

(activators Cpd **3** and **4** from *Burns et al. 2014 PNAS*)

- In contrast to SOS-activator Cpd **4**, **BAY-293** disrupts KRAS-SOS1 interaction, despite of both cpds addressing a similar binding site on SOS1
- MoA confirmed for analogs of **BAY-293** by SPR and 2D-NMR (*Hillig 2019 PNAS*)

Cellular mechanistic data

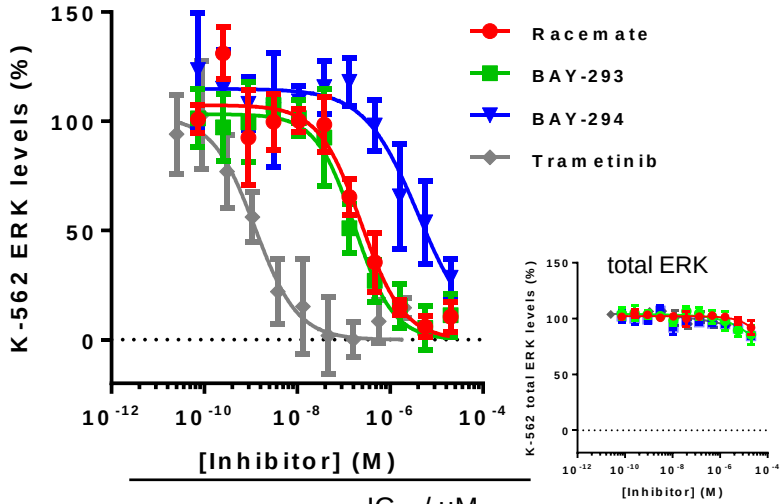
Active RAS HeLa (KRAS-wt)



	IC_{50} / µM
Racemate	**1.3**
BAY-293	**0.41**
BAY-294	**18.6**
Erlotinib	**0.84**

45 x

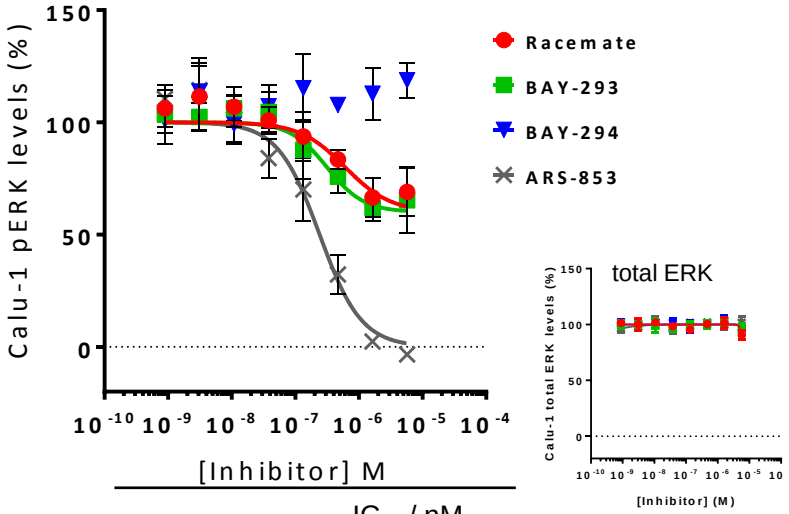
pERK K-562 (KRAS-wt)



	IC_{50} / µM
Racemate	**0.26**
BAY-293	**0.18**
BAY-294	**4.6**
Trametinib	**0.002**

25 x

pERK Calu-1 (KRAS-G12C$^{+/+}$)



	IC_{50} / nM
Racemate	**0.36**
BAY-293	**0.15**
BAY-294	**>20.0**
ARS-853	**0.25**

> 133 x

- **BAY-293** inhibits RAS-activation and pERK in cells with IC_{50} < 1 µM
- Inactive **BAY-294** with significantly less activity (25 - 133 fold compared to active **BAY-293**)
- Complete inhibition of pERK in wildtype KRAS cells, only partial in KRAS-G12C

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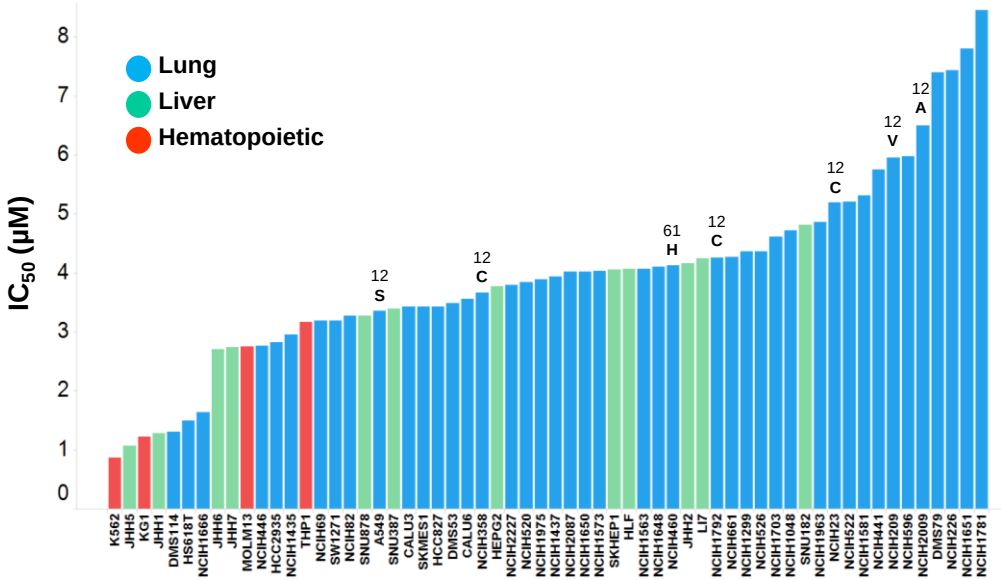
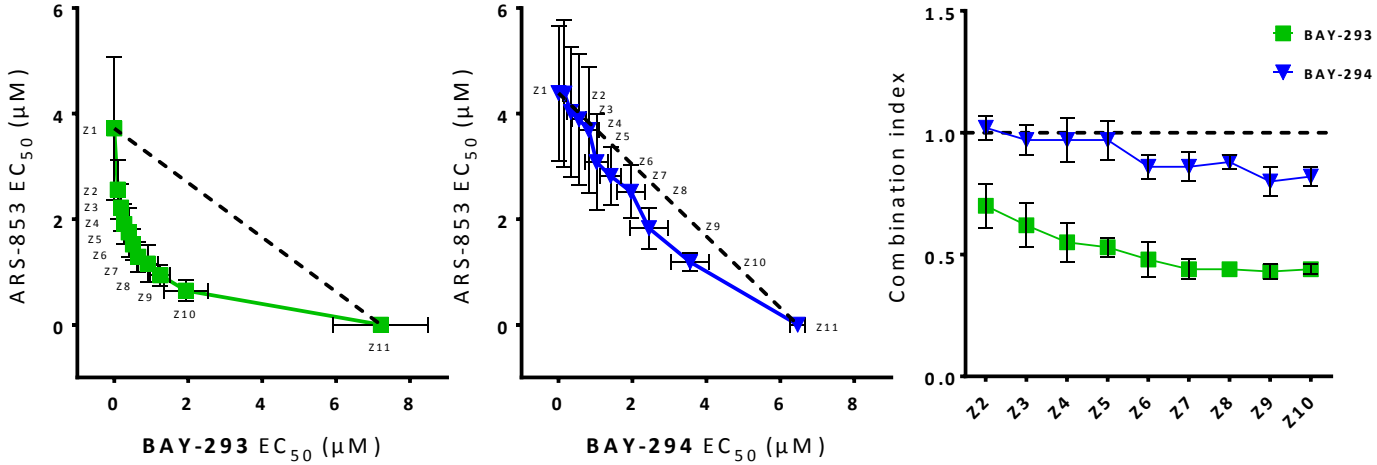
Cellular proliferation data

Proliferation panel (60 tumor cell lines)



Cpd #	KRAS wild-type		KRAS^{G12C}	
	K-562 IC_{50} (nM)	**MOLM-13** IC_{50} (nM)	**H358** IC_{50} (nM)	**Calu-1** IC_{50} (nM)
Racemate	1,100 ± 180	1,320 ± 520	2,660 ± 230	2,050 ± 270
BAY-293	1,090 ± 170	995 ± 400	3,480 ± 100	3,190 ± 50
BAY-294	7,500 ± 620	7,570 ± 1,140	3,390 ± 70	1,840 ± 400

Synergistic combination with **ARS-853** in H358 (KRAS-G12C$^{+/-}$)



- Racemate is active in a range of cell lines with high share of hematopoietic cells in top 10
- 7-fold differential activity of active and inactive probe in KRAS-wildtype cells, but not in KRAS^{G12C} cells
- Synergistic activity of **BAY-293**, but not **BAY-294** with the covalent KRAS^{G12C} inhibitor **ARS-853** in H358 cells

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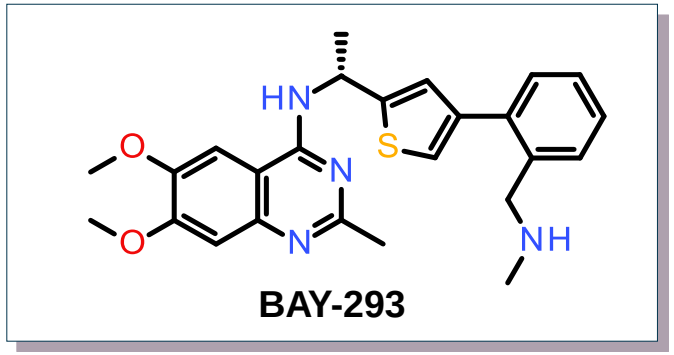
Profile active **BAY-293**



BAY-293

▪ **Pharmacology**

SOS1 - KRAS^{G12C} interaction	0.02 µM
SOS2 - KRAS^{G12C} activation	>20 µM
LLE^{logD}	5
Active RAS HeLa	0.41 µM
pERK K-562	0.18 µM
Proliferation K-562	1.1 µM
Kinases Eurofins	clean
Lead Profiler	16 GPCRs, 4 transporters
TSA dTm	+ 3.2°
ITC K_D	0.04 µM

▪ **Molecular Properties**

MW [g/mol]	449
MWcorr [g/mol]	449
TPSA [Å2]	68
Rotatable bonds	8

▪ **PhysChem**

Sw $^{pH\ 6.5}$ [mg/L]	> 448
log D (pH 7.5)	2.1

▪ **In vitro PK**

		Clint [L/h/kg]	Fmax [%]
LM	Human	0.9	30
	Mice	4.2*	22*
	Rat	2.3*	45*
Hep	Rat	3.0	28

Caco2	A-B [nm/s]	B-A [nm/s]	Ratio
	< 1	21	> 21

* for racemate

- Acceptable PhysChem properties and solubility
- Low to moderate metabolic stability
- Low permeability and strong efflux

SOS1 Probe BAY-293

Summary / Conclusion

Probe criteria	BAY-293
Inhibitory biochemical potency: goal < 100 nM (based on $IC_{50,}$ Kd)	**Surpasses criteria** **IC_{50} (SOS1 interaction assay) = 21 nM**
Selectivity within target family: goal > 30-fold (based on biochemical IC_{50}, Kd)	**Surpasses criteria** **GEFs: IC_{50} > 20000 nM on SOS2 and DBS**
Selectivity outside target family: describe the off-targets	**358 kinases** at 1µM compound concentration > 67% remaining activity **Lead profiling screen** (77 targets): BAY-293 binds to several aminergic GPCRs and transportes (see backup slide)
On target cell activity for cell-based targets: goal < 1 µM	**Surpasses criteria** Inhibition of RAS-activation and pERK in cells with IC_{50} < 1 µM
Negative control: *in vitro* potency →100-fold less than probe	**Surpasses criteria** **IC_{50} (SOS1 interaction assay) = 2340 nM (> 100 fold)**
Link to publication of BAY-293	https://www.pnas.org/content/early/2019/01/24/1812963116

We ask for acceptance of SOS1 inhibitor **BAY-293** as chemical probe, accompanied by **BAY-294** as negative control.

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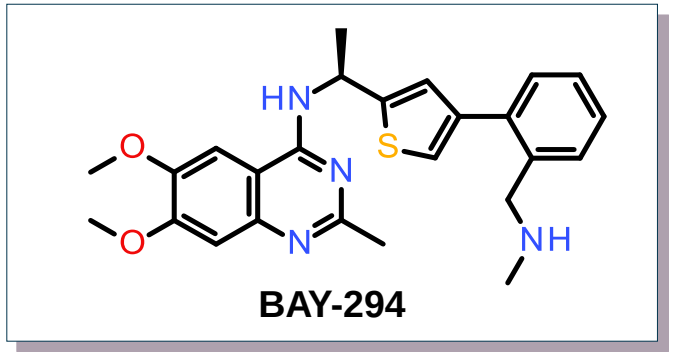
Thank you!

Profile inactive **BAY-294**



BAY-294

▪ **Molecular Properties**

MW [g/mol]	449
MWcorr [g/mol]	449
TPSA [Å2]	68
Rotatable bonds	8

▪ **PhysChem**

Sw $^{pH\ 6.5}$ [mg/L]	> 448
log D (pH 7.5)	2.1

▪ **Pharmacology**

SOS1 - KRAS^{G12C} interaction	2.34 µM
SOS2 - KRAS^{G12C} activation	>20 µM
LLE^{logD}	5
Active RAS HeLa	18.6 µM
pERK K-562	4.6 µM
Proliferation K-562	7.5 µM
Kinases Eurofins	n.d. for inactive
Lead Profiler	n.d. for inactive
TSA dTm	+ 0.3°
ITC K_D	no bdg.

▪ **In vitro PK**

		Clint [L/h/kg]	Fmax [%]
LM	Human	0.8*	40*
	Mice	4.2*	22*
	Rat	2.3*	45*
Hep	Rat	2.2*	48*

Caco2	A-B [nm/s]	B-A [nm/s]	Ratio
	< 1	21	> 21

* for racemate

- Acceptable PhysChem properties and solubility
- Low to moderate metabolic stability
- Low permeability and strong efflux

Lead profiler data

Cat #	Assay Name	Species	Conc.	% Inh.
203100	Adrenergic α_{1A}	rat	10 µM	106
203630	Adrenergic α_{2A}	hum	10 µM	99
203710	Adrenergic α_{2B}	hum	10 µM	85
203810	Adrenergic α_{2C}	hum	10 µM	99
219500	Dopamine D_1	hum	10 µM	85
219600	Dopamine D_{2L}	hum	10 µM	67
219700	Dopamine D_{2S}	hum	10 µM	71
219800	Dopamine D_3	hum	10 µM	88
239710	Histamine H_2	hum	10 µM	99
252610	Muscarinic M_1	hum	10 µM	94
260210	Opiate κ(OP2, KOP)	hum	10 µM	105
260410	Opiate µ(OP3, MOP)	hum	10 µM	93
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	hum	10 µM	81
271650	Serotonin (5-Hydroxytryptamine) 5-HT_{2A}	hum	10 µM	110
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	hum	10 µM	100
271800	Serotonin (5-Hydroxytryptamine) 5-HT_{2C}	hum	10 µM	104
202000	Transporter, Adenosine	gp	10 µM	62
220320	Transporter, Dopamine (DAT)	hum	10 µM	90
226400	Transporter, GABA	rat	10 µM	71
204410	Transporter, Norepinephrine (NET)	hum	10 µM	86

- 77 targets tested (GPCRs, transporters, nuclear receptors, enzymes)
- Racemate inhibits several aminergic GPCRs and transporters
- Results not considered causative for on-target and downstream cellular effects or antiproliferative activity

Kinase panel Eurofins

kinase	% residual kinase activity @ 1 µM
CK2α2(h)	67
DYRK1A(h)	68
IGF-1R(h)	74
LTK(h)	75
Syk(h)	79
TrkB(h)	79
TSSK1(h)	79
CHK2(h)	80
TrkC(h)	80
Ret(h)	81
Flt4(h)	82
PASK(h)	84
PDHK2(h)	85
CDK1/cyclinB(h)	87
PEK(h)	87
TTBK1(h)	87
Axl(h)	88
Flt1(h)	88
Lyn(h)	88
MST4(h)	88
PAK6(h)	88
PKCε(h)	88
ATR/ATRIP(h)	88
EphA3(h)	89
PhKγ2(h)	89
Pim-1(h)	89
Rse(h)	89

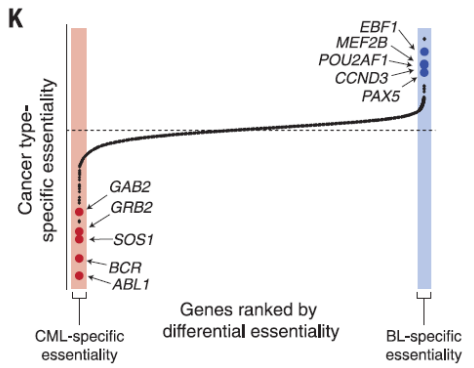
- 358 kinases tested at 1 µM compound concentration
- All tested kinases retain activity > 67%
- Racemate shows very clean profile

top kinases sorted by % residual kinase activity

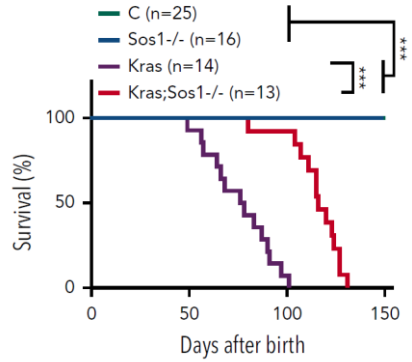
SOS1/2: Relevance in Cancer

- Tumors requiring SOS-dependent RAS^{WT}-activation are expected to be sensitive to SOS-inhibitors, e.g. tumors with:
  - enhanced upstream signalling (e.g. EGFR mutants, BCR-Abl)
    SOS1 was identified as an essential gene for chronic myeloic leukemia (CML) by a CRISPR genome-wide screen (Wang 2013)
  - loss-of-function of GTPase activating proteins like NF1 (Nichols 2018)
  - class 3 BRAF mutants (Yao 2017)
- RAS mutants which depend on nucleotide cycling (e.g. G12C, G12D) may require SOS1 for activation and thus be sensitive to SOS1 inhibition (Huang 2014, Hunter 2015)
- SOS1 mediates mutant KRAS induced cross-activation of N-Ras and H-Ras, SOS1-/- KO attenuates KRAS^{G12D}-induced myeloproliferative neoplasm (MPN) and prolongs survival of KRAS^{G12D} mice (You 2018)
- Novel SOS-mutations support role of SOS1 as oncogenic driver in lung adenocarcinoma (Cai 2019)
- Small molecule SOS-activators lead to inhibition of pERK signalling by a feedback mechanism (Burns 2014, Abbott 2018)

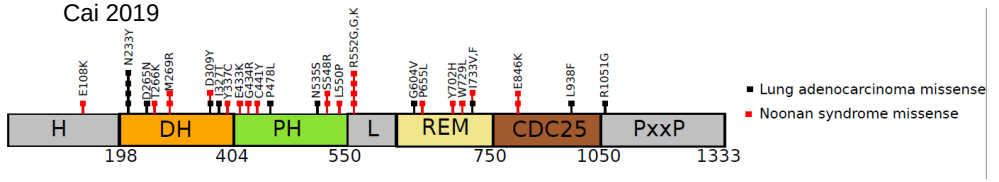
SOS1 essentiality in CML
Wang 2013



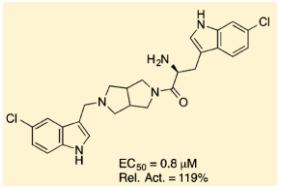
SOS1 required for KRAS^{G12D} MPN
You 2018

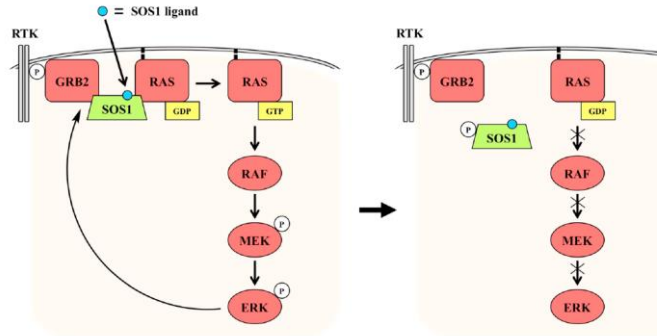


SOS1 mutants
Cai 2019



SOS1 SMOL activators
Abbott 2018





- Role of SOS1/2 inhibition in cancer so far only studied by genetic approaches
- Published tool compounds induce activation of SOS1