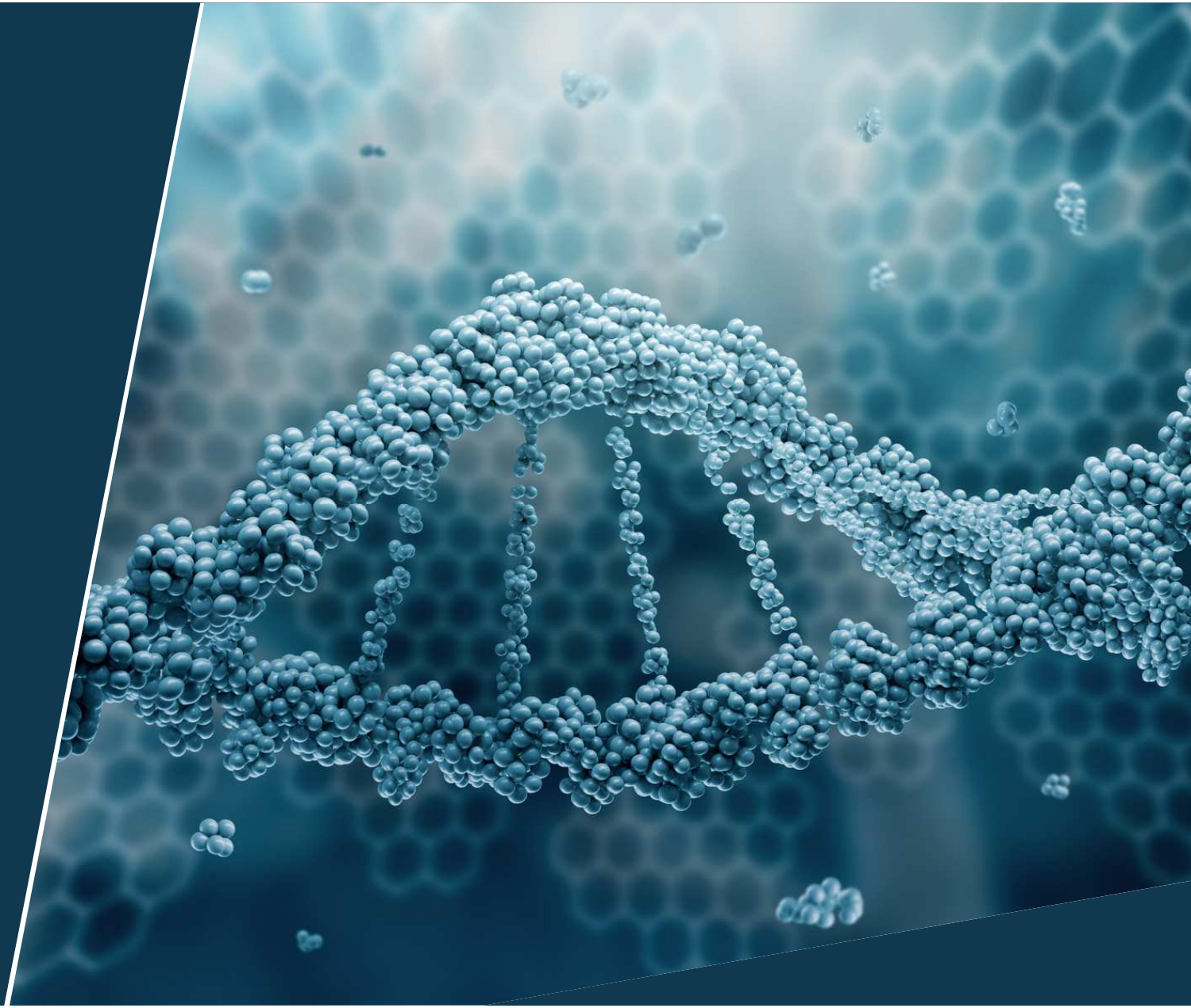
Donated Chemical Probe

Dual BCAT1/2 chemical probe Probe BAY-069

June, 2th 2020

Presenters:
BCAT team

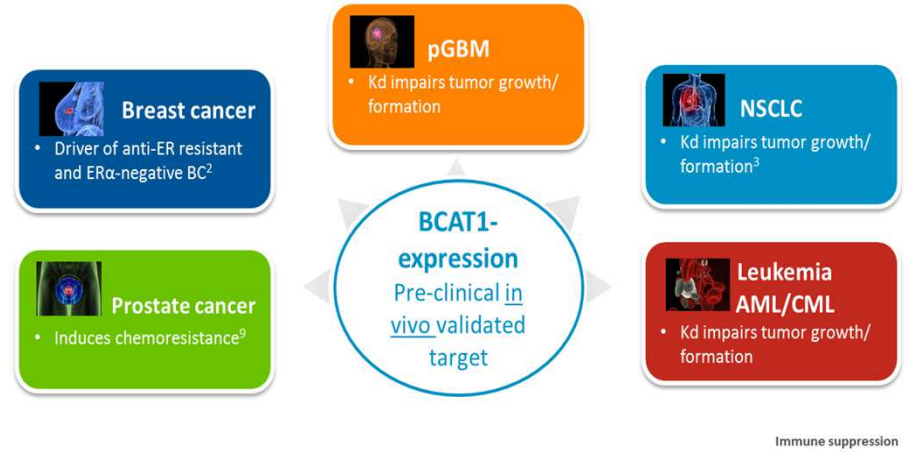


Dual BCAT1/2 Probe BAY-069

Scientific rationale

- BCAAs are **essential amino acids** that animals cannot synthesize de novo
- **Highly tumor specific**:
  - BCAT1 is c-Myc driven
  - **De novo expression** in tumors (besides brain*, testis and uterus)
  - (*) Overexpressed in IDH1 wt brain tumors (e.g pGBM)
  - Correlated with worse survival prognosis
- BCAA release in skeletal muscle → **cancer cachexia?**
- BCAT1 expression is **tumorigenic** by inhibiting α-KG dependent enzymes by substrate depletion.
- BCKA excretion **inhibits local macrophage immune function**

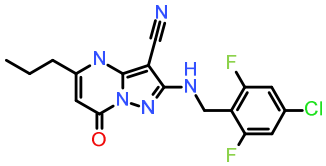
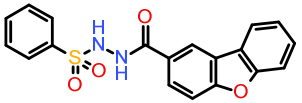
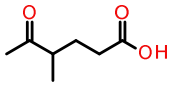
Broad indication space



➢ BCAT1 (Branched Chain Amino Acid Transaminase 1) is a validated target for the targeted treatment of different major cancers entities

Dual BCAT1/2 Probe BAY-069

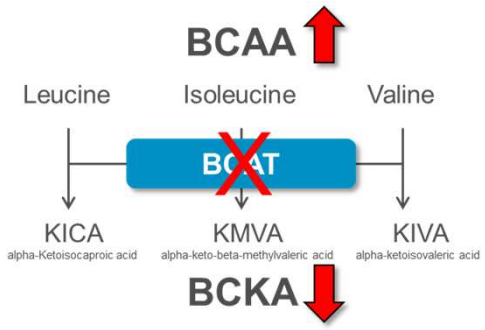
literature known compounds

	CAS: 1800024-45-6 compound 61 (GSK)	Pfizer	ERG240 Ergon
			
BACT1 / BCAT2	92 nM / 26 nM	0.8 µM / 4.3 µM	50 µM / 50 µM
BCAT1 $IC_{50}^{cell\text{-}MDA\ MB231}$	452 nM	>30 µM	> 30 µM
BCAT1 $IC_{50}^{cell\text{-}U87MG}$	4.9 µM	-	-
Caco2 (Papp (A-B)/ Papp (B-A) / efflux	116 / 131 / 1.1	207 / 69 / 0.33	-
Solubility [mg/L]	151	-	-
indication	obesity	neurodegenerative disorder	
Literature	*J. Med. Chem.* **2015**, *58*, 18, 7140-7163	*Bioorg. Med: Chem. Lett.*, **2006**, *16*, 2337-2340	US 2016/0368862 A1

- Other BACT1/2 inhibitors are known in the literature; the most potent one is the GSK inhibitor
- GSK inhibitor is from a different chemical class

Dual BCAT1/2 Probe BAY-069

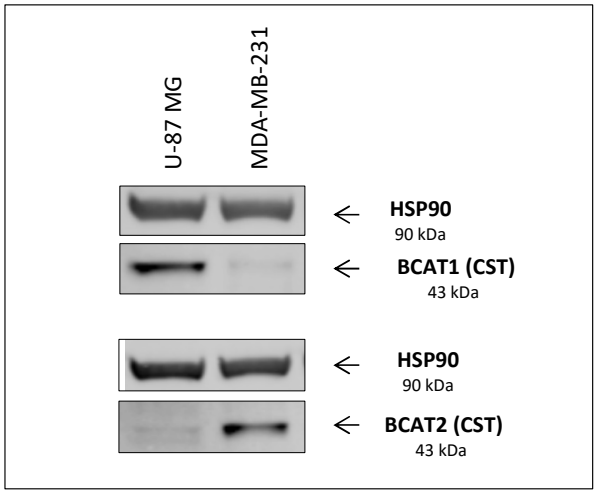
Cellular mechanistic assay – BCAA measurement



- Enzymatic detection of BCAA in cell culture
- **Medium without FCS**
- Increase of BCAAs indicates inhibition of BCAT
- 20 h sampling, Intra- and extracellular

	MDA-MB-231	U-87-MG
BCAT protein expression	Highly BCAT2	Highly BACT1
BAY-069	874 nM	358 nM

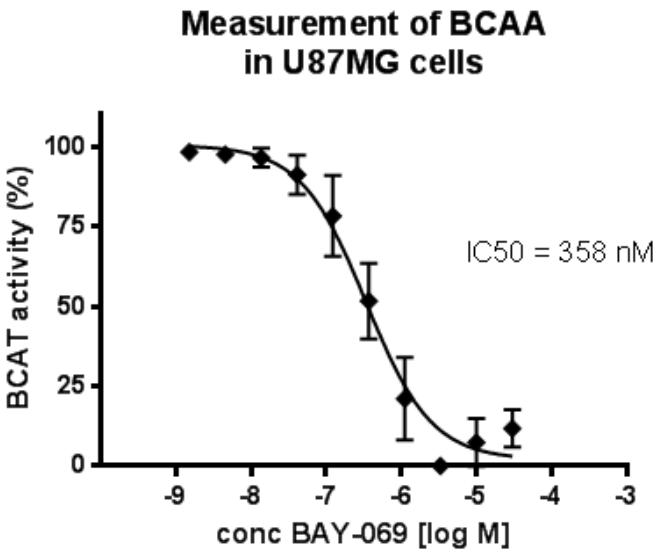
BCAT1 and BCAT2 protein expression (Western Blot)

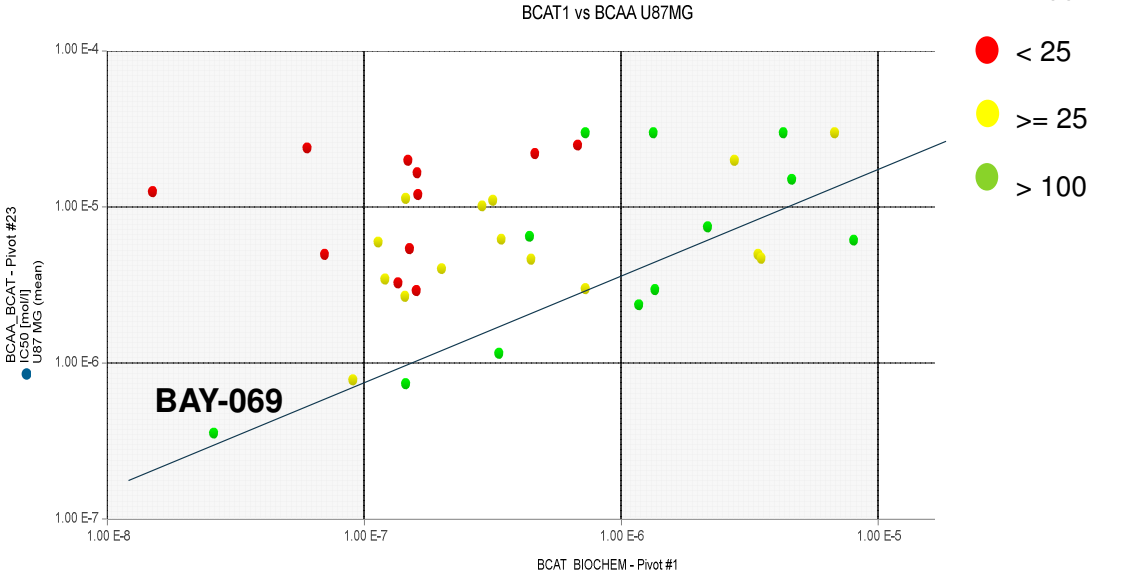


- Cellular mechanistic assay validated with BAY-069 in 2 cell lines
- BAY-069 leads to increased BCAA levels in U-87-MG (high BCAT1 expressing) cells and MDA-MB-231 (high BCAT2 expressing) cells
- These results confirm on-target (BCAT1 and BCAT2) cellular activity

Dual BCAT1/2 Probe BAY-069

Cellular mechanistic assay – BCAA measurement

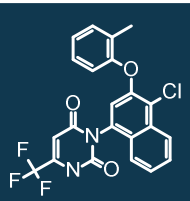




- BAY-069 leads to increased BCAA levels in U-87-MG (high BCAT1 expressing) cells
- These results confirm on-target cellular activity
- Correlation between biochemical BCAT1 IC_{50} and cellular activity (BCAA in U-87-MG)
- Outliers (red) show low Caco-2 permeability (Papp A-B)

Dual BCAT1/2 Probe BAY-069

Cellular functional assay - 2D cell proliferation experiments*

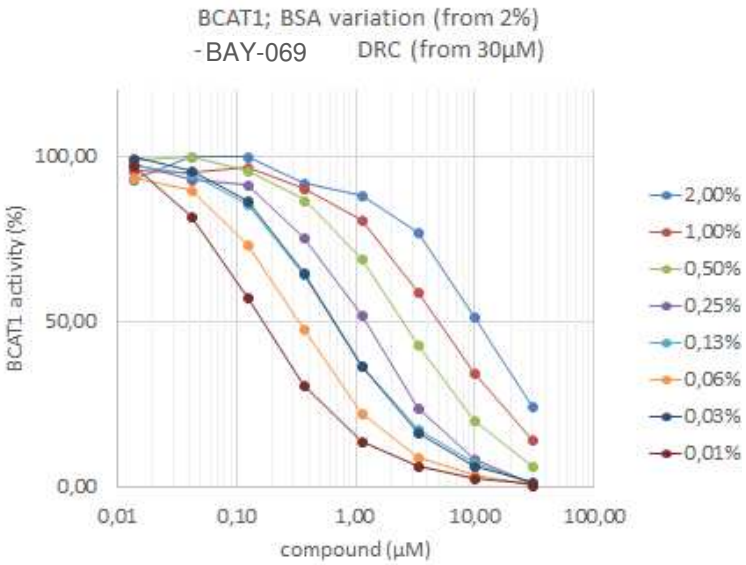
	MDA-MB-231		U-87-MG		SEM		CAL 51		HC33		NCI-H2110	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
 BAY-069	>50 µM	>50 µM	>50 µM	>50 µM	>50 µM	>50 µM	47.7 µM	50 µM	50 µM	50 µM	50 µM	50 µM

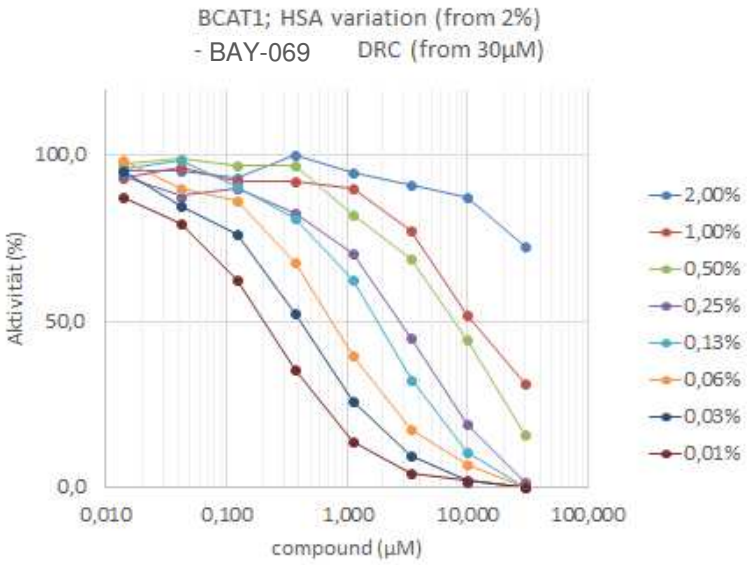
*proliferation experiments done in cell culture medium with 10% FBS

- BAY-069 shows no anti-proliferative activity in different cell lines after 72 h
- The cellular mechanistic assay does not translate in a proliferative activity.

Dual BCAT1/2 Probe BAY-069

Biochemical assay – effect of protein binding

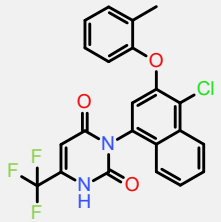




- Strong loss of potency in the presence of serum albumin for BAY-069
- Might explain lack of effect in in-vitro assays with FCS, e.g. proliferation assays

Dual BCAT1/2 Probe BAY-069

Technical profile



BAY-069 (en1)

POTENCY (IC_{50} [nM])	
BACT1 IC_{50}	27 nM
BACT2 IC_{50}	130 nM
LLE / BEI	4.8 / 19.4
Mechan. IC_{50} MDA-MB-231	874 nM
Mechan. IC_{50} U-87-MG	358 nM

Properties & Physchem	
LogD @ pH 7.5 / pka	2.4 / 5.7
fu [%] Williams_E / brain / Mouse	0.81 / 0.47 / 0.14
Sw powder @ pH 6.5 [mg/L]	140
MW / TPSA [g*mol / Å^2]	389 / 59
Stability (r /h plasma, 4h) [%]	Stable in r and h

***in vitro* DMPK Properties**

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	252		122		0.48	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	Human liver mics		0.11		92	
	rat hepatocytes		1.8		56	
In vivo rat PK	Low CLb, moderate Vss, intermediate half-life, high oral bioavailability					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	0.87	0.64	>20	4.1	3.2
PXR	red					

Selectivity

In-house kinase panel (#21)	clean
Eurofins safety panel	clean

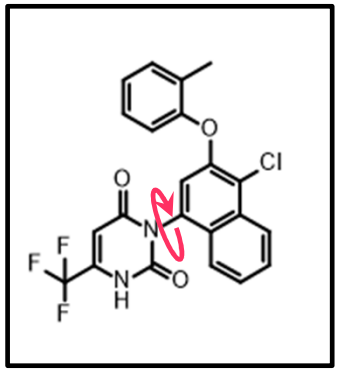
SAFETY	
Cytotox	n.d.
hERG IC_{50} [µM]	>10 µM

- BAY-069 is a dual BCAT1/2 inh.
- BAY-069 shows high Caco-2 permeability and high solubility
- Stock availability: 250 mg are available

Dual BCAT1/2 Probe BAY-069

Atropisomerism

- Annulated phenyl induces axial chirality
  - H-bond to Gln244 in X-ray allows unequivocal assignment of stereochemistry in active isomer
  - Atropisomers has been separated via column chromatography
  - Atropisomers show different biochemical profiles:

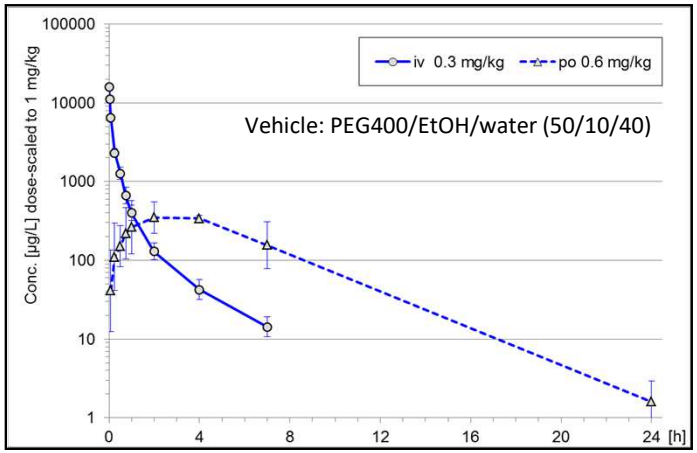


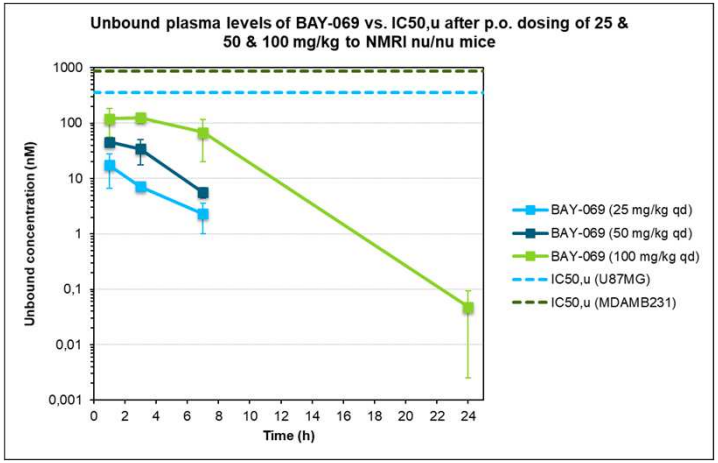
	BAY-069 (atrop 1)	BAY-252 (atrop 2)	BAY-5000 (rac)
IC_{50} BACT1	26 nM	2 µM	333 nM
IC_{50} BACT2	130 nM	2 µM	22 nM
IC_{50} BCAT1 cell-MDA MB231	874 nM	2 µM	564 nM
IC_{50} BCAT1 cell-U87-MG	358 nM	-	5 µM
Caco2 (Papp (A-B)/ Papp (B-A) / efflux	252 / 122 / 0.48	260 / 152 / 0.58	137 / 146 / 1.1

- Both atropisomers show different activity profiles -> BAY-069 displays the best profile
- Currently studies ongoing in order to determine the energy barrier

Dual BCAT1/2 Probe BAY-069

Low dose *In vivo* rat PK and High dose In vivo mouse PK





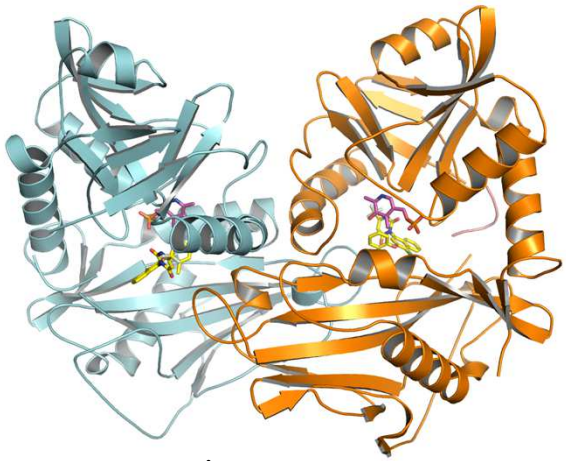
BAY Number	BAY-069	BAY-069	BAY-069
Dose	25 mg/kg	50 mg/kg	100 mg/kg
$AUC_{0\text{-tlast}}$	16 h*mg/L	53 h*mg/L	270 h*mg/L
$AUC_{0\text{-tlast,norm}}$	0,63 h*kg/L	1,1 h*kg/L	2,7 h*kg/L
$AUC_{0\text{-tlast,u}}$	0,022 h*mg/L	0,074 h*mg/L	0,38 h*mg/L
$C_{max,u}$	17 nM	46 nM	130 nM
IC_{50} Assay	U87MG	U87MG	U87MG
IC_{50}	358 nM	358 nM	358 nM
$IC_{50,u}$	358 nM	358 nM	358 nM
$C_{max,u}$ / $IC_{50,u}$	0,047	0,13	0,36

Admin Route		iv bolus	po
Dose Admin	[mg/kg]	0.30	0.60
AUC_{norm}	[kg·h/L]	2.9	2.5
$C_{max,norm}$	[kg/L]	16	0.48
CL_{blood}	[L/h/kg]	0.64	
t_{max}	[h]		2.0
V_{ss}	[L/kg]	0.25	
$t_{1/2}$	[h]	1.6	2.2
F	[%]		89

- Low CL_b, moderate Vss, intermediate half-life, high oral bioavailability
- High dose PK study with oral dosing in mouse (female NMRI nude) up to 100 mg/kg showed exposure which did reach the levels of the determined cellular IC_{50}
- BAY-069 may be suitable for *in vivo* studies at higher concentrations

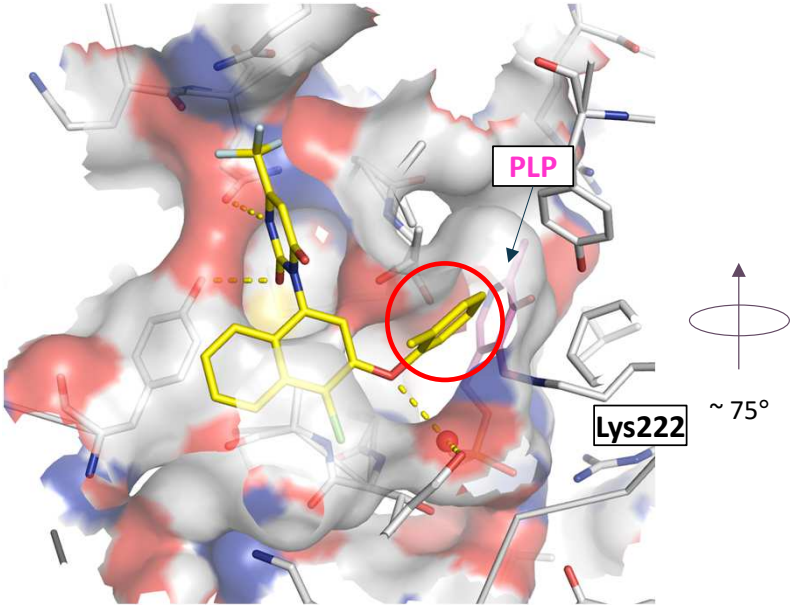
Dual BCAT1/2 Probe BAY-069

X-ray structure of BAY-069

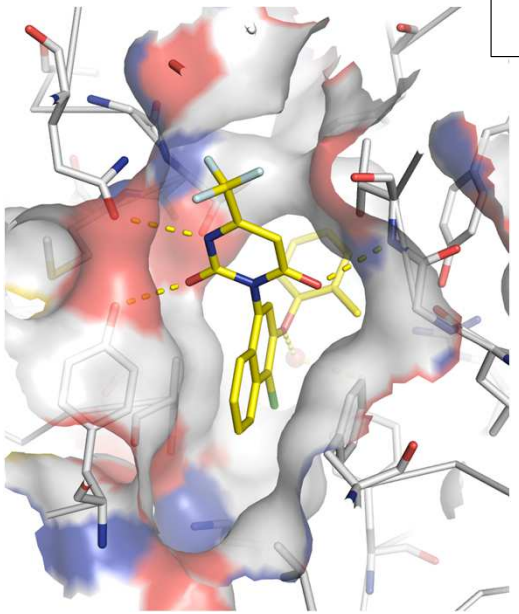


Resolution 1.85 Å,

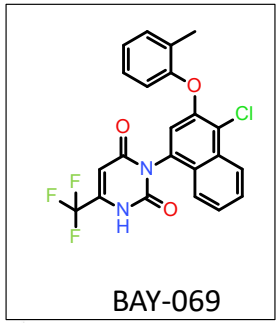
R_{work}/R_{cryst} = 0.184 / 0.227



~ 75°



BAY-069 bound to BCAT1, chain A



BAY-069

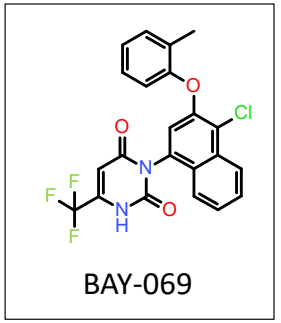
BAY-069 soaked into BCAT1 crystals grown with co-factor PLP (covalently linked to Lys222) & substrate mimic (3-phenylpropionic acid).

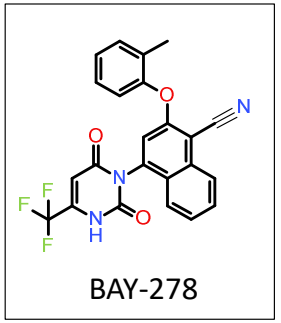
- BAY-069 bound in substrate site in front of co-factor PLP (magenta), in both active sites of the BCAT1 homodimer.
- Methyl-phenoxy-moiety occupies hydrophobic binding site for BCAA side chains (red circle) & replaces substrate mimic 3PP
- Chloro-naphthyl moiety fills canyon in outer part of substrate binding site; pyrimidine-dion occupies orthogonal subpocket
- Multiple H bonds to BCAT1, formed by phenoxy-oxygen and by all polar atoms of the pyrimidine-dion ring.

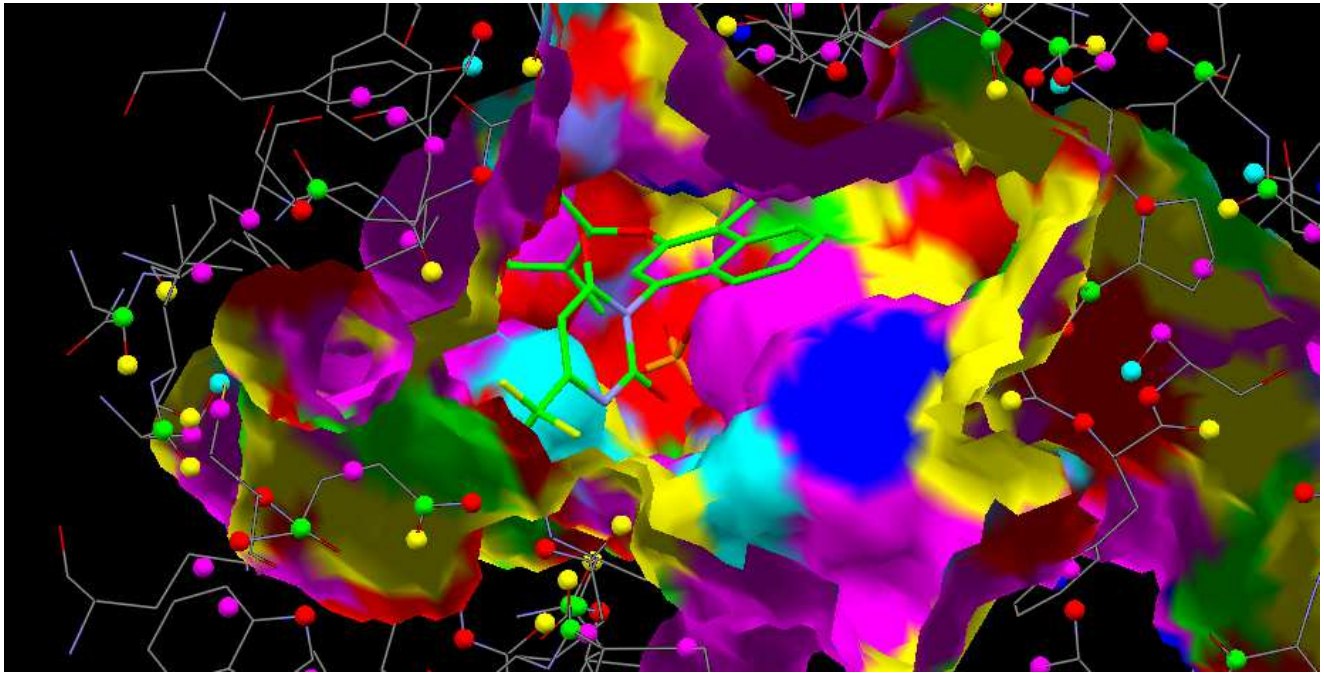
Selectivity

Dual BCAT1/2 Probe BAY-069

Cavbase with close congener BAY-278







- **Searching across the X-ray characterized proteome (pocketome) with closely related variant BAY-278**
  - Using in-house co-crystal structure with BAY-278 (identical binding mode)
  - pick all pseudocenters (donor/acceptor/pi/aromatic/aliphatic) within a 4 Å radius of co-crystallized ligand BAY-278

- Little is known about overall selectivity of this target family, therefore we looked at selectivity profile in more detail using CavBase search for possible co/anti-targets (cavity search) for BAY-278.
- No hints for any cross reactivity of probe BAY-069 (based on X-ray of close analogue BAY-278) were found .

Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: broader panels

- **Other Transaminases**
  - Aspartate transaminase: GOT1/2: IC_{50} > 50 µM

- **In-house protease panel (# 30 proteases)**
  - All IC_{50} > 10 µM; one hit at 6 µM

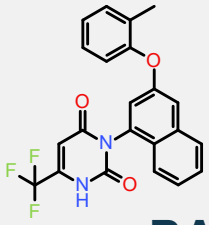
- **In-house kinase panel (# 30 kinases)**
  - All IC_{50} > 7 µM; one hit at 2 µM

- **Safety screen (Eurofins, # 77 targets)**
  - Clean (no relevant activity >50%)
  - Hits:
    - Thromboxane synthase: 45%
    - GABA transporter: 46%

➢ BAY-069 shows an overall clean profile (for more information, see backup)

Dual BCAT1/2 Probe BAY-069

In vitro technical profile of Negative Control BAY-771



BAY-771

POTENCY (IC_{50} [nM])	
BACT1 IC_{50}	6.5 µM
BACT2 IC_{50}	10.8 µM
Mechan. IC_{50} MDA-MB-231	n.d.
Mechan. IC_{50} U-87-MG	6.2 µM (17fold)

Properties & Physchem	
LogD @ pH 7.5	2.2
fu [%] Williams_E / rat / Mouse	-
Sw @ pH 6.5 [mg/L]	>412
MW / TPSA [g*mol / Å^2]	412 / 59
Stability (r /h plasma, 4h) [%]	-

***in vitro* DMPK Properties**

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	275		171		0.62	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	Human liver mics		0.79		40	
	rat hepatocytes		2.5		40	
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
PXR						

Selectivity

In-house kinase panel (#21)	Clean (> 7 µM) FLT3: 5 µM

SAFETY

Cytotox	Not available
hERG IC_{50} [µM]	Not available

- BAY-771 was suggested as negative control
- Further profiling could be undertaken after probe acceptance
- Stock availability: only DMSO solution, resynthesis necessary

Dual BCAT1/2 Probe BAY-069

Summary / Conclusion

Probe criteria	
Inhibitor potency: goal is < 100 nM (IC_{50}, Kd)	meets criteria for BACT1; slightly above for BCAT2
Selectivity within target family: goal is > 30-fold	meets criteria; selective to aspartate transaminase
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Surpasses criteria**
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Surpasses criteria**
Additional structural information	X-ray available
Neg ctrl: in vitro potency – > 100-fold less; Cell activity – >100-fold less potent than the probe	Neg control available; 17-fold less in cellular assay

We ask for acceptance of BAY-069 as chemical probe, accompanied by BAY-771 as negative control

Dual BCAT1/2 Probe BAY-069

Project Team / Acknowledgement

dkfz.

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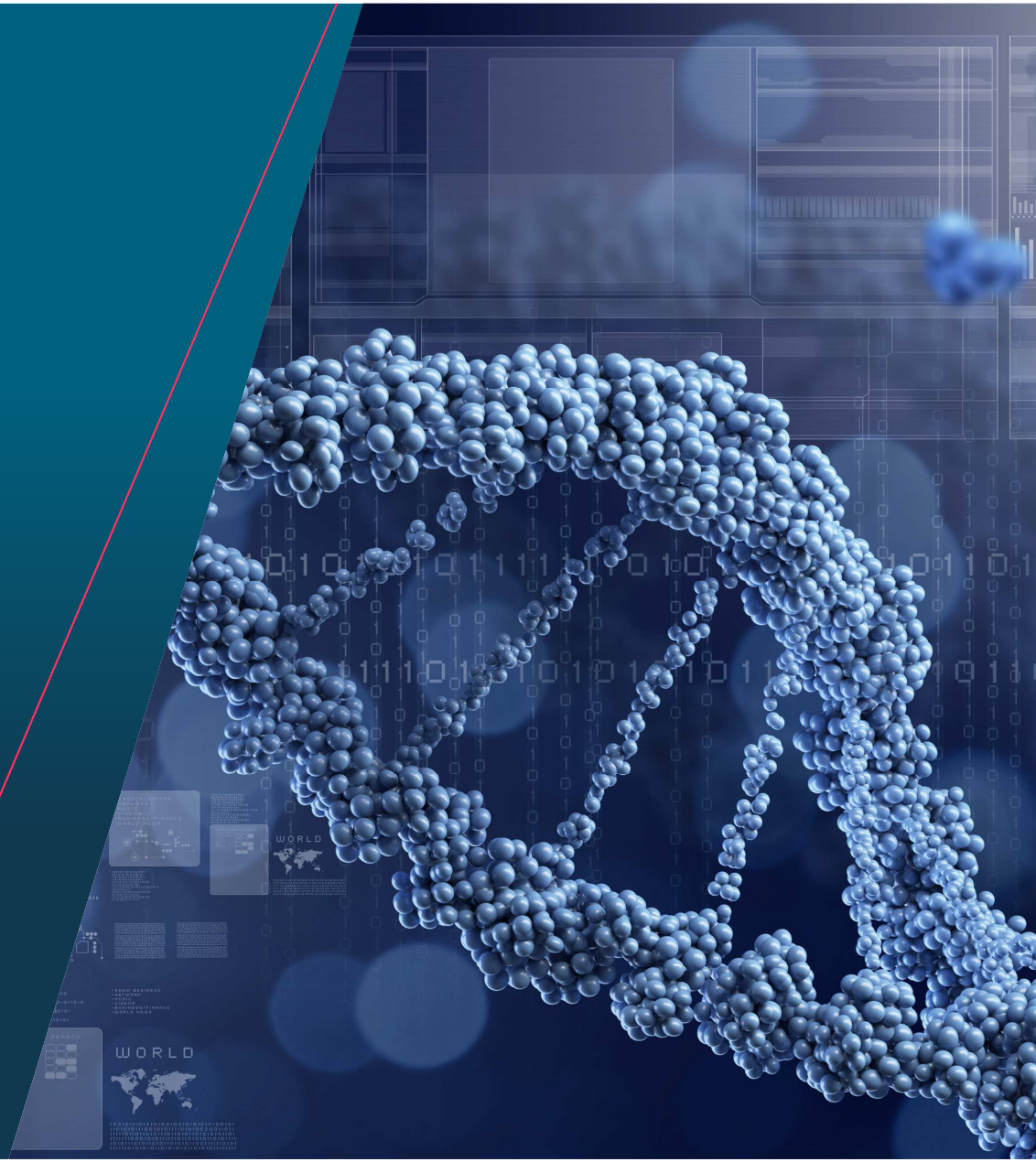
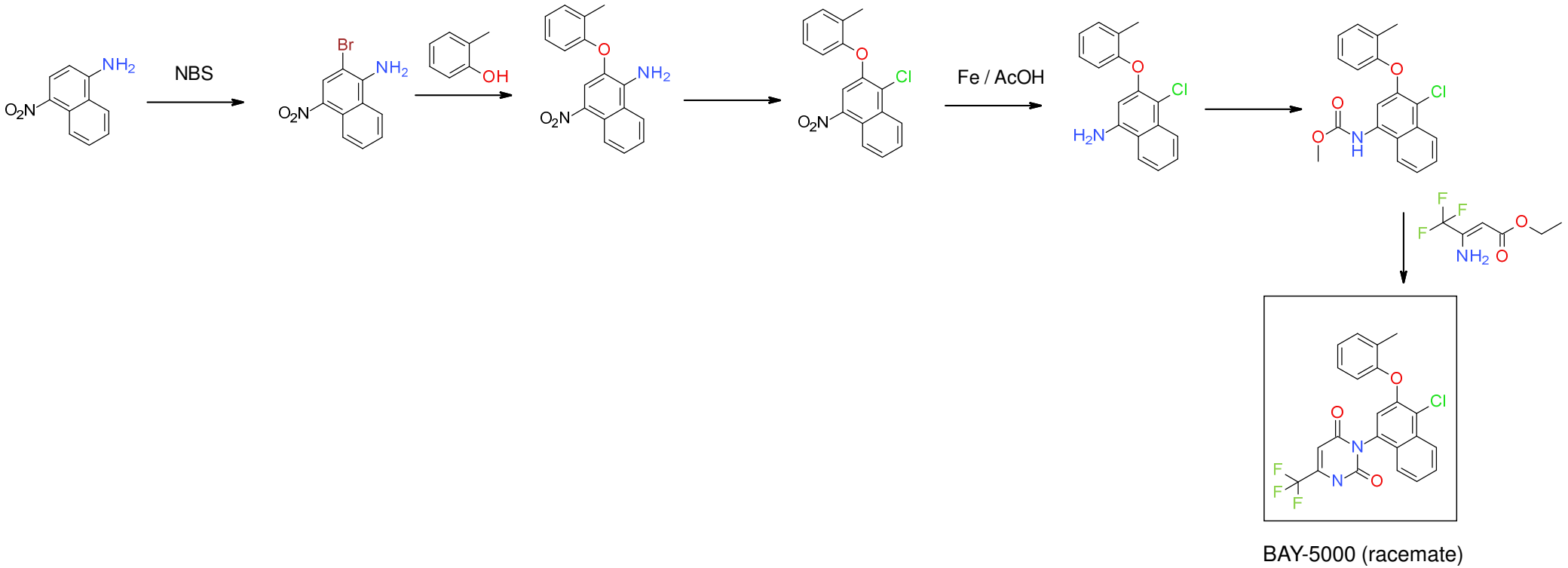
Roman Hillig
Clara Lemos
Judith Guenther
Roland Neuhaus
Christian Lechner
Roland Neuhaus

Thank You



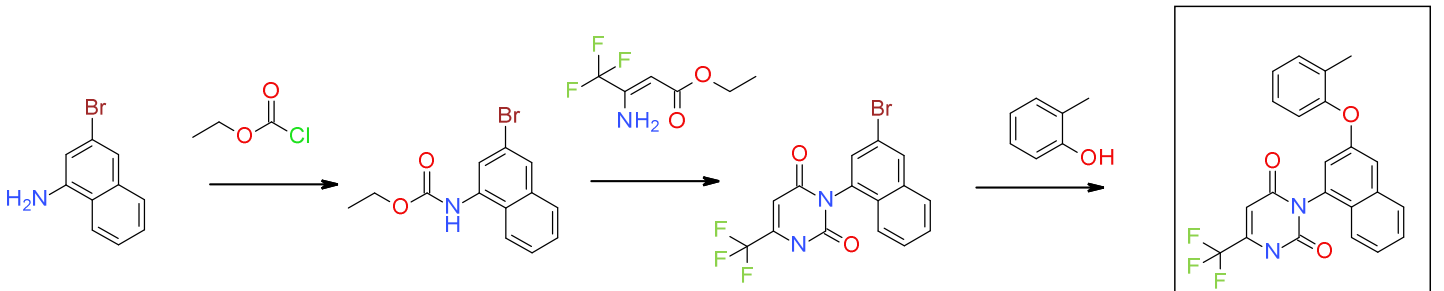
Dual BCAT1/2 Probe BAY-069

Synthesis of BAY-069



Dual BCAT1/2 Probe BAY-069

Synthesis of negative Control BAY-771



BAY-771 (racemate)

Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: use of **Cavbase** search for possible co/anti-targets for BAY-278

Search Results 1-15 of 100

Select	Cavity	Score	Normalised Score	Matched Centres	RMS	Protein Homology	Cavity Homology	Header	Title
☑	pdb1gyp.5	7.0	17.9	9	1.188	22.6	unknown	OXIDOREDUCTASE (ALDEHYDE(D)-NAD+(A))	CRYSTAL STRUCTURE OF GLYCOSOMAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM LEISHMANIA MEXICANA: IMPLICATIONS FOR STRUCTURE-BASED DRUG DESIGN AND A NEW POSITION FOR THE INORGANIC PHOSPHATE BINDING SITE
☐	pdb2jkv.34	6.6	16.9	8	1.317	29.2	unknown	OXIDOREDUCTASE	STRUCTURE OF HUMAN PHOSPHOGLUCONATE DEHYDROGENASE IN COMPLEX WITH NADPH AT 2.53A
☐	pdb2gah.11	6.6	16.8	8	1.311	29.7	unknown	OXIDOREDUCTASE	HETEROTETRAMERIC SARCOSINE: STRUCTURE OF A DIFLAVIN METALOENZYME AT 1.85 A RESOLUTION
☐	pdb1xah.5	6.5	16.6	8	1.129	21.3	unknown	LYASE	CRYSTAL STRUCTURE OF STAPHLYOCOCCUS AUREUS 3-DEHYDROQUINATE SYNTHASE (DHQS) IN COMPLEX WITH ZN2+ AND NAD
☐	pdb2zea.10	6.1	15.7	8	1.552	42.9	1.4	OXIDOREDUCTASE	CRYSTAL STRUCTURE OF ALCALIGENES FAECALIS D-3 HYDROXYBUTYRATE DEHYDROGENASE IN COMPLEX WITH NAD+ AND ACETATE
☐	pdb2vuu.18	6.1	15.7	7	0.898	21.4	unknown	TRANSCRIPTION	CRYSTAL STRUCTURE OF NADP-BOUND NMRA-AREA ZINC FINGER COMPLEX
☐	pdb3ru7.2	6.1	15.6	7	1.161	39.1	2.7	ISOMERASE	SPECIFIC RECOGNITION OF N-ACETYLATED SUBSTRATES AND DOMAIN

// **Results:**

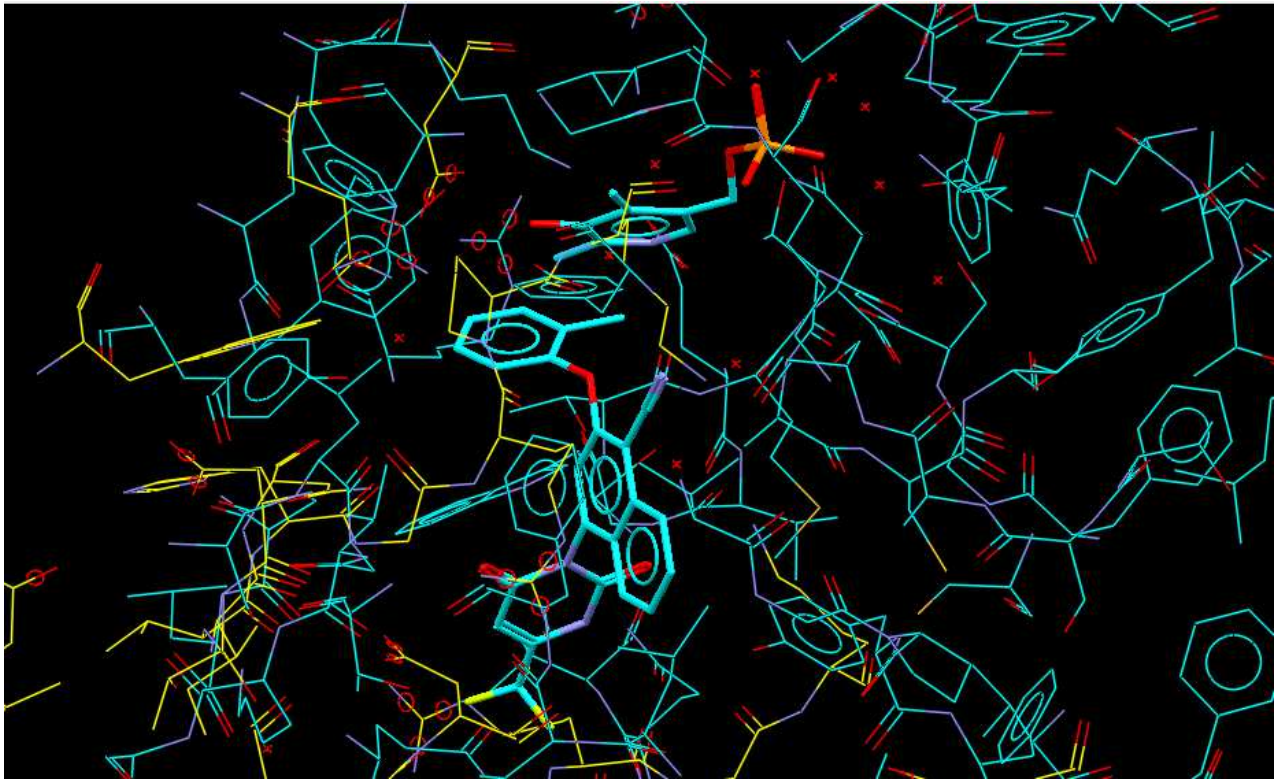
// All hits identified have very low scores for pocket match to query

// (see backup for more detail)

- No hints for any cross reactivity of probe BAY-069 (based on X-ray of close analogue BAY-278) found
- Search space is restricted to X-ray characterized part of proteome
- Outside this space, no conclusions on possible other targets can be drawn based on the query setup.

Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: use of **Cavbase** search for possible co/anti-targets for BAY-078



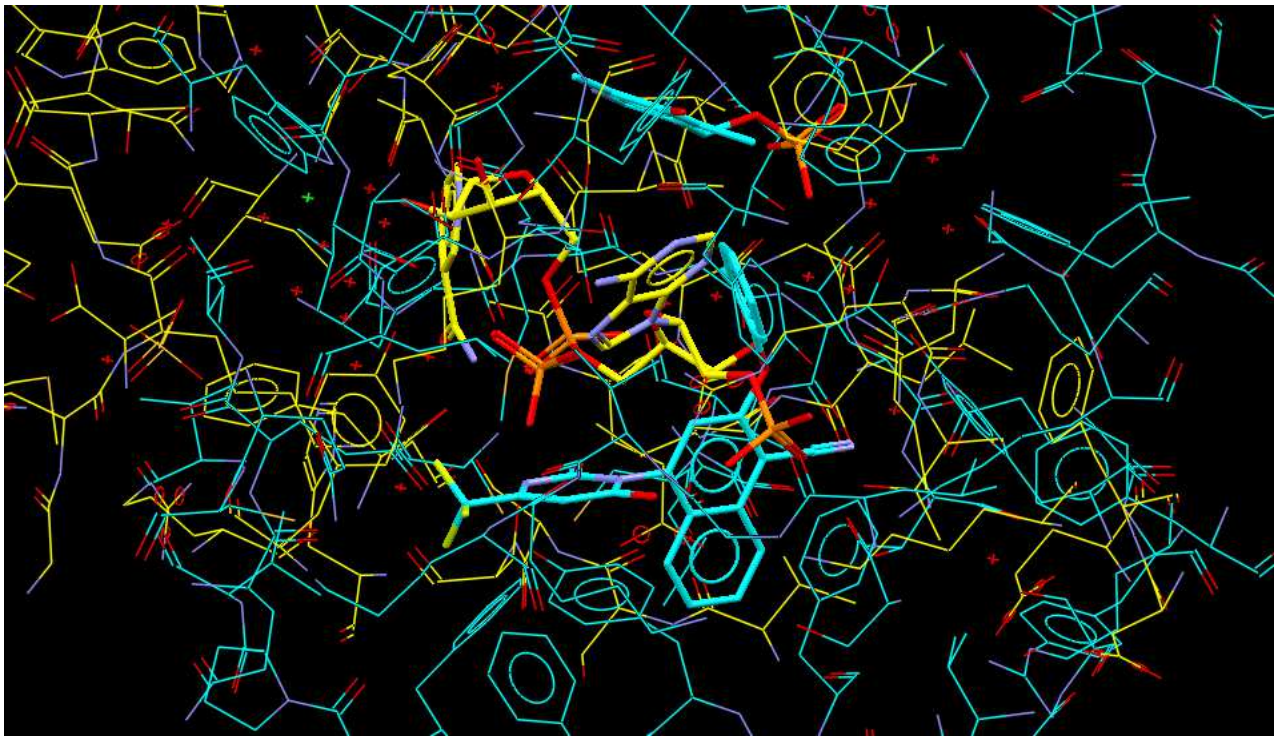
Top Scorer:

BCAT1 (X647) ↔
PDB1GYP Glycosomal Glyceraldehyde-3-Phosphate Dehydrogenase from LEISHMANIA MEXICANA

- **Results:**
  - Visual inspection confirms irrelevance to match

Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: use of **Cavbase** search for possible co/anti-targets for BAY-078



Top **human** Scorer:

BCAT1 (X647) ↔
PDB2JKV Human Phosphogluconate Dehydrogenase

// **Results:**

- // Visual inspection confirms irrelevance to match

Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: safety screen (Eurofins, # 77 targets)

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Compound: CHH026-2019, PT #: 1224239						
107000	Aldose Reductase	433410	rat	2	10 µM	-1
107710	ATPase, Na^+/K^+, Heart, Pig	433438	pig	2	10 µM	-13
112020	Carbonic Anhydrase II	433411	hum	2	10 µM	1
104010	Cholinesterase, Acetyl, ACES	433409	hum	2	10 µM	8
116020	Cyclooxygenase COX-1	433651	hum	2	10 µM	-10
118010	Cyclooxygenase COX-2	433652	hum	2	10 µM	7
124010	HMG-CoA Reductase	433419	hum	2	10 µM	0
132000	Leukotriene LTC_4 Synthase	433418	gp	2	10 µM	29
199017	Lipoxygenase 15-LO	433427	hum	2	10 µM	15
140010	Monoamine Oxidase MAO-A	433440	hum	2	10 µM	8
140120	Monoamine Oxidase MAO-B	433441	hum	2	10 µM	8
142000	Nitric Oxide Synthase, Neuronal (nNOS)	433420	rat	2	10 µM	-4
199010	Nitric Oxide Synthetase, Inducible (iNOS)	433425	mouse	2	10 µM	14
107300	Peptidase, Angiotensin Converting Enzyme	433437	rabbit	2	10 µM	1
152000	Phosphodiesterase PDE3	433452	hum	2	10 µM	-8
154420	Phosphodiesterase PDE4D2	433454	hum	2	10 µM	2
156000	Phosphodiesterase PDE5	433453	hum	2	10 µM	-19
194020	Thromboxane Synthase	433426	hum	2	10 µM	45
200510	Adenosine A_1	433503	hum	2	10 µM	27
200610	Adenosine A_{2A}	433505	hum	2	10 µM	-6
200720	Adenosine A_3	433689	hum	2	10 µM	-2
203100	Adrenergic α_{1A}	433443	rat	2	10 µM	5
203630	Adrenergic α_{2A}	433428	hum	2	10 µM	2
203710	Adrenergic α_{2B}	433429	hum	2	10 µM	-2
203810	Adrenergic α_{2C}	433430	hum	2	10 µM	6
204010	Adrenergic β_1	433456	hum	2	10 µM	3
204110	Adrenergic β_2	433457	hum	2	10 µM	-11
204200	Adrenergic β_3	433459	hum	2	10 µM	15
206000	Androgen (Testosterone)	433476	hum	2	10 µM	9
210030	Angiotensin AT_1	433527	hum	2	10 µM	7
210120	Angiotensin AT_2	433528	hum	2	10 µM	6
212520	Bradykinin B_1	433509	hum	2	10 µM	-8
212620	Bradykinin B_2	433464	hum	2	10 µM	1
217030	Cannabinoid CB_1	433671	hum	2	10 µM	16

No significant results noted.

Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: safety screen (Eurofins, # 77 targets)

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
217100	Cannabinoid CB_2	433530	hum	2	10 µM	-5
219500	Dopamine D_1	433525	hum	2	10 µM	7
219600	Dopamine D_{2L}	433523	hum	2	10 µM	-5
219700	Dopamine D_{2S}	433524	hum	2	10 µM	-5
219800	Dopamine D_3	433525	hum	2	10 µM	14
224010	Endothelin ET_A	433548	hum	2	10 µM	-2
224110	Endothelin ET_B	433549	hum	2	10 µM	-9
226010	Estrogen ERα	433601	hum	2	10 µM	6
226810	$GABA_A$, Chloride Channel, TBOB	433540	rat	2	10 µM	15
226600	$GABA_A$, Flunitrazepam, Central	433465	rat	2	10 µM	4
228510	$GABA_B$, Non-Selective	433506	rat	2	10 µM	-15
232030	Glucocorticoid	433479	hum	2	10 µM	13
232600	Glutamate, AMPA	433538	rat	2	10 µM	11
232710	Glutamate, Kainate	433539	rat	2	10 µM	-4
232810	Glutamate, NMDA, Agonism	433532	rat	2	10 µM	11
232910	Glutamate, NMDA, Glycine	433536	rat	2	10 µM	-2
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	433558	hum	2	10 µM	1
239610	Histamine H_1	433467	hum	2	10 µM	-2
239710	Histamine H_2	433541	hum	2	10 µM	-2
239820	Histamine H_3	433654	hum	2	10 µM	3
243000	Insulin	433555	rat	2	10 µM	15
252200	Motilin	433504	hum	2	10 µM	4
252610	Muscarinic M_1	433435	hum	2	10 µM	8
252710	Muscarinic M_2	433435	hum	2	10 µM	4
252810	Muscarinic M_3	433436	hum	2	10 µM	-7
252910	Muscarinic M_4	433442	hum	2	10 µM	-5
258730	Nicotinic Acetylcholine α3β4	433470	hum	2	10 µM	-8
260130	Opiate δ_1 (OP1, DOP)	433461	hum	2	10 µM	-3
260210	Opiate κ (OP2, KOP)	433462	hum	2	10 µM	-7
260410	Opiate µ (OP3, MOP)	433463	hum	2	10 µM	0
299005	Progesterone PR-B	433474	hum	2	10 µM	-4
299036	Purinergic P2X	433451	rat	2	10 µM	11
268810	Purinergic P2Y	433697	rat	2	10 µM	-4
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	433545	hum	2	10 µM	9
271650	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	433484	hum	2	10 µM	1

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
gp=Guinea pig; hum=Human

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	433519	hum	2	10 µM	1
271800	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	433544	hum	2	10 µM	3
202020	Transporter, Adenosine	433553	hum	2	10 µM	28
220320	Transporter, Dopamine (DAT)	433478	hum	2	10 µM	33
226400	Transporter, GABA	433531	rat	2	10 µM	46
204410	Transporter, Norepinephrine (NET)	433477	hum	2	10 µM	22
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	433471	hum	2	10 µM	3
287530	Vasopressin V_{1A}	433516	hum	2	10 µM	7

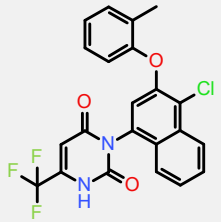
Dual BCAT1/2 Probe BAY-069

Selectivity Profile in more detail: in-house protease panel

Protease	#
Metallo protease	8
Serine protease	15
Cysteine protease	4
Aminopeptidase	3

Dual BCAT1/2 Probe BAY-069

Solubility

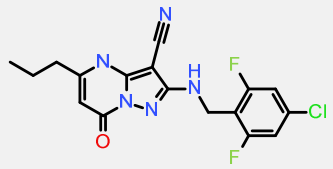


BAY-069 (en1)

Properties & Physchem	
Sw @ pH 6.5 [mg/L]	<0.10
Sw @ pH 6.5 PBS [mg/L]	93
Sw @ pH 4 citrate buffer [mg/L]	4.4
Sw in PEG400/EtOH/H2O 60/10/30 [mg/L]	>830
Sw in PEG400/H2O 60/40 [mg/L]	>790

Compound 61 (GSK) as additional BCAT1/2 probe

Technical profile



Compound 61

POTENCY (IC_{50} [nM])	
BACT1 IC_{50}	92 nM
BACT2 IC_{50}	26 nM
Mechan. IC_{50} MDA-MB-231	564 nM
Mechan. IC_{50} U-87-MG	5.3 µM

Properties & Physchem	
LogD @ pH 7.5 / pka	1.93 / 6.3
fu [%] Williams_E	6.2 / 6.2 / 0.47
Sw powder @ pH 6.5 [mg/L]	151
MW / TPSA [g*mol / Å^2]	377.8 /
Stability (r /h plasma, 24h) [%]	Stable

***in vitro* DMPK Properties**

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	115.98		130.99		1.13	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	Human liver mics		0.05		96	
	rat hepatocytes		0.1		98	
In vivo rat PK	Low CLb, moderate Vss, intermediate half-life, high oral bioavailability					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
PXR						

Selectivity

In-house kinase panel (#37)	>10 µM
Eurofins safety panel	Aldone reductase 72% Available (see next slide)

SAFETY

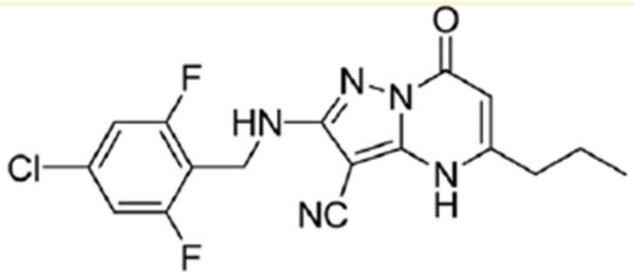
Cytotox	-
hERG IC_{50} [µM]	>10 µM

- Compound 61 is a dual BCAT1/2 inh.
- BAY-069 shows high Caco-2 permeability and high solubility
- Stock availability: 250 mg are available

Compound 61 (GSK) as additional BCAT1/2 probe

Literature

GSK data (Bertrand et al., 2015)
J. Med. Chem. 2015, 58, 7140−7163



61

BCATm pIC_{50} = **7.3**, chromlog$D_{7.4}$ = 3.12
LE = 0.39, LLE_{AT} = 0.33
cell pIC_{50} = **6.8**
solid sol. 500 µg/mL
Cl_b 0.3 mL/min/kg; $T_{1/2}$ 9 h; F% 100

- Enzyme inhibition (BCAT2) activity comparable to in-house data (50 nM vs 26 nM)
- Cellular data slightly better (160 nM vs 452 nM) but measured in different cell type (primary adipocyte)

Compound 61 (GSK) as additional BCAT1/2 probe

Selectivity Profile in more detail: safety screen (Eurofins, # 77 targets)

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H	R
Compound: CHH036-2018, PT #: 1217295										
107000	Aldose Reductase	419005	rat	2	10 µM	72				
107710	ATPase, Na^+/K^+, Heart, Pig	419157	pig	2	10 µM	-9				
112020	Carbonic Anhydrase II	419006	hum	2	10 µM	7				
104010	Cholinesterase, Acetyl, ACES	419032	hum	2	10 µM	11				
116020	Cyclooxygenase COX-1	419236	hum	2	10 µM	-2				
118010	Cyclooxygenase COX-2	419237	hum	2	10 µM	-19				
124010	HMG-CoA Reductase	419158	hum	2	10 µM	13				
132000	Leukotriene LTC_4 Synthase	419009	gp	2	10 µM	-8				
199017	Lipoxygenase 15-LO	419128	hum	2	10 µM	-14				
140010	Monoamine Oxidase MAO-A	419125	hum	2	10 µM	23				
140120	Monoamine Oxidase MAO-B	419126	hum	2	10 µM	10				
142000	Nitric Oxide Synthase, Neuronal (nNOS)	419159	rat	2	10 µM	-10				
199010	Nitric Oxide Synthetase, Inducible (iNOS)	419011	mouse	2	10 µM	16				
107300	Peptidase, Angiotensin Converting Enzyme	419156	rabbit	2	10 µM	-4				
152000	Phosphodiesterase PDE3	419198	hum	2	10 µM	11				
154000	Phosphodiesterase PDE4	419197	hum	2	10 µM	20				
156000	Phosphodiesterase PDE5	419199	hum	2	10 µM	34				
194020	Thromboxane Synthase	419127	hum	2	10 µM	7				
200510	Adenosine A_1	419204	hum	2	10 µM	26				
200610	Adenosine A_{2A}	419205	hum	2	10 µM	-2				
200720	Adenosine A_3	419054	hum	2	10 µM	39				
203100	Adrenergic α_{1A}	419069	rat	2	10 µM	-7				
203630	Adrenergic α_{2A}	419028	hum	2	10 µM	-11				
203710	Adrenergic α_{2B}	419072	hum	2	10 µM	3				
203810	Adrenergic α_{2C}	419073	hum	2	10 µM	-2				
204010	Adrenergic β_1	419207	hum	2	10 µM	4				
204110	Adrenergic β_2	419132	hum	2	10 µM	-4				
204200	Adrenergic β_3	419104	hum	2	10 µM	-1				
206000	Androgen (Testosterone)	419252	hum	2	10 µM	7				
210030	Angiotensin AT_1	419057	hum	2	10 µM	3				
210120	Angiotensin AT_2	419058	hum	2	10 µM	8				
212520	Bradykinin B_1	419081	hum	2	10 µM	-2				
212620	Bradykinin B_2	419189	hum	2	10 µM	-1				
217030	Cannabinoid CB_1	419208	hum	2	10 µM	-7				
217100	Cannabinoid CB_2	419131	hum	2	10 µM	9				
219500	Dopamine D_1	419105	hum	2	10 µM	-7				
219600	Dopamine D_{2L}	419190	hum	2	10 µM	0				
219700	Dopamine D_{2S}	419191	hum	2	10 µM	-14				
219800	Dopamine D_3	419106	hum	2	10 µM	8				
224010	Endothelin ET_A	419107	hum	2	10 µM	-8				
224110	Endothelin ET_B	419239	hum	2	10 µM	12				
226010	Estrogen ERα	419055	hum	2	10 µM	-18				
226810	$GABA_A$, Chloride Channel, TBOB	419137	rat	2	10 µM	8				
226600	$GABA_A$, Flunitrazepam, Central	419095	rat	2	10 µM	-2				
228510	$GABA_B$, Non-Selective	419345	rat	2	10 µM	-1				
232030	Glucocorticoid	419192	hum	2	10 µM	14				
232600	Glutamate, AMPA	419194	rat	2	10 µM	7				
232710	Glutamate, Kainate	419059	rat	2	10 µM	-9				
232810	Glutamate, NMDA, Agonism	419035	rat	2	10 µM	16				
232910	Glutamate, NMDA, Glycine	419035	rat	2	10 µM	2				
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	419071	hum	2	10 µM	12				
239610	Histamine H_1	419036	hum	2	10 µM	3				
239710	Histamine H_2	419062	hum	2	10 µM	-1				
239820	Histamine H_3	419094	hum	2	10 µM	-6				
243000	Insulin									
252200	Motilin									
252610	Muscarinic M_1									
252710	Muscarinic M_2									
252810	Muscarinic M_3									
252910	Muscarinic M_4									
258590	Nicotinic Acetylcholine									
260130	Opiate δ_1 (OP1, DOP)	419294	hum	2	10 µM					
260210	Opiate κ (OP2, KOP)	419215	hum	2	10 µM					
260410	Opiate µ (OP3, MOP)	419101	hum	2	10 µM					
299005	Progesterone PR-B	419061	hum	2	10 µM					
268700	Purinergic P2X	419217	rabbit	2	10 µM					
268810	Purinergic P2Y	419211	rat	2	10 µM					
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	419196	hum	2	10 µM					
271650	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	419052	hum	2	10 µM					

Compound 61 (GSK) as additional BCAT1/2 probe

Selectivity Profile in more detail: safety screen (Eurofins, # 77 targets)

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	433519	hum	2	10 µM	1
271800	Serotonin (5-Hydroxytryptamine) 5-HT_{2C}	433544	hum	2	10 µM	3
202020	Transporter, Adenosine	433553	hum	2	10 µM	28
220320	Transporter, Dopamine (DAT)	433478	hum	2	10 µM	33
226400	Transporter, GABA	433531	rat	2	10 µM	46
204410	Transporter, Norepinephrine (NET)	433477	hum	2	10 µM	22
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	433471	hum	2	10 µM	3
287530	Vasopressin V_{1A}	433516	hum	2	10 µM	7

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	419203	hum	2	10 µM	1
271800	Serotonin (5-Hydroxytryptamine) 5-HT_{2C}	419123	hum	2	10 µM	11
202000	Transporter, Adenosine	419206	gp	2	10 µM	-9
220320	Transporter, Dopamine (DAT)	419027	hum	2	10 µM	20
226400	Transporter, GABA	419138	rat	2	10 µM	15
204410	Transporter, Norepinephrine (NET)	419027	hum	2	10 µM	46
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	419075	hum	2	10 µM	-4
287530	Vasopressin V_{1A}	419066	hum	2	10 µM	-3

Cat #	Assay Name	Species	Conc.	% Inh.
107000	Aldose Reductase	rat	10 µM	72