Donated Chemical Probe

BUB1 Inhibitor BAY 1816032

June 21st, 2022
(including post-meeting data)

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on behalf of the team

BUB1 Inhibitor BAY 1816032

Target rationale

Bub1 is a mitotic protein that ensures accurate segregation of chromosomes by ...

- recruitment of essential spindle assembly checkpoint (SAC) proteins,
- accurate positioning of the chromosomal passenger complex (CPC),
- recruitment of shugoshin proteins (Sgo) to secure centromere cohesion

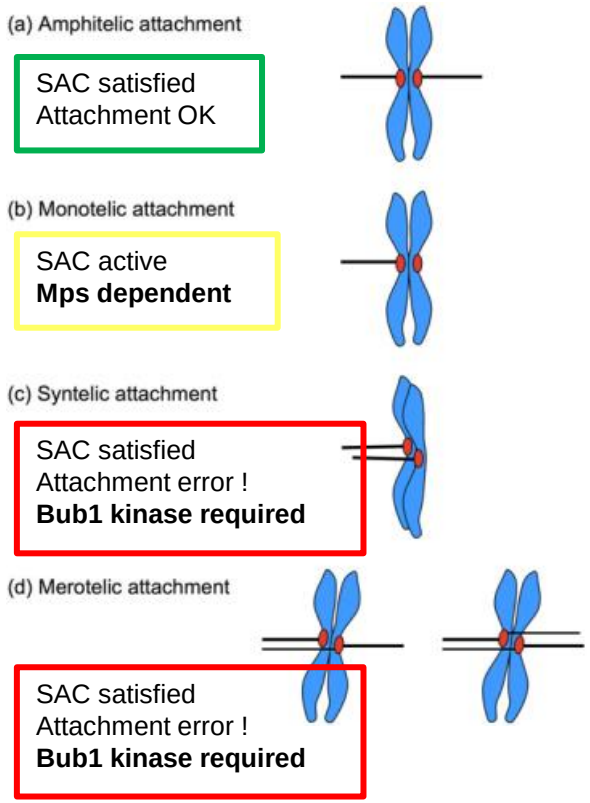
Bub1 kinase function:

- phosphorylates histone H2A at kinetochores

➢ correct positioning of the CPC for attachment error correction

➢ protection of centromer cohesion

- potential role in ATM-dependent DNA damage response



➢ Bub1 was validated as mitotic breakthrough target

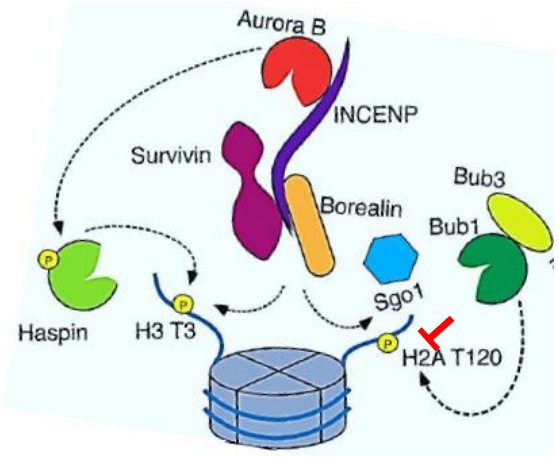
BUB1 Inhibitor BAY 1816032

Literature / publications

Publication: Siemeister et al. Clin Cancer Res 2019;
DOI: 10.1158/1078-0432.CCR-18-0628

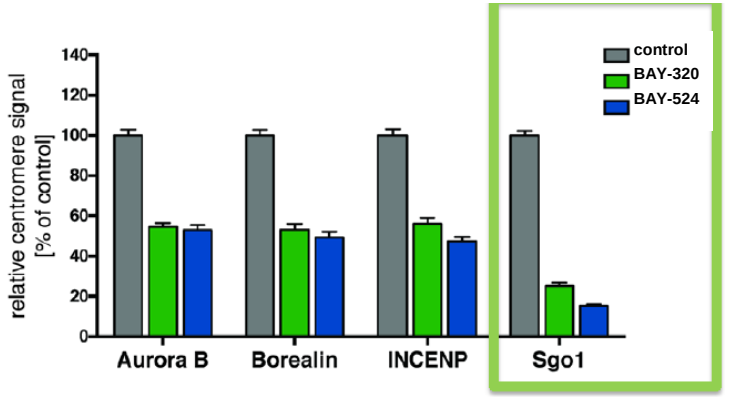
Ricke et al (J Cell Biol. Dec 2012):

- Knock-in mice expressing kinase-inactive Bub1 variant from the endogenous locus.
- Mice were without obvious phenotype. No increase in spontaneous or carcinogen-induced tumor incidence.
- Bub1 kinase activity required for microtubule-kinetochor attachment error correction.
- Bub1 phosphorylates H2A-T121 at the inner centromere & creates a high affinity binding site for AurB.



Baron, Nigg et al. (eLife 2016;5:e12187):

- Inhibition of Bub1 kinase by Bayer compounds results in **reduced levels of chromosomal passenger complex proteins** (AurB, Borealin, INCENP) and of Sgo1 at unattached kinetochors. Cooperativity with inhibition of Haspin kinase.



➢ The availability of complementary inhibitor BAY 1816032 will help to better understand pharmacology

BUB1 Inhibitor Probe BAY 1816032

Technical *in vitro* profile

BAY 1816032

POTENCY (IC_{50} [nM])	
Bub1 low ATP / high ATP (2mM) (w/o preinc.) [nM]	6.5 / 1900
Bub1 ePCA [nM]	1.0
Target Residence Time [min]	87
P-H2A (form./ abrog.) HeLa [nM]	43 / 29

Properties & Physchem	
logD @ pH 7.5	3.0
MW / MW_{corr}	535 / 507
Sw @ pH 6.5 [mg/L]	0.15
TPSA [g*mol / Å^2]	116
Stability (r /h plasma, 2 h)	stable

in vitro DMPK Properties

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	4.4		3.0		0.7*	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	mouse liver mics		3.8		29	
	rat hepatocytes		1.8		56	
	human hepatocytes		0.7		46	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 5	4.2	5	> 5	2.1	TDI TDI observed in rhCYP3A4. Further assessment in Cocktail assay with HHeps, considered not critical.
CYP induction NOEL [µM]	1A2 ≥ 1111; 3A4 > 10000					

Selectivity

In-house kinase panel (#20)	≥1.000x
DiscoverX panel (403 kinases) • except LOK/STK10	>250x 17x
Panlabs Eurofins lead profiling screen of 89 targets * except human Adenosine transporter	no relevant off-target activity 51x

SAFETY

Cytotox / MNT / Ames	negative
hERG IC_{20} [µM]	≥ 10

- BAY 1816032 is a potent and highly selective Bub1 kinase inhibitor.
- Single digit µM antiproliferative activity.

*High permeation through Caco-2 monolayer, in presence of albumin inhibitory potential towards Pgp observed

BUB1 Inhibitor BAY 1816032

In vitro profile

Proliferation Assays on 43 tumor cell lines:

- **median IC_{50}: 1.4E-06 M** (range 4.7E-07 – 5.8E-06 M**)**

Indication	N of Cell lines	IC_{50} in M
Breast	5	6.4E-07 – 1.2E-06
Bladder	1	4.9E-06
Cervix	2	1.1E-06 -1.7E-06 6.3E-08 (in presence of 3 nM Tx)
Colon	4	7.0E-07 – 2.6E-06
Mantle cell lymphoma	1	1.1E-06
Melanoma	2	7.6E-07 – 1.3E-07
Gastric	2	3.0E-06 – 3.5E-06
Glioblastoma/Neuroglioma	1	9.3E-07
NSCLC	16	4.7E-07 – 5.8E-06
Ovary	2	1.0E-06 – 2.2E-06
Pancreas	2	3.0E-06 – 3.3E-06
Prostate	3	7.1E-07 – 3.4E-06
Skin	1	3.0E-06
Mouse melanoma	1	2.0E-06

- Single digit µM antiproliferative activity in many cell lines.

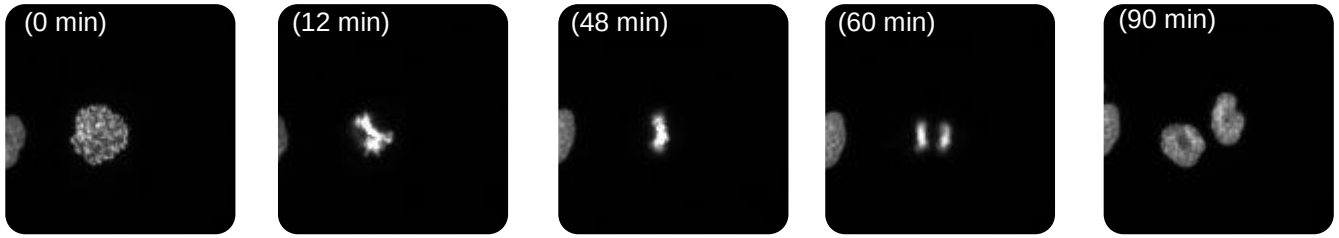
Proliferation Assay

Indication	Cell line	(IC_{50} in M)
Breast	MDA-MB-436	1.2E-06
	MDA-MB-453	1.2E-06
	MDA-MB-468	1.0E-06
	SK-BR-3	1.1E-06
	SUM-149	6.4E-07
Bladder	HT-1197	4.9E-06
Cervix	HeLa	1.7E-06
	HeLa	6.3E-08 (in presence of 3 nM Tx)
	HeLa-MaTu-ADR	1.1E-06
Colon	Caco2	1.0E-06
	HCT116	1.3E-06
	Lovo	7.0E-07
	OUMS-23	2.6E-06
Mantle cell lymphoma	GRANTA-519	1.1E-06
Melanoma	A375	7.6E-07
	HT-144	1.3E-06
Gastric	KATO III	3.0E-06
	MKN-45	3.5E-06
Glioblastoma/Neuroglioma	H4	9.3E-07
NSCLC	Calu-6	4.7E-07
	NCI-H460	1.1E-06
	NCI-H1299	2.4E-06
	NCI-H1395	5.4E-06
	NCI-H1437	2.8E-06
	NCI-H1651	2.7E-06
	NCI-H1703	9.2E-07
	NCI-H1755	1.4E-06
	NCI-H1792	1.6E-06
	NCI-H1838	2.9E-06
	NCI-H1944	3.0E-06
	NCI-H2009	2.1E-06
	NCI-H2087	8.7E-07
	NCI-H-2228	1.9E-06
	NCI-H23	1.0E-06
	NCI-H2347	5.8E-06
Ovary	Colo-704	1.0E-06
	SK-OV-3	2.2E-06
Pancreas	MIA Paca-2	3.0E-06
	Panc-1	3.3E-06
Prostate	DU145	1.1E-06
	PC-3	3.4E-06
	22RV1	7.1E-07
Skin	A431	3.0E-06
Mouse melanoma	B16F10	2.0E-06

BUB1 Inhibitor BAY 1816032

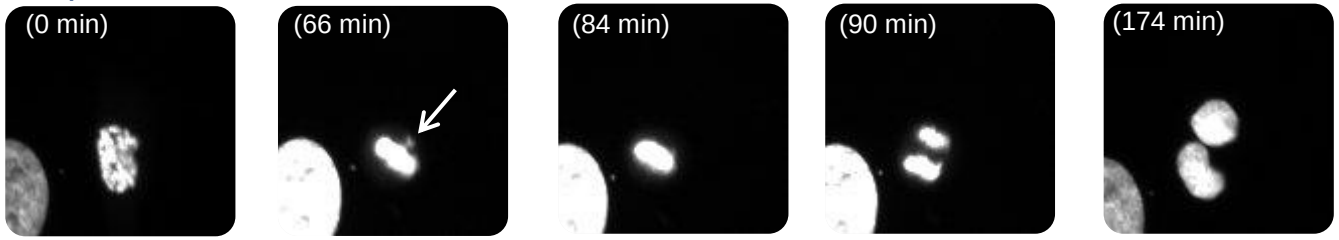
Phenotype/MoA of Paclitaxel/Bub1i Combo in vitro

3 nM Paclitaxel



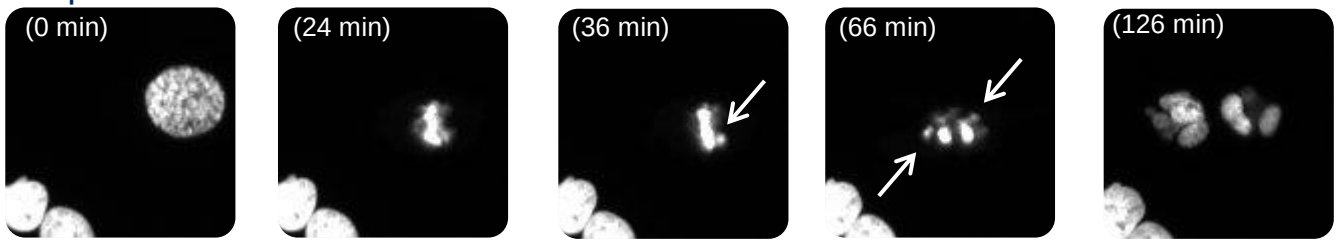
no effect at 3 nM

1.1 µM BAY 1816032

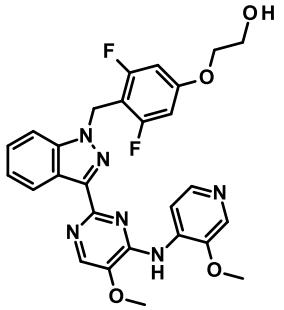


lagging chromosomes get captured

1.1 µM BAY 1816032 + 3 nM Paclitaxel



persistent lagging chromosomes ⇔ missegregation



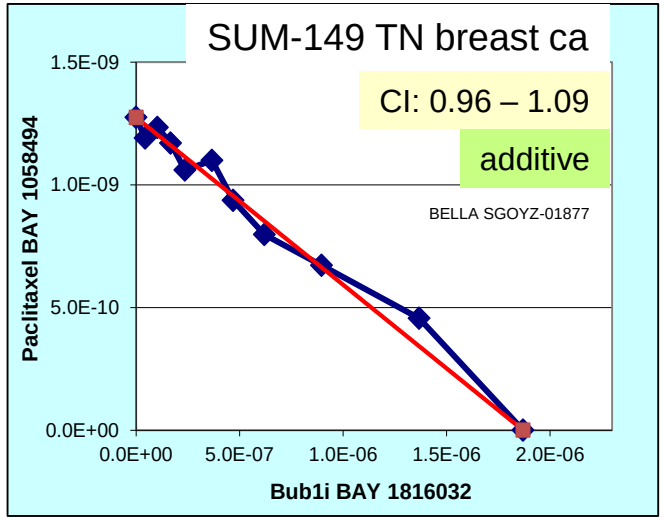
BAY 1816032

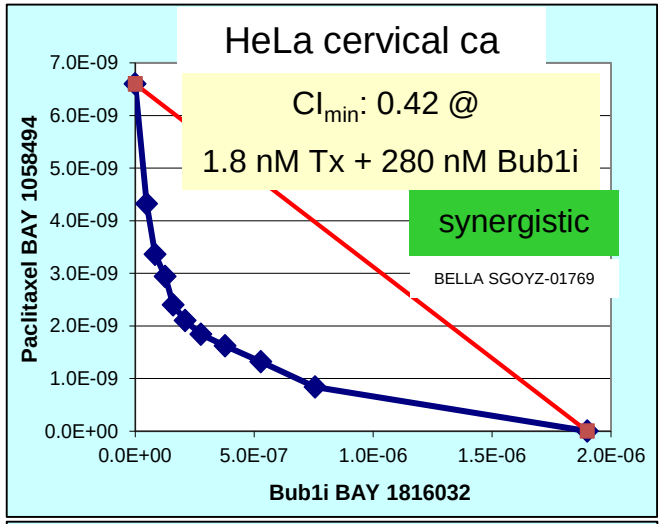
* images taken from sequence within the first 24hrs after compound addition (incubation time >= 4.4hrs). Times are relative to entry into prophase (= 0 min). Sebastian Räse, LDB

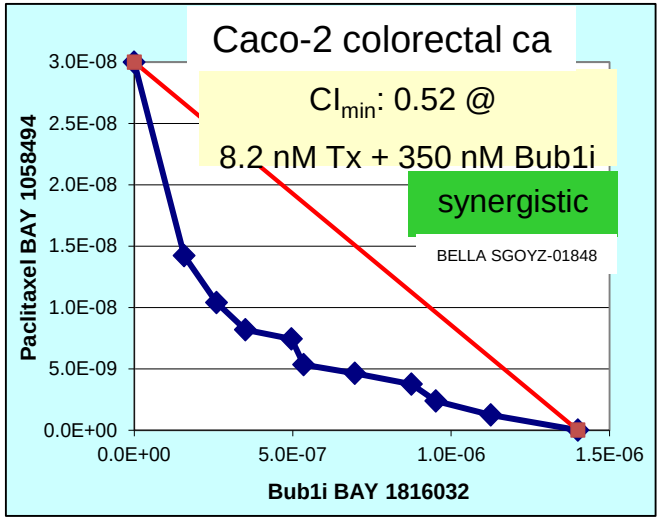
- Live cell imaging reveals cooperative effect of Bub1i with paclitaxel: lagging chromosomes & incorrect chromosome distribution

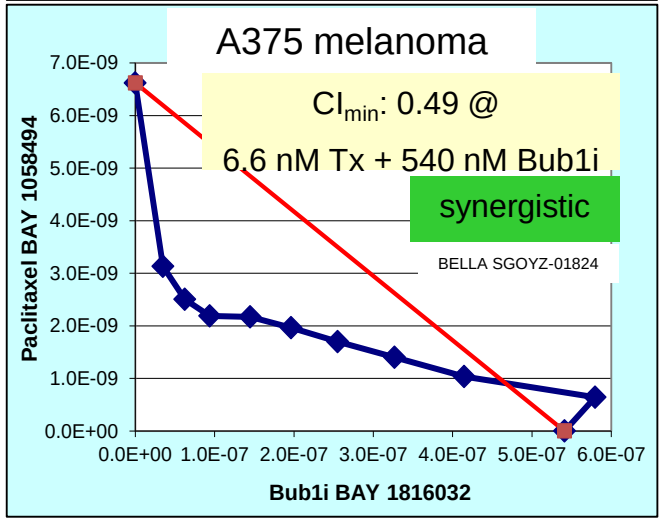
BUB1 Inhibitor BAY 1816032

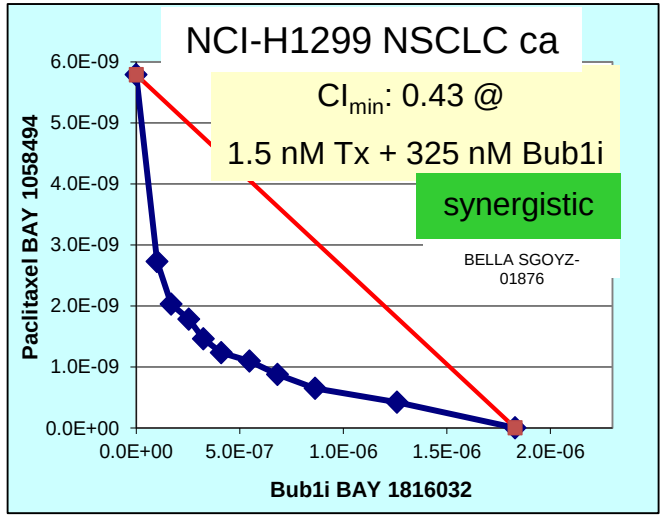
Paclitaxel combination in vitro











- Synergistic/ additive interaction of BAY 1816032 with paclitaxel confirmed in several cell lines.

BUB1 Inhibitor BAY 1816032

Summary of in vitro combination assays

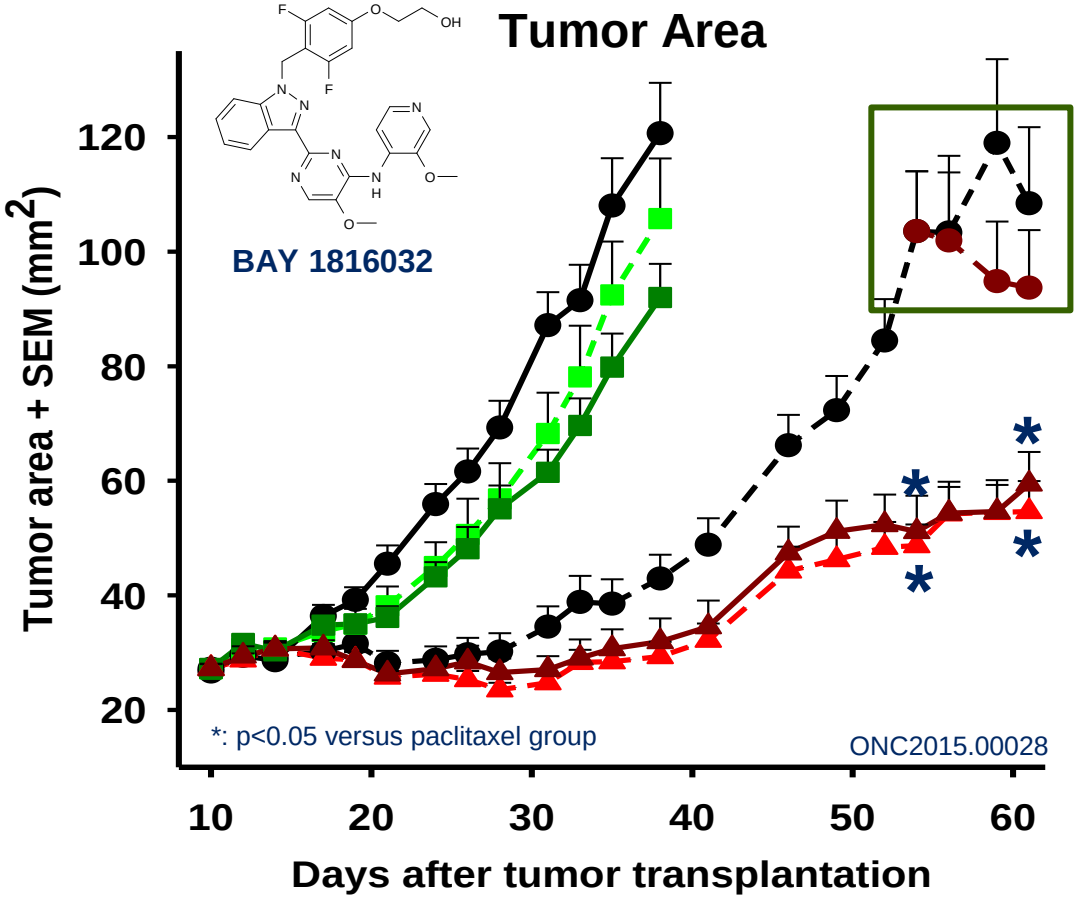
Proliferation Combination Assays: • **Bub1i + paclitaxel (PTx) or** • **Bub1i + docetaxel (DTx)**	
color code	>additive (synergistic)
	additive
	antagonistic

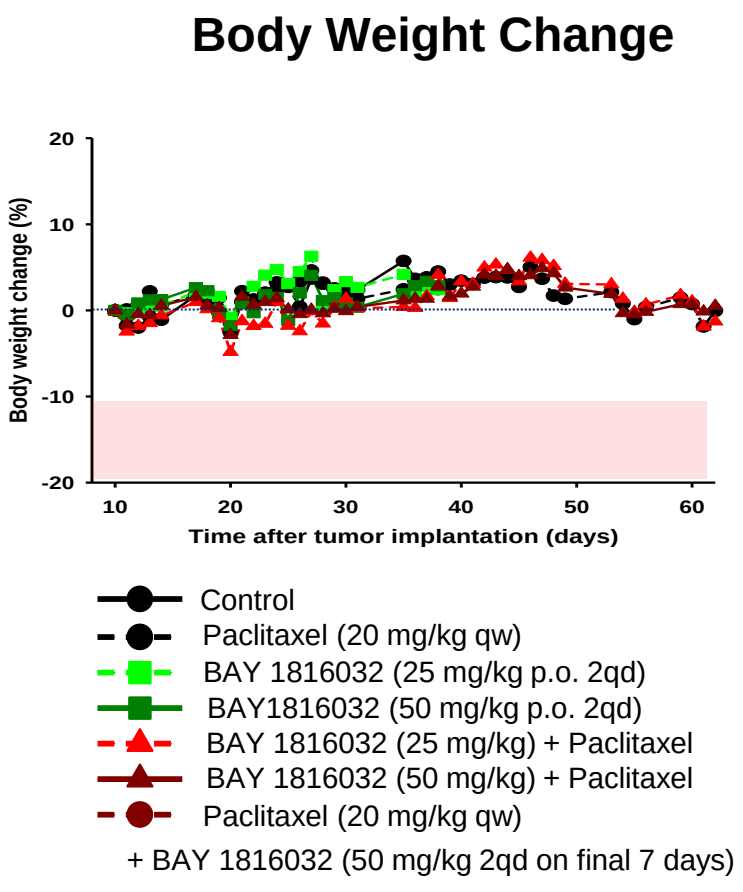
Indication	**Cell line**	**PTx**	**DTx**	Comment
TN breast	MDA-MB-436	X		
	MDA-MB-231	X		
	SUM-149	X		
Prostate	22RV1	X	X	
	PC3	X	X	
CRC	Caco-2	X		
	Colo-205	X		
	HT29	X		
Gastric	MKN-45	X		
	Hs746T	X		
	Snu-5	X		
GBM	H4	X	X	
	42-MG-BA	X		
	U87-MG	X		
Melanoma	A375	X		
	LOX IMVI	X		
Cervix	HeLa	X		
Pancreas	MIA Paca-2	X		
Ewing sarcoma	SK-ES-1	X		
NSCLC	NCI-H1299	X	X	
	NCI-H1755	X	X	
	NCI-H1792	X	X	
	NCI-H1437	X	X	
	NCI-H1651	X	X	PTx insensitive
	NCI-H1944	X	X	
	NCI-H1703	X	X	dep. on DTx/Bub ratio
	NCI-H1838	X	X	
	NCI-H2009	X	X	
	NCI-H2087	X	X	
	NCI-H23	X	X	
	NCI-H2347	X	X	
Bladder	UM-UC-3	X		

- Predominantly synergistic/additive interaction with paclitaxel & docetaxel.

BUB1 Inhibitor BAY 1816032

Paclitaxel combotherapy in SUM-149 (TN breast) in nude mice - additive

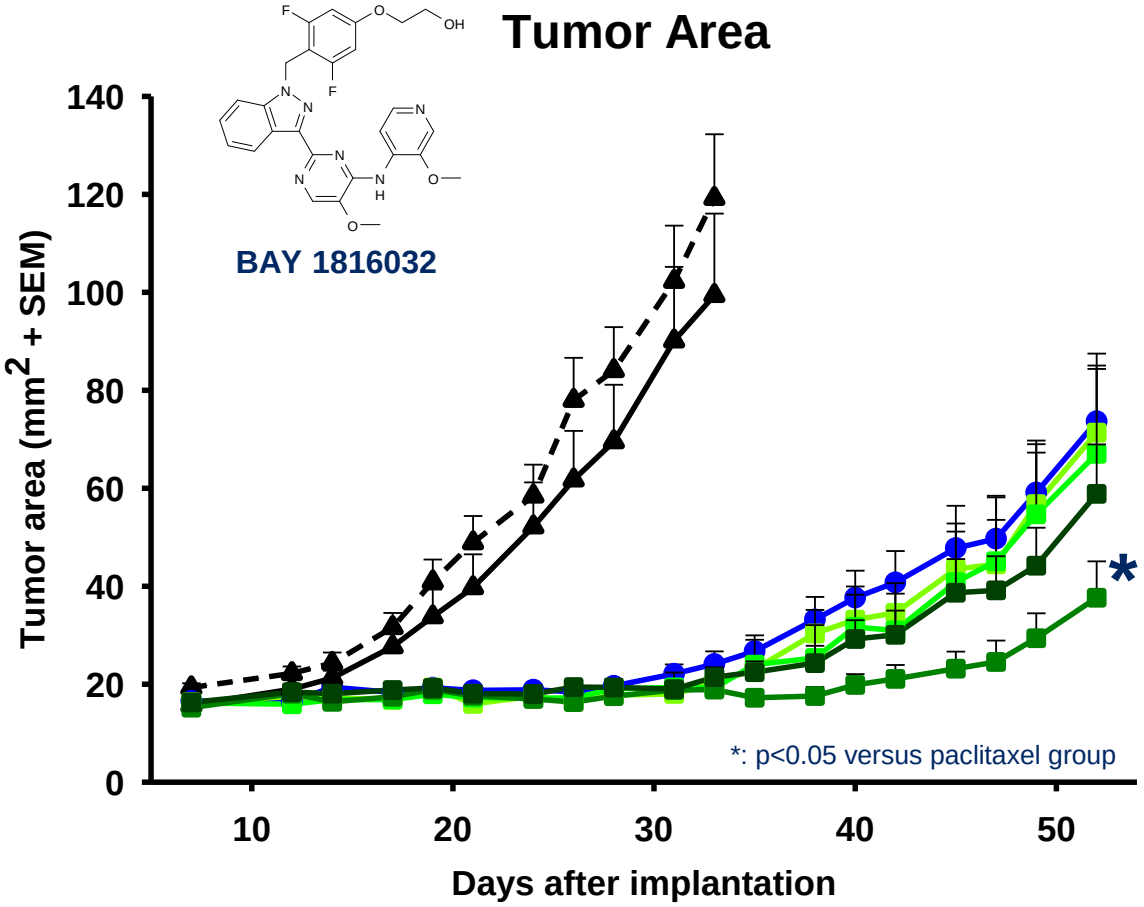


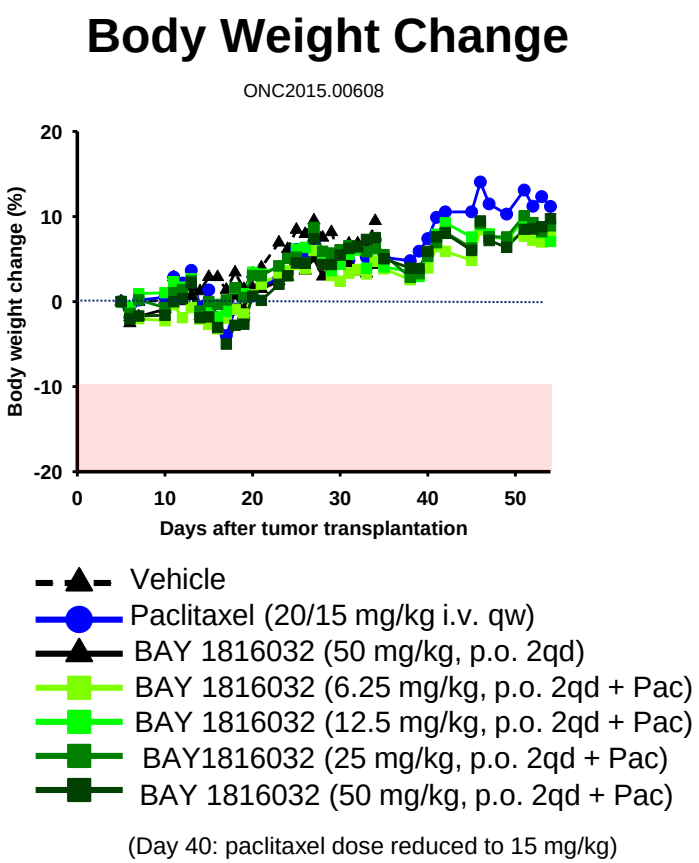


- Significantly improved efficacy as compared to paclitaxel mono
- BAY 1816032 is well tolerated, no effects on paclitaxel level

BUB1 Inhibitor BAY 1816032

Paclitaxel combotherapy in NCI-H1299 (NSCLC) in nude mice - synergistic





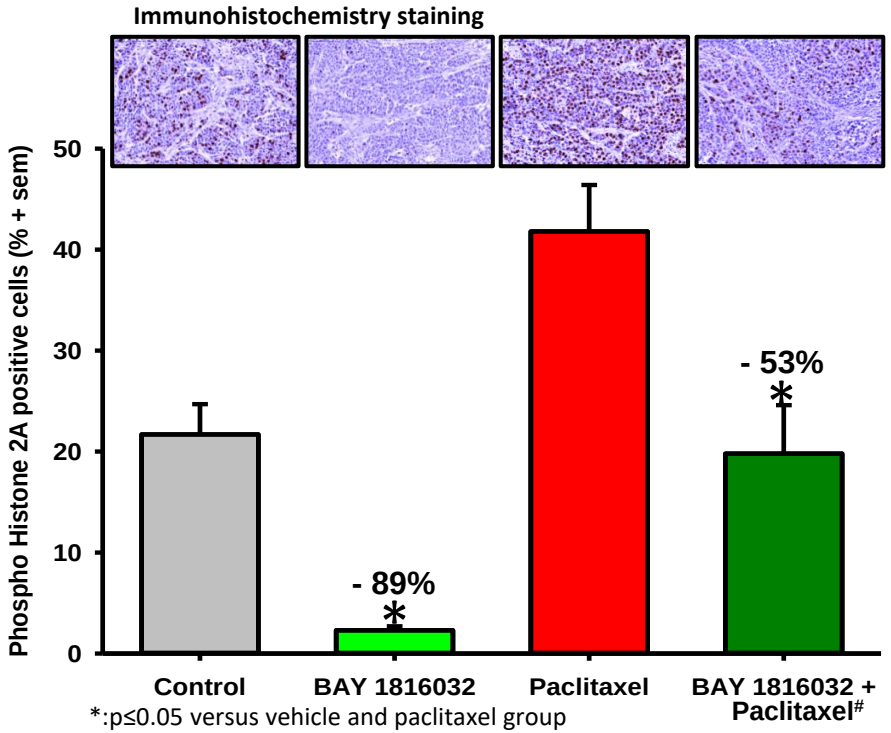
(Day 40: paclitaxel dose reduced to 15 mg/kg)

- Significantly improved efficacy as compared to paclitaxel mono
- BAY 1816032 is well tolerated

BUB1 Inhibitor BAY 1816032

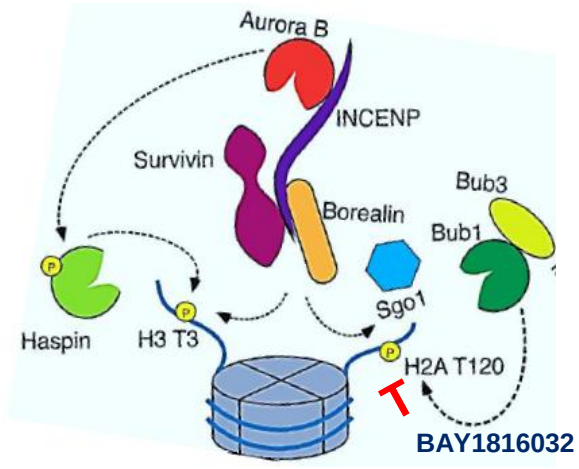
In vivo MoA study in HeLa-MaTu (cervix)

HeLa-MaTu cervical ca xenograft on nude mice

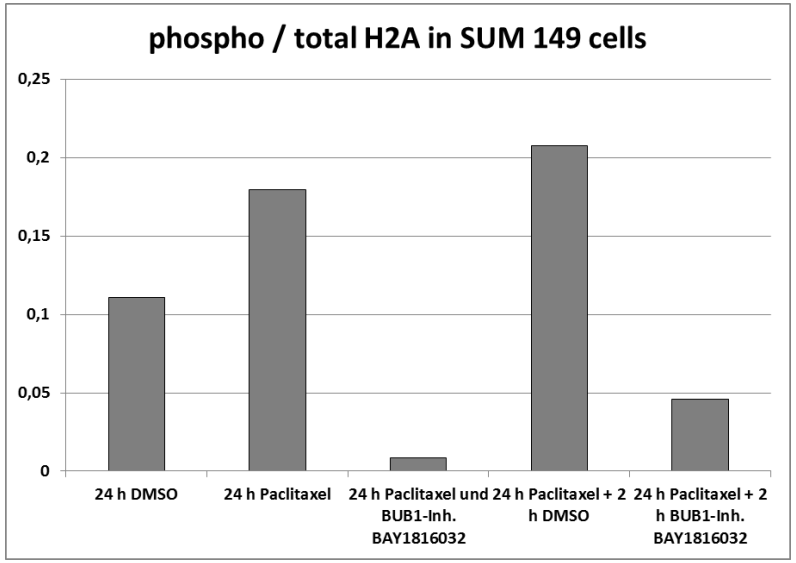


*:p≤0.05 versus vehicle and paclitaxel group

#: Concurrent treatment: 30 mg/kg paclitaxel i.v. once and 150 mg/kg BAY 1816032 p.o., 3x, every 8h; Necropsy 2 h after 3rd administration



BAY 1816032 blocks phosphorylation of Thr121 of the conserved C-tail of H2A, which is crucial for centromeric localization of shugoshin (Sgo1)



- Strong inhibition of Histone 2A (Thr-121) phosphorylation in HeLa-MaTu xenografts
- Conclusion: BAY 1816032 acts as Bub 1 kinase inhibitor in vivo.

BUB1 Inhibitor BAY 1816032

Summary of *in vivo* combination studies

Indication	Model	Dose [mg/kg] Bub1-I: 2QD PTx: QD 1on/6off	Response Bub1i + PTx	Significant improvement over PTx mono [at study end]
TN breast	SUM-149	Bub1-I: 25 / 50 mg/kg PTx: 20 mg/kg	growth delay	Yes
	MDA-MB-436	Bub1-I: 25 mg/kg PTX: 20 mg/kg	growth delay	Yes
NSCLC	NCI-H1299	Bub1-I: 25 mg/kg PTX: 20 / 15 mg/kg	growth delay	Yes
Cervix	HeLa-MaTu	Bub1-I: 25 / 50 mg/kg PTx: 10 mg/kg	growth delay	Yes
Melanoma	A375	Bub1-I: 100 mg/kg 1QD PTx: 12 mg/kg	growth delay	Yes
Bladder	UM-UC-3	Bub1-I: 25 / 50 mg/kg PTx: 20 mg/kg	growth delay	No improvement by Bub1i

- Conclusion: BAY 1816032 acts as Bub 1 kinase inhibitor in vivo in different in vivo models.

BUB1 Inhibitor BAY 1816032

Safety pharmacology

Off-target activity (Panlabs Eurofins)

- No off-target activity @ 10 µM at selected receptors, ion channels, transporters, enzymes (n=87) except of binding to hum **Adenosine transporter (IC_{50} 0.37 µM)**

  = Multiples of exposure (MoEs) ~50 regarding IC_{50} at Bub1

and slight interaction with COX-1, PDE4, Adenosine A_{2A}, $GABA_A$, and Na channel (<70%)

Cardiac Re-/Depolarization (voltage clamp)

- No inhibition of hERG K^+, hNav1.5 and Cav1.2 current, IC_{20} >10 µM

  = MoEs >10 regarding IC_{50} in cellular assay

- BAY 1816032 shows a favorable safety profile

BUB1 Inhibitor BAY 1816032

Toxicological Characterization

	***In vitro* toxicity**
Genotoxicity	• Mini Ames screen: No mutagenic potential • In vitro micronucleus screen: no mutagenic potential
Cytotoxicity	• Not cytotoxic

	2-week Repeat-Dose Rat Toxicity Study
Design	• 6 males/group • 0 – 25 – 50 - 100 mg/kg/d, once daily administration • vehicle: PEG400/water: 60/40, v/v; 5 mL/kg
Results	• No clinical signs, no effects on body weight or food consumption • No treatment related findings in hematology, clinical biochemistry, histopathology

- Clean toxicity profile

Negative Control BAY-283

In vitro technical profile

BAY-283

POTENCY (IC_{50} [nM])	
Bub1 low ATP / high ATP (2 mM) (w/o preinc.) [nM]	1710 / > 20,000

Properties & Physchem	
logD @ pH 7.5	4.00
MW / MW_{corr}	548 / 520
Sw @ pH 6.5 [mg/L]	< 0.1
TPSA [g*mol / Å^2]	104
Stability (r /h plasma, 4h) [%]	no data

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	pending		pending		pending	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	Human liver mics		no data		no data	
	rat hepatocytes		1.4		66	
	human hepatocytes		no data		no data	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	-	-	-	-	-	-
PXR	-					

Selectivity	
In-house kinase panel (#)	IC_{50} >20 µM for 25 tested kinases
Eurofins safety panel	no data

SAFETY	
Cytotox	no data
hERG IC_{50} [µM]	no data

- BAY-283 is suggested as negative control
- Further profiling could be undertaken after probe acceptance

BUB1 Inhibitor BAY 1816032

Summary / Conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 50 nM (IC_{50}, Kd)	**Meets criteria;** IC_{50} (Bub1 low ATP) = 6.5 nM IC_{50} (P-H2A (form./ abrog.) HeLa) = 43 / 29 nM
Selectivity within target family: goal is > 30-fold	**Meets criteria;** DiscoverX panel (403 kinases) > 250-fold Except LOK/STK10 = 17-fold
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Meets criteria;** no relevant off-target activity in Panlabs Screen (89 targets) Except human Adenosine transporter = 51-fold Cytotox / MNT / Ames – all negative
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Meets criteria;** Cellular target engagement demonstrated: IC_{50} (different tumor cell lines) = 0.1 – 58 µM.
Suitability for in vivo studies	**Meets criteria;** significant improved *in vivo* efficacy in paclitaxel combotherapy in SUM-149 (TN-breast) as compared to paclitaxel mono
Neg ctrl: in vitro potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Meets criteria;** BAY-283 as negative control available IC_{50} (Bub1 low ATP) = 1710 nM (260-fold less potent than probe)

We ask for acceptance of BAY 1816032 as chemical probe, accompanied by BAY-283 as negative control

BUB1 Inhibitor BAY 1816032

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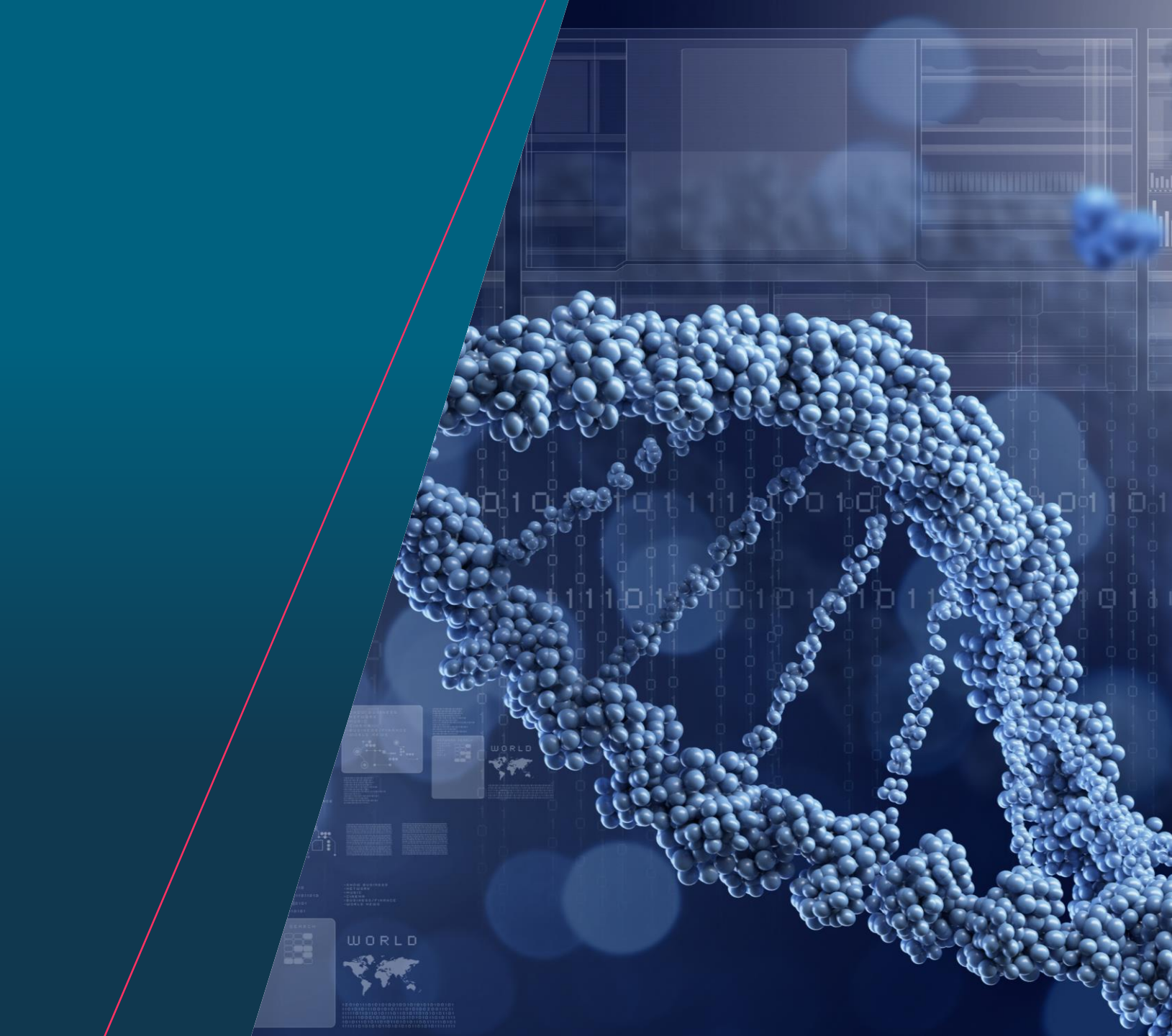
Thank you to the whole team!

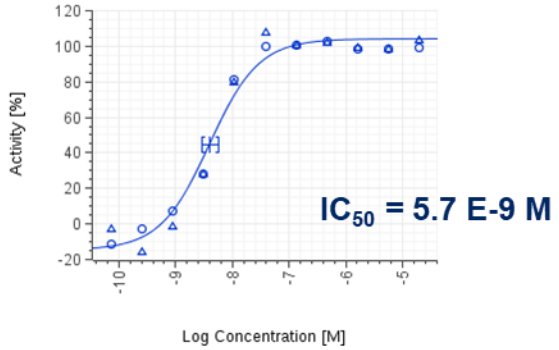
Thank You



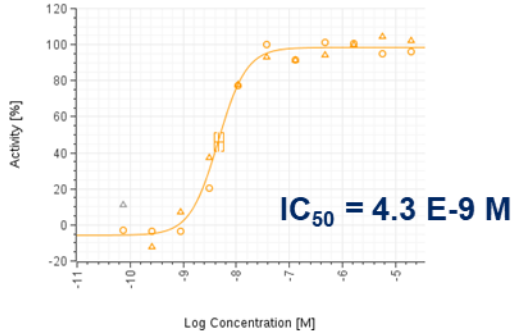
BUB1 Inhibitor BAY 1816032

Biochemical activity

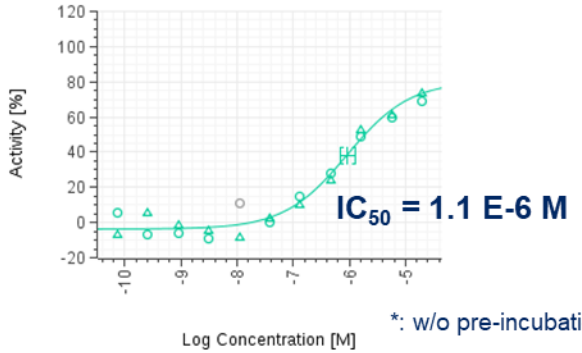
Bub1 Kinase (10 µM ATP)



Bub1 Kinase (2 mM ATP)

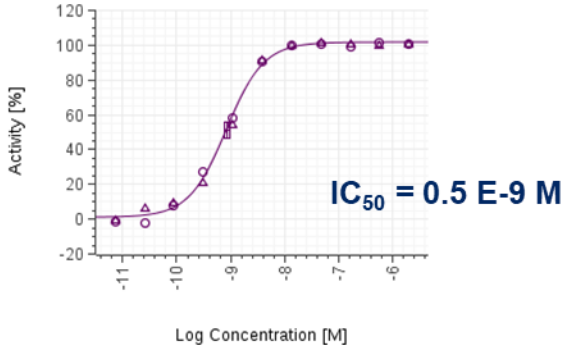


Bub1 Kinase (2 mM ATP*)

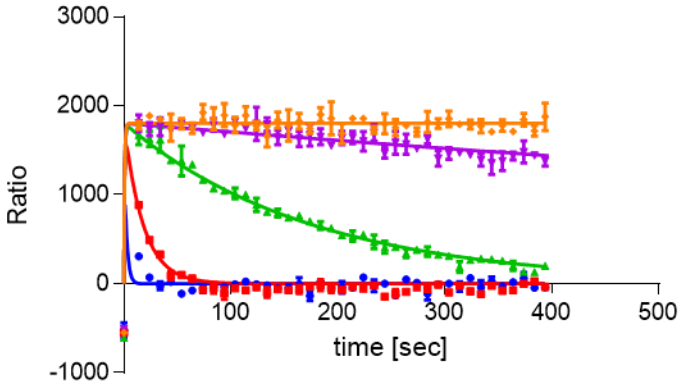


*: w/o pre-incubation

Probe Competition Assay (ePCA)



Kinetic Probe Competition Assay (kPCA)



K_{on} = 4.1±0.5E5 $M^{-1} * s^{-1}$

K_{off} = 2.2±1.0E-4 s^{-1} (RT = 76‘)

$K_{D\ kin}$ = 1.3±0.9E-9 M

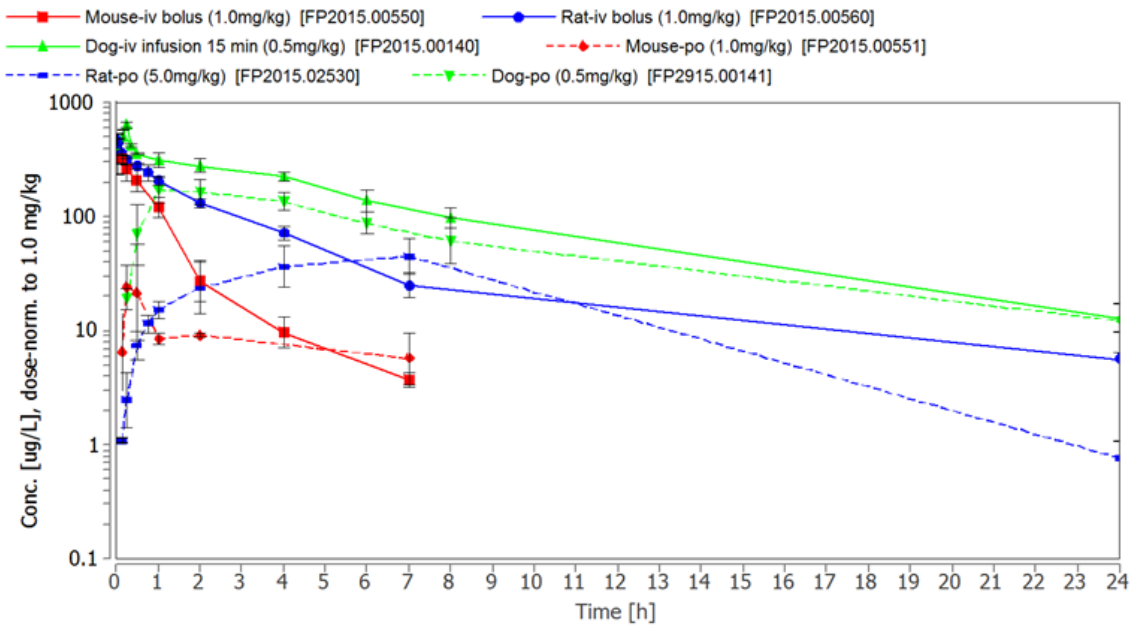
$K_{D\ eq}$ = 5.3±0.3E-10 M

- BAY 1816032 is a potent inhibitor with slow binding kinetics and long residence time

BUB1 Inhibitor BAY 1816032

In vivo PK

In *vivo* PK in different species

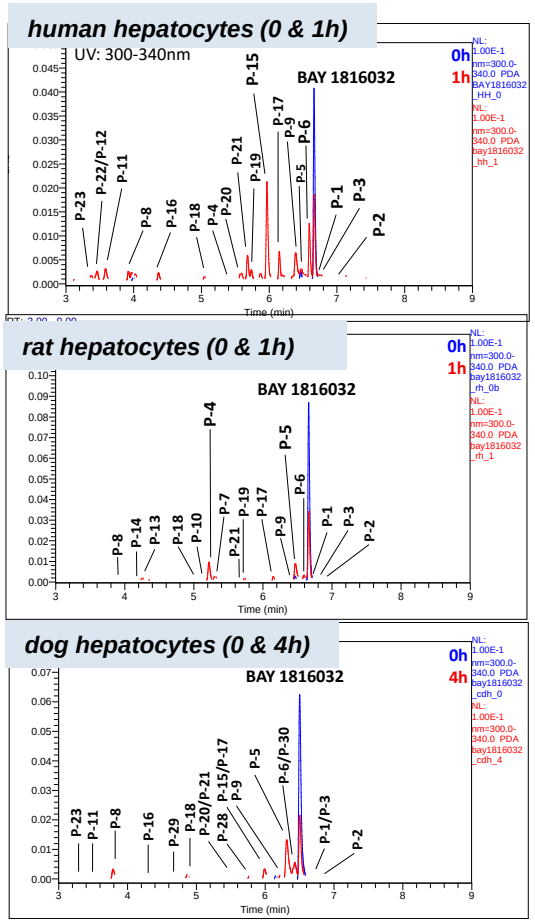


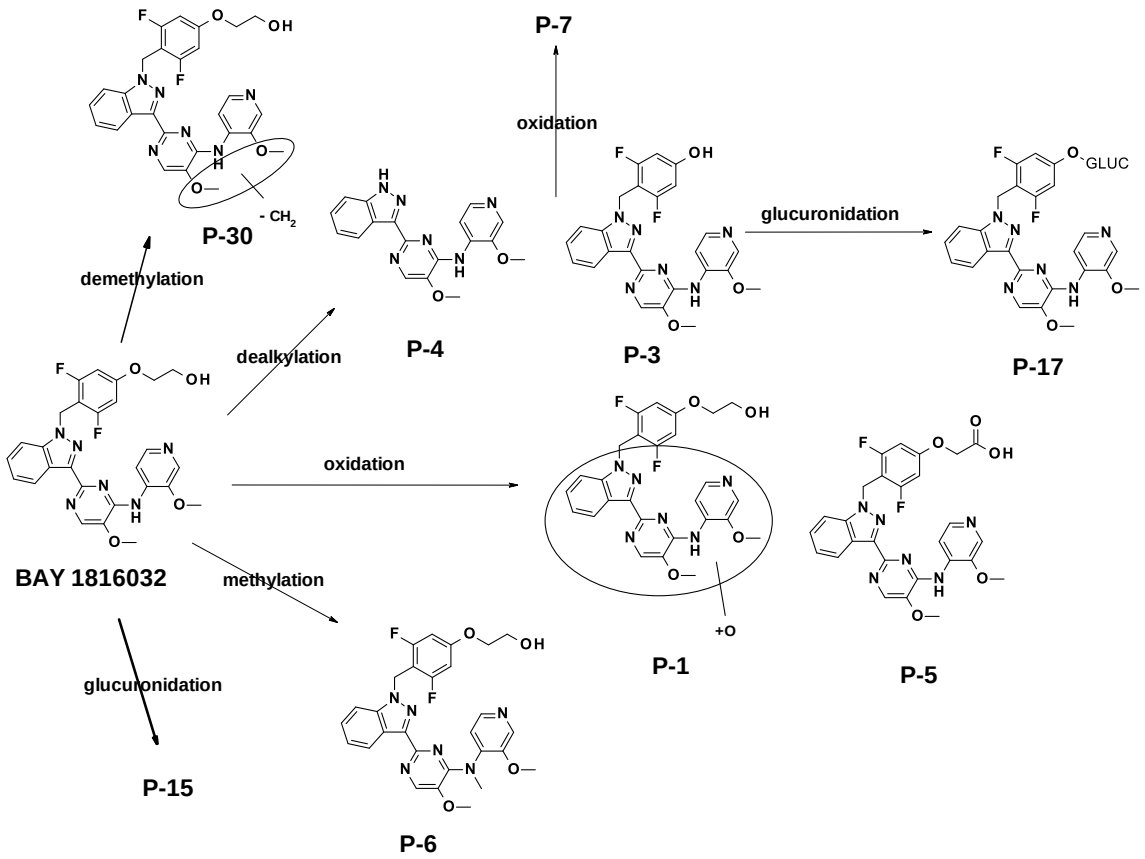
iv	Mouse	Rat	Dog
AUC_{norm} [kg·h/L]	0.34	0.99	2.4
CL_{plasma}	3.0	1.0	0.42
CL_{blood} [L/h/kg]	3.7	1.7	0.56
V_{ss} [L/kg]	4.0	4.5	2.5
$t_{1/2}$ [h]	1.8	3.9	4.2
po			
AUC_{norm} [kg·h/L]	0.075	0.59	1.4
$Cmax_{norm}$ [kg/l]	0.024	0.054	0.17
Tmax [h]	0.25	7.0	1.0
$t_{1/2}$ po [h]	n.a.	5.8	4.7
F [%]	22	60	59

- Species differences in CL with low in rat and dog and moderate in mouse which is line with Clint suggesting hepatic metabolism to be the main clearance mechanism
- High volume of distribution (V_{ss})
- Intermediate to high half life ($t_{1/2}$)
- Oral bioavailability is consistent with in vivo CL in all species,

BUB1 Inhibitor BAY 1816032

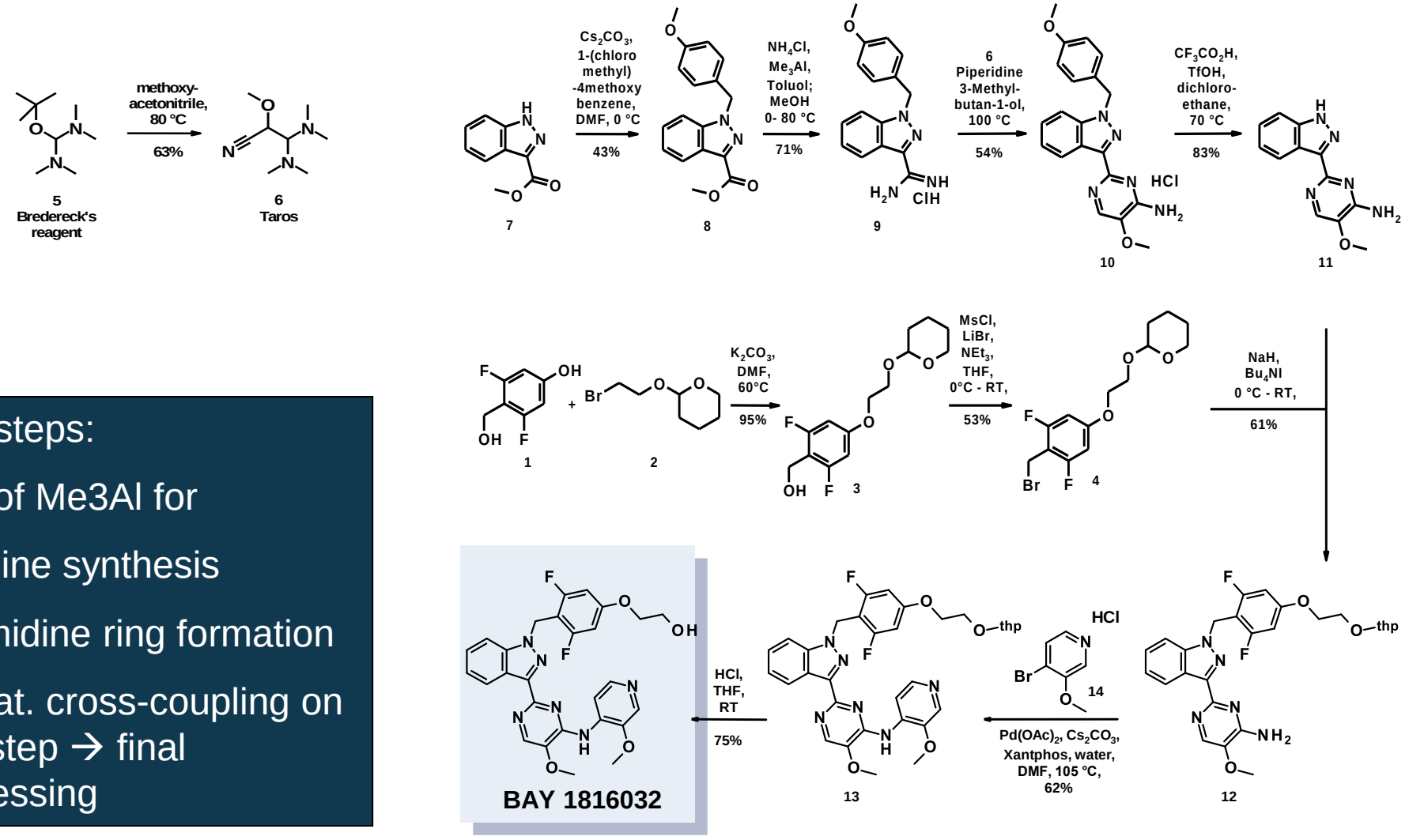
Drug metabolism: In vitro MetID & Species comparison in hepatocytes





- Main metabolic pathways in human hepatocytes:
  - direct glucuronidation (P-15), dealkylation (P-3), methylation (P-6), various oxidation (e.g. P-1, P-5)
- Complex phase I metabolism. Similar primary pathways in all species. However, in rat hepatocytes no direct glucuronidation observed.

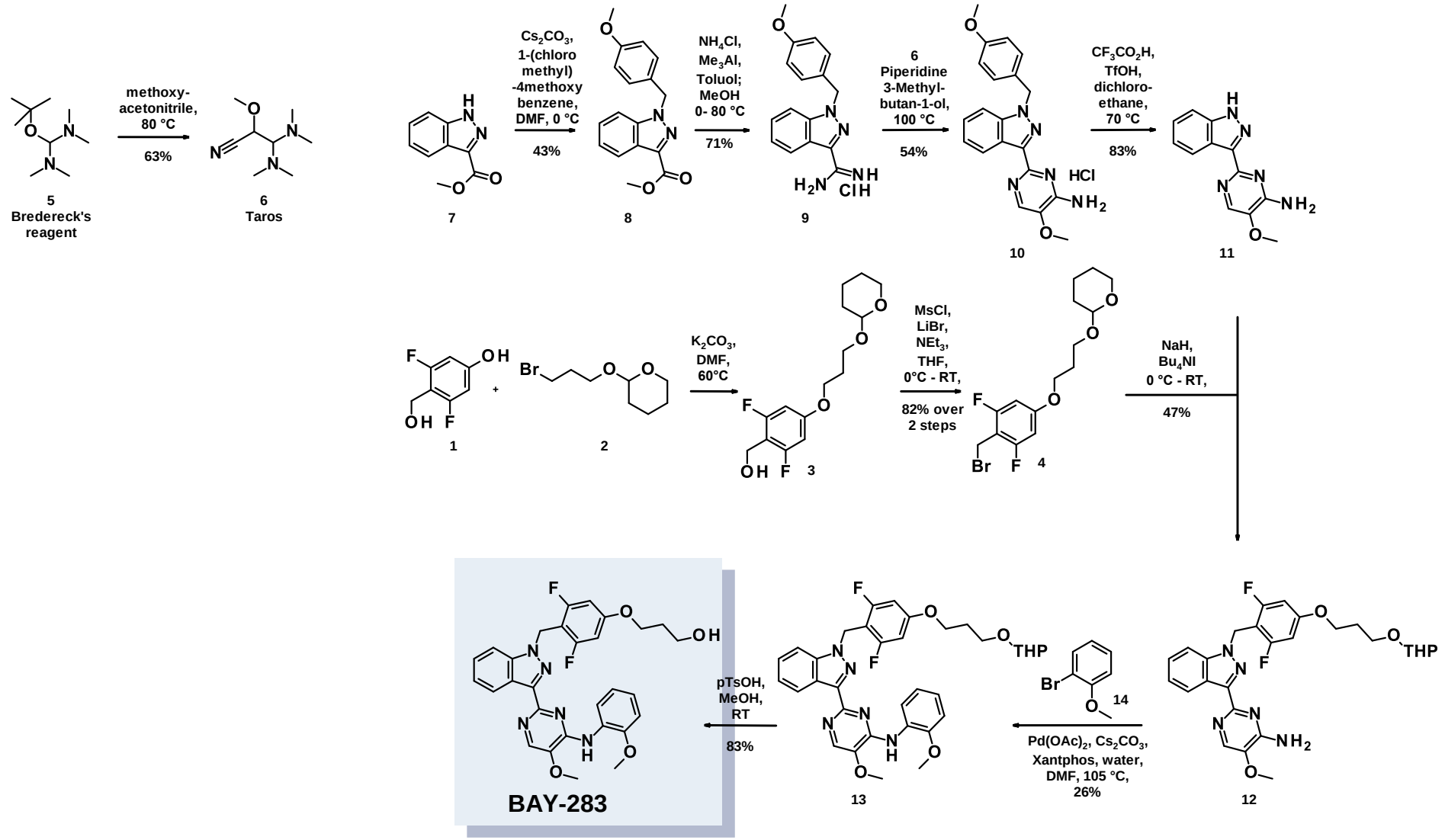
Synthesis of BUB1 Inhibitor BAY 1816032



Critical steps:

- Use of Me3Al for
- Amidine synthesis
- Pyrimidine ring formation
- Pd-cat. cross-coupling on late step → final processing

Synthesis of Negative Control BAY-283



BUB1 Inhibitor BAY 1816032

Discoverx KINOMEscan profiling service; #468

Target	MC 1171524		MC 1177655		MC 1464074	
Gene Symbol	%Ctrl @ 100nM	%Ctrl @ 1000nM	%Ctrl @ 1nM	%Ctrl @ 100nM	%Ctrl @ 100nM	%Ctrl @ 1000nM
CSNK1G2	100	95	93	100	90	78
CSNK1G3	100	100	100	100	100	100
CSNK2A1	100	95	100	49	100	100
CSNK2A2	43	38	42	4.5	46	48
CTK	98	100	100	63	100	97
DAPK1	100	100	100	100	100	100
DAPK2	78	89	93	95	82	85
DAPK3	81	95	83	86	85	84
DCAMKL1	100	100	100	100	100	100
DCAMKL2	83	99	85	94	71	90
DCAMKL3	100	100	100	100	100	100
DDR1	36	36	46	29	28	21
DDR2	100	98	100	74	98	93
DLK	79	89	53	63	88	87
DMPK	100	100	100	88	100	100
DMPK2	100	100	100	100	77	22
DRAK1	88	99	98	11	100	95
DRAK2	99	100	66	64	91	83
DYRK1A	81	100	34	0	82	96
DYRK1B	28	11	19	0	39	6.7
DYRK2	100	100	90	7.8	100	100
EGFR	100	100	100	100	100	100
EGFR(E746-A750del)	86	98	76	90	100	78
EGFR(G719C)	100	100	100	93	100	98
EGFR(G719S)	97	100	96	90	96	100
EGFR(L747-E749del, A750P)	100	100	100	100	100	100
EGFR(L747-S752del, P753S)	100	100	97	98	100	72
EGFR(L747-T751del,Sins)	91	99	88	92	99	100
EGFR(L858R)	100	100	100	100	100	100
EGFR(L858R,T790M)	100	100	100	90	100	100
EGFR(L861Q)	92	100	92	87	91	100
EGFR(S752-I759del)	83	94	93	84	97	98
EGFR(T790M)	77	86	86	54	74	76
EIF2AK1	95	86	86	84	92	87
EPHA1	71	77	65	48	77	65
EPHA2	100	100	83	95	100	100
EPHA3	100	100	100	100	100	85
EPHA4	99	96	94	97	89	96
EPHA5	83	93	82	92	88	78
EPHA6	93	86	93	90	85	89
EPHA7	97	100	95	69	100	98
EPHA8	96	100	100	83	100	100
EPHB1	100	87	93	80	99	100
EPHB2	100	100	100	100	100	100
EPHB3	95	91	89	100	84	95

LeadHunter DISCOVERY SERVICES

DiscoveRx

Table 1 - Assay Matrix (continued).

Target	MC 1171524		MC 1177655		MC 1464074	
Gene Symbol	%Ctrl @ 100nM	%Ctrl @ 1000nM	%Ctrl @ 1nM	%Ctrl @ 100nM	%Ctrl @ 100nM	%Ctrl @ 1000nM
BRK	79	90	79	100	100	91
BRSK1	95	100	100	100	100	93
BRSK2	93	96	91	100	88	83
BTK	92	100	54	83	42	100
BUB1	92	89	93	94	0.45	0.05
CAMK1	56	92	51	94	87	52
CAMK1B	95	92	100	87	96	95
CAMK1D	78	97	71	100	99	83
CAMK1G	100	97	70	36	87	99
CAMK2A	83	92	77	8.4	86	58
CAMK2B	94	97	97	36	83	100
CAMK2D	100	100	100	32	100	100
CAMK2G	95	100	80	18	96	100
CAMK4	100	100	100	100	100	68
CAMKK1	100	100	47	0.7	90	100
CAMKK2	100	100	55	1.1	98	100
CASK	79	76	69	78	69	78
CDC2L1	85	93	91	57	85	91
CDC2L2	91	98	90	47	80	100
CDC2L5	100	100	100	5.9	100	100
CDK11	86	95	90	99	98	78
CDK2	79	85	82	1.6	82	89
CDK3	100	100	100	2.2	100	99
CDK4	100	100	100	88	99	100
CDK4-cyclinD1	91	100	69	6.7	47	100
CDK4-cyclinD3	100	100	93	10	100	100
CDK5	92	96	89	57	97	81
CDK7	97	99	73	0.95	73	100
CDK8	77	76	84	90	88	69
CDK9	97	96	89	0.05	85	99
CDKL1	83	84	73	5.2	81	81
CDKL2	99	90	88	0	95	90
CDKL3	94	93	86	1.6	90	89
CDKL5	99	100	93	5.9	98	100
CHEK1	89	90	92	100	90	93
CHEK2	100	100	50	100	100	100
CIT	100	79	84	3.3	96	52
CLK1	100	100	100	37	100	100
CLK2	94	84	90	55	88	100
CLK3	100	100	100	100	100	100
CLK4	100	94	100	18	96	83
CSF1R	74	100	90	57	97	99
CSF1R-autoinhibited	80	83	74	5.5	95	86

BUB1 Inhibitor BAY 1816032

Discoverx KINOMEscan study report in detail

LeadHunter DISCOVERY SERVICES

BPA136-01-s-00001 Study Results

Table 1 - Matrix of Kds for BPA136-01-s-00001.

Target	MC 1464074
Gene Symbol	Kd (nM)
BUB1	3.3
LOK	57

Kd Legend

x<100nM	100nM≤x<1uM	x≥1uM	No Binding	Not Requested

LeadHunter DISCOVERY SERVICES

BPA161-01-s-00001 Study Results

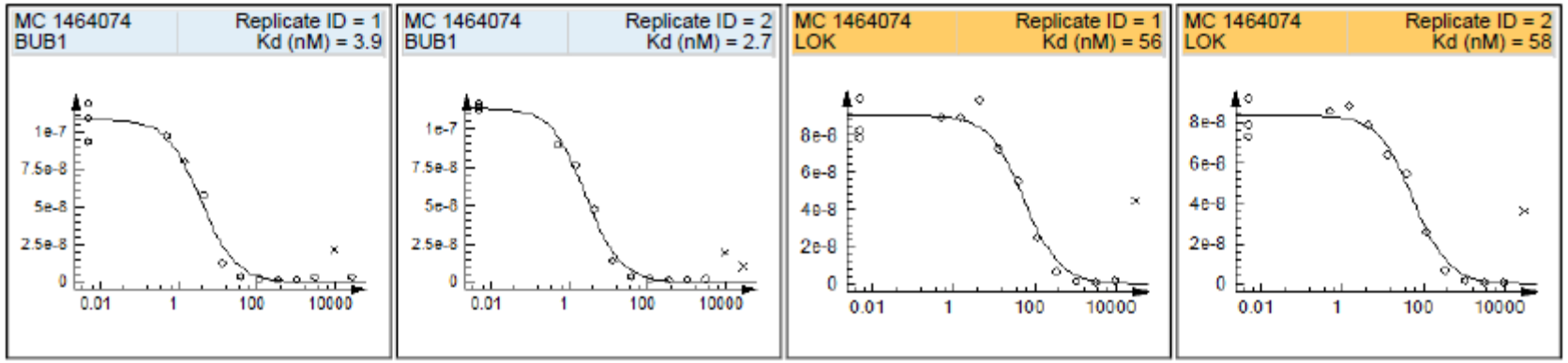
Table 1 - Matrix of Kds for BPA161-01-s-00001.

Target	MC 1464074
Gene Symbol	Kd (nM)
DDR1	2300
DMPK2	850
DYRK1B	>30000
GSK3A	>30000

Kd Legend

x<100nM	100nM≤x<1uM	x≥1uM	No Binding	Not Requested

Table 2 - Curve Images for BPA136-01-s-00001. The amount of kinase measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis). Data points marked with an "x" were not used for Kd determination.



BUB1 Inhibitor BAY 1816032

BUB1 was investigated in Eurofins Cerep Panlabs – SafetyScreen # of Assays: 87

Significant responses (≥ 50% inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}*	K_i	n_H
116020	Cyclooxygenase COX-1	hum	10 µM	54			
154000	Phosphodiesterase PDE4	hum	10 µM	52			
200610	Adenosine A_{2A}	hum	10 µM	67			
226810	$GABA_A$, Chloride Channel, TBOB	rat	10 µM	60			
279510	Sodium Channel, Site 2	rat	10 µM	53			
202000	Transporter, Adenosine	gp	10 µM	71			

- BAY 1816032 - not relevant off-target activity except human Adenosine transporter (51x).

BUB1 Inhibitor BAY 1816032

BUB1 was investigated in Eurofins Cerep Panlabs – SafetyScreen # of Assays: 87

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
Compound: CHH241-2014, PT #: 1188210							
107710	ATPase, Na^+/K^+, Heart, Pig	364727	pig	2	10 µM	21	
104010	Cholinesterase, Acetyl, ACES	364725	hum	2	10 µM	2	
116020	Cyclooxygenase COX-1	364999	hum	2	10 µM	54	
118010	Cyclooxygenase COX-2	365000	hum	2	10 µM	-9	
140010	Monoamine Oxidase MAO-A	364729	hum	2	10 µM	7	
140120	Monoamine Oxidase MAO-B	364851	hum	2	10 µM	-15	
107300	Peptidase, Angiotensin Converting Enzyme	364726	rabbit	2	10 µM	-4	
112510	Peptidase, CTSG (Cathepsin G)	364728	hum	2	10 µM	-15	
152000	Phosphodiesterase PDE3	364731	hum	2	10 µM	4	
154000	Phosphodiesterase PDE4	364732	hum	2	10 µM	52	
178010	Protein Serine/Threonine Kinase, PKC, Non-Selective	364859	rat	2	10 µM	-12	
174990	Protein Tyrosine Kinase, Insulin Receptor	364733	hum	2	10 µM	-12	
176020	Protein Tyrosine Kinase, LCK	364734	hum	2	10 µM	6	
200510	Adenosine A_1	364682	hum	2	10 µM	9	
200610	Adenosine A_{2A}	364682	hum	2	10 µM	67	
203100	Adrenergic α_{1A}	364810	rat	2	10 µM	8	
203200	Adrenergic α_{1B}	364811	rat	2	10 µM	-6	
203400	Adrenergic α_{1D}	364812	hum	2	10 µM	24	
203630	Adrenergic α_{2A}	364745	hum	2	10 µM	2	
203710	Adrenergic α_{2B}	364664	hum	2	10 µM	-15	
204010	Adrenergic β_1	364652	hum	2	10 µM	4	
204110	Adrenergic β_2	364655	hum	2	10 µM	10	
206000	Androgen (Testosterone)	364762	hum	2	10 µM	9	
299020	Angiotensin AT_1	365501	hum	2	10 µM	-2	
299021	Bradykinin B_2	365498	hum	2	10 µM	0	
214510	Calcium Channel L-Type, Benzothiazepine	364943	rat	2	10 µM	-10	
214600	Calcium Channel L-Type, Dihydropyridine	364763	rat	2	10 µM	8	
299019	Calcium Channel L-Type, Phenylalkylamine	365512	rat	2	10 µM	14	
216000	Calcium Channel N-Type	364693	rat	2	10 µM	10	
217030	Cannabinoid CB_1	364694	hum	2	10 µM	-6	
217100	Cannabinoid CB_2	364696	hum	2	10 µM	31	
217510	Chemokine CCR1	364750	hum	2	10 µM	-4	
299023	Chemokine CXCR2 (IL-8R$_B$)	365504	hum	2	10 µM	-3	
218030	Cholecystokinin CCK_1 (CCK_A)	364750	hum	2	10 µM	-19	
218130	Cholecystokinin CCK_2 (CCK_B)	364750	hum	2	10 µM	1	
219500	Dopamine D_1	364747	hum	2	10 µM	5	
219600	Dopamine D_{2L}	364748	hum	2	10 µM	4	
219700	Dopamine D_{2S}	364748	hum	2	10 µM	17	
299027	Endothelin ET_A	365502	hum	2	10 µM	-23	
226010	Estrogen ERα	364658	hum	2	10 µM	10	
226810	$GABA_A$, Chloride Channel, TBOB	364806	rat	2	10 µM	60	
226600	$GABA_A$, Flunitrazepam, Central	364680	rat	2	10 µM	7	
226630	$GABA_A$, Ro-15-1788, Hippocampus	364699	rat	2	10 µM	22	
228610	$GABA_{B1A}$	364700	hum	2	10 µM	-2	
232030	Glucocorticoid	364673	hum	2	10 µM	-24	
232600	Glutamate, AMPA	364753	rat	2	10 µM	7	
232700	Glutamate, Kainate	364749	rat	2	10 µM	-1	
237000	Glutamate, Metabotropic, $mGlu_5$	364982	hum	2	10 µM	17	
232810	Glutamate, NMDA, Agonism	364819	rat	2	10 µM	4	
232910	Glutamate, NMDA, Glycine	364754	rat	2	10 µM	-2	
233000	Glutamate, NMDA, Phencyclidine	364755	rat	2	10 µM	2	
234000	Glutamate, NMDA, Polyamine	364807	rat	2	10 µM	0	
239000	Glycine, Strychnine-Sensitive	364756	rat	2	10 µM	0	
239610	Histamine H_1	364669	hum	2	10 µM	12	
299012	Histamine H_2	365506	hum	2	10 µM	-13	
250460	Leukotriene, Cysteinyl $CysLT_1$	364709	hum	2	10 µM	32	
251100	Melanocortin MC_1	364791	hum	2	10 µM	4	
299024	Melanocortin MC_4	365505	hum	2	10 µM	10	
299029	Muscarinic M_1	365508	hum	2	10 µM	32	
252710	Muscarinic M_2	364685	hum	2	10 µM	-20	
252810	Muscarinic M_3	364685	hum	2	10 µM	-5	
252910	Muscarinic M_4	364794	hum	2	10 µM	8	
299011	Neuropeptide Y Y_1	365503	hum	2	10 µM	-11	
258590	Nicotinic Acetylcholine	364711	hum	2	10 µM	-15	
258700	Nicotinic Acetylcholine α1, Bungarotoxin	364712	hum	2	10 µM	14	
260130	Opiate δ_1 (OP1, DOP)	364676	hum	2	10 µM	-6	
260210	Opiate κ(OP2, KOP)	364678	hum	2	10 µM	26	
260410	Opiate µ(OP3, MOP)	364842	hum	2	10 µM	-37	

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
265010	Platelet Activating Factor (PAF)	364761	hum	2	10 µM	28	
265600	Potassium Channel [K_{ATP}]	364714	ham	2	10 µM	4	
265900	Potassium Channel hERG	364716	hum	2	10 µM	23	
267500	PPARγ	364715	hum	2	10 µM	-8	
299005	Progesterone PR-B	364675	hum	2	10 µM	-12	
299013	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	365510	hum	2	10 µM	0	
271230	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1B}$	364659	hum	2	10 µM	15	
299014	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	365511	hum	2	10 µM	22	
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	364660	hum	2	10 µM	22	
271800	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	364662	hum	2	10 µM	21	
299022	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_3$	365500	hum	2	10 µM	-3	
279510	Sodium Channel, Site 2	364650	rat	2	10 µM	53	
255520	Tachykinin NK_1	364649	hum	2	10 µM	19	
202000	Transporter, Adenosine	364809	gp	2	10 µM	71	
299026	Transporter, Dopamine (DAT)	365499	hum	2	10 µM	-5	
226400	Transporter, GABA	364820	rat	2	10 µM	1	
204410	Transporter, Norepinephrine (NET)	364666	hum	2	10 µM	26	
299025	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	365513	hum	2	10 µM	4	
299018	Vasopressin V_{1A}	365509	hum	2	10 µM	11	