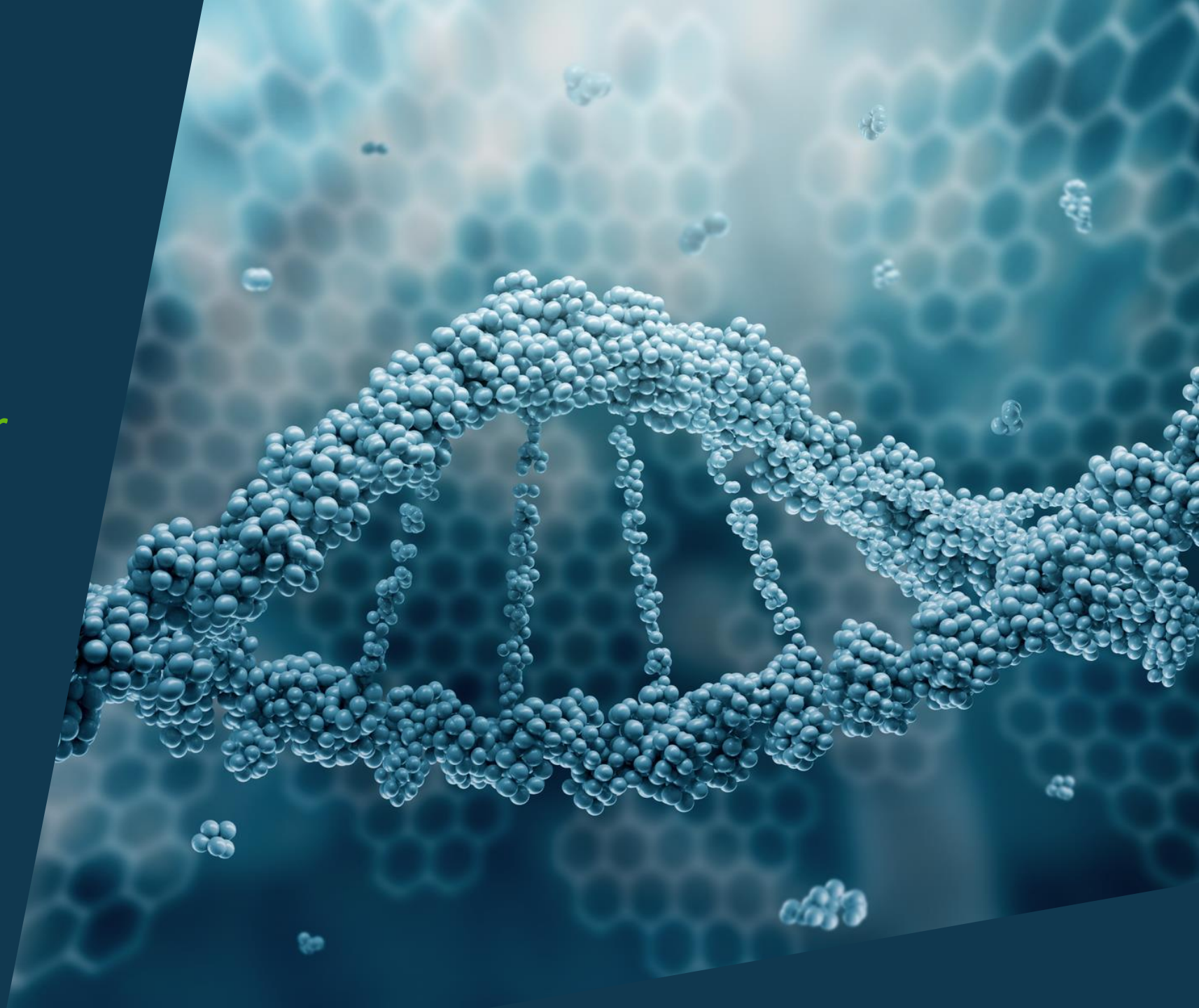
Donated Chemical Probe

Complex 1 Inhibitor Probe BAY-179



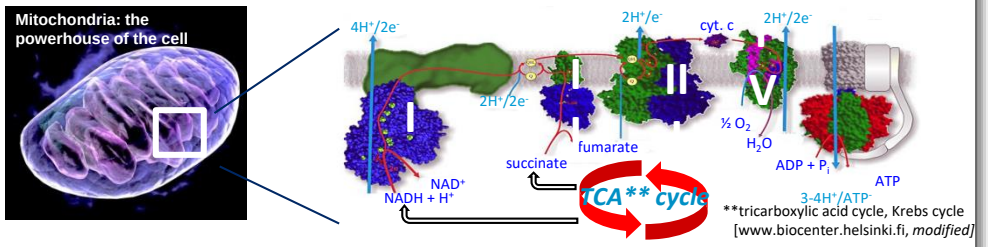
November, 2019

Sven Christian, Jeffrey Mowat, Roman Hillig

Complex 1 probe BAY-179

Target rationale and cancer

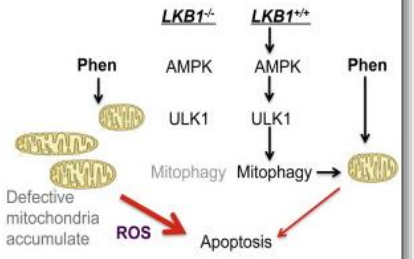
Physiological role of complex-1



- The electron transport chain is located in the inner mitochondrial membrane
- It transfers electrons from electron donors to electron acceptors via redox reactions (Complex I-IV), which is coupled to the transfer of protons (H^+ ions) across the membrane. This creates an electrochemical proton gradient that drives ATP synthesis (Complex V). The final acceptor of electrons in the electron transport chain is molecular oxygen.
- Mitochondria are key regulators of energy supply & cell death

Target Rationale

- Oncogenic mutations have been described to sensitize tumor cells to Complex I inhibition (e.g. LKB1 -/-)



Shackelford et al. 2014

- Inhibition of $BRAF^{V600E}$ by Vemurafenib increases PGC1a, MITF and OXPHOS [Haq et al., 2013, Cancer Cell] and therefore sensitizes to Complex I inhibition
- A subset of diffuse large B-cell lymphoma (Oxphos-DLBCL) has been characterized by enhanced Oxphos [Caro et al., 2012, Cancer Cell]
- Former HIF-FR project showed that Complex 1 inhibitors have enhanced efficacy when used in combination with Radiation

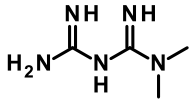
Genetic mutations and consequent metabolic changes have been shown to sensitive cancer cells to Complex 1 inhibition

Complex 1 probe BAY-179

Known Complex-1 inhibitors

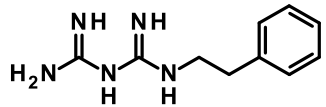
Metformin

- Oral antidiabetic drug in the biguanide class. The first-line drug of choice for the treatment of type 2 diabetes
- approved in Canada in 1972, approval by FDA for type 2 diabetes in 1994
- IC_{50} >10 µM
- slow membrane-potential-driven accumulation of the drug within the mitochondria



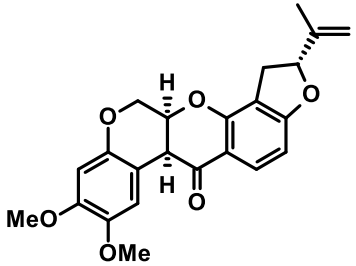
Phenformin

- Biguanide class
- marketed as DBI by Ciba-Geigy, but was withdrawn from most markets in the late 1970s due to a high risk of lactic acidosis, which was fatal in 50% of cases
- IC_{50} >1 µM



Rotenone

- broad-spectrum insecticide, piscicide, and pesticide
- IC_{50} = 20 nM

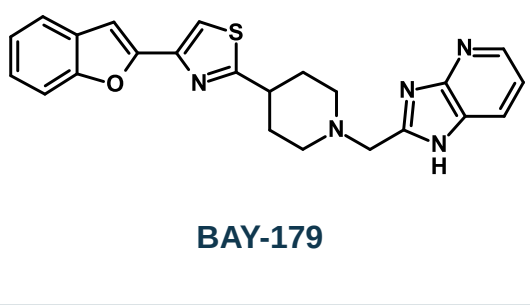


- Rotenone is neurotoxic and induces a Parkinson-like phenotype in mice
- In house data show that toxicity can not be rescued by expression of NDI1 => Complex 1 independent effect

No potent and selective Complex 1 inhibitor commercially available

Complex 1 probe BAY-179

Overall profile



BAY-179

Pharmacology

IC_{50} H1299 HTS ATP (human)	79 nM
IC_{50} H1299 CTG ATP (human)	33 nM
IC_{50} 4T1 CTG ATP (mouse)	38 nM

Safety

hERG IC_{50} volt clamp	3.0 µM
Off-targets	Serotonin 5HT2b in-house data Agonism: no effect Antagonism: IC_{50}= 600 nM (sensitive set-up, 10nM 5HT agonist)

Molecular Properties

MWcorr [g/mol]	416
i.s.o. PS (0-10)	3
TPSA [Å2]	71
clogP / cLogD@pH7.5	4.0/3.6
Rotatable bonds	4

PhysChem

Sw solid @pH 6.5 [mg/L]	1.0
Log D (pH 7.5)	2.8
Stability (plasma h,r,m)	stable
Stability (pH 1,7,10) [% remaining after (1h)]	100/100/100

In vitro PK

		CL_B [L/h/kg]	Fmax [%]
LM	Human	0.55	58
	Mouse (f)	1.9	65
	Rat	0.77	82
	Dog	0.53	75
Hep	Rat	0.81	81
	Dog	1.1	47
	Mouse (f)	1.5	72
	Human	0.64	52

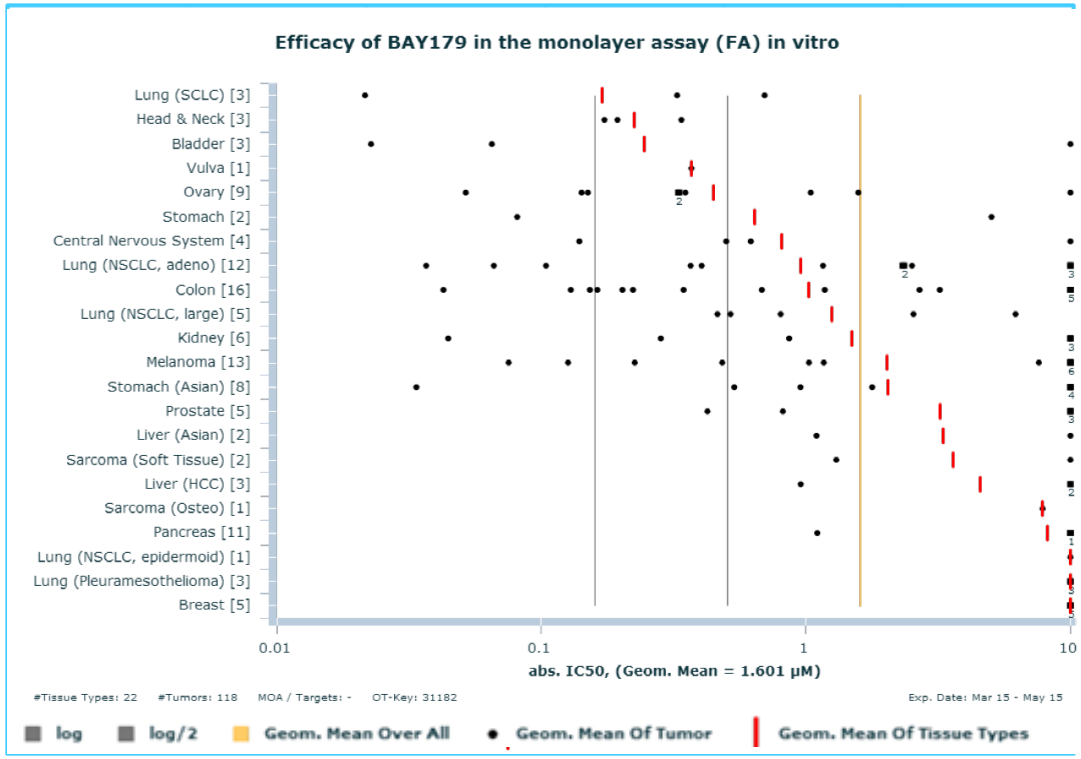
CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
	92	41	0.44

Cyp Inh profile [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc
	> 20	7.8	15	> 20	> 20	> 20

Cyp Ind [µM]	PXR EC_{50}	PXR Rating	CYP Ind NOEL
	>2µM (7.8% Emax)	green	3A4: NOEL 123µg/l loss of cell viability @ higher doses

Complex 1 probe BAY-179

Cellular data - proliferation



Indication	Cell line	Patient-derived	IC_{50}[µM]	IC_{70}[µM]
Bladder	BXF1352	x	0,023	0,048
Gastric	MKN1		0,034	0,085
Bladder	BXF1218	x	0,065	0,104
Colon	LS174T		0,043	0,109
Gastric	GXF251	x	0,081	0,147
Lung	LXFA629	x	0,066	0,195
Ovary	BG1		0,142	0,207
Colon	Caco-2		0,129	0,209
Melanoma	SK-MEL-28		0,075	0,215
Colon	RKO		0,153	0,251
Melanoma	LOX		0,126	0,335

Unbiased approach to identify more sensitive tumor models (oncotest):

- Several cancer cell lines were identified to be highly sensitive towards Complex 1 inhibition
- Selected cell lines were tested in house in vivo
- Bioinformatic analysis identifies KRASmut to be enriched in resistant tumor cell lines

Complex 1 probe BAY-179

DMPK data of BAY-179

- ***In vitro* PK**

		LM CL_{blood} [L/h/kg]	HEP CL_{blood} [L/h/kg]
Metabolic Stability	Mouse	1.9	1.5
	Rat	0.77	0.81
	Dog	0.53	1.1
	Human	0.55	0.64

Fraction unbound [%]	Williams_E	Mouse	Rat	Dog	Human
	24	0.43	0.26	0.37	0.48

Caco-2	A-B [nm/s]	B-A [nm/s]	Ratio
	92	41	0.44

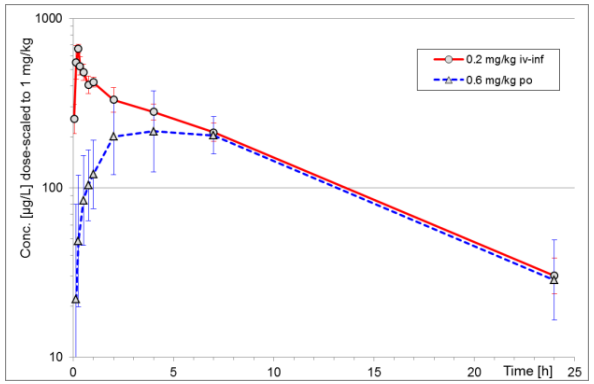
CYP Inh [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc
	>20	7.8	15	>20	>20	>20

PXR Assay	Rating:	green

CYP Induction	1A2: NOEL 41µg/l 3A4: NOEL 123µg/l loss of cell viability @ higher doses

- ***In vivo* PK**

Species		Rat	
Route		iv inf.	po
Dose	[mg/kg]	0.2	0.6
AUC_{norm}	[kg·h/L]	4.1	3.1
$Cmax_{norm}$	[kg/L]		0.23
t_{max}	[h]		4 to 7
CL_{matrix}	[L/h/kg]	0.25	
CL_{blood}	[L/h/kg]	0.40	
V_{ss}	[L/kg]	2.2	
$t_{1/2}$	[h]	6.2	6.2
F	[%]		76



- *In vitro* CL: low to moderate in hepatocytes and liver microsomes
- High protein binding in all species
- *In vivo* rat PK: Low CL_{blood}, high V_{ss}, long $t_{1/2}$, high oral bioavailability

BAY-179 is suitable for in vivo studies

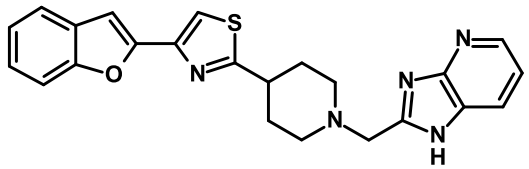
Complex 1 probe BAY-179

GPCR data of BAY-179

The results of the current Eurofins-Cerep GPCR-Panel:

BAY-179

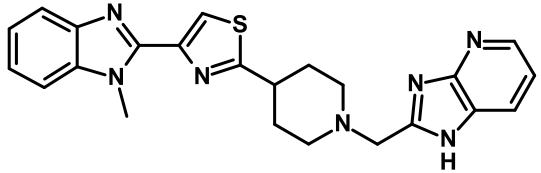
A2B (h) (antagonist effect) 57.8%
H2(h) (antagonist effect) 64.4%
MC4(h) (antagonist effect) 67.7%
M1(h) (antagonist effect) 53.6%
μ (MOP) (h) (antagonist effect) 51.3%
5-HT2B(h) (antagonist effect) 73.5%



BAY-179

BAY-070

5-HT2B(h) (antagonist effect) 59.9%

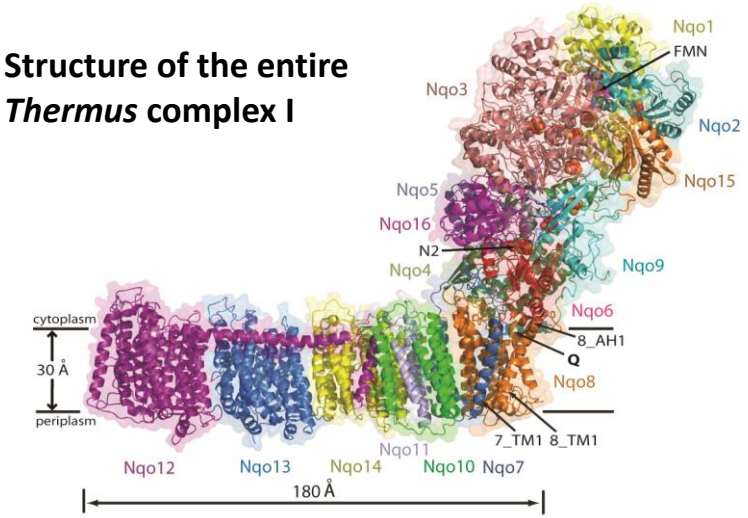


BAY-070

Complex 1 probe BAY-179

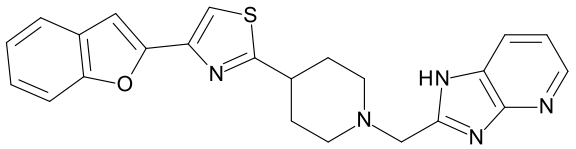
Bacterial Complex 1 as crystallization surrogate (Prof. Leonid Sazanov, IST Austria)

Structure of the entire *Thermus* complex I



Baradaran, Berrisford, Minhas and Sazanov, *Nature*, 2013

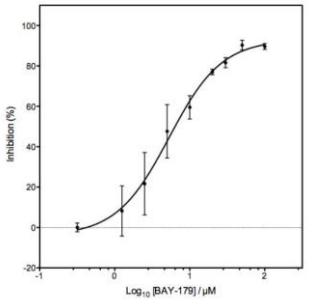
Probe candidate: BAY-179



Chemically related compound-1

(*structure cannot be disclosed*)

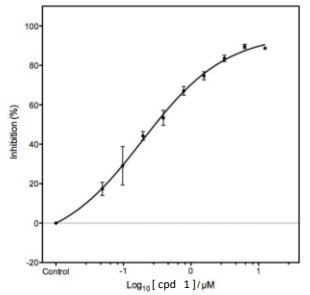
Dose response in biochemical activity assay with bacterial complex I*:



IC50 (human) = 79 nM

IC50 (bact.) = 5.3 µM

→ **too weak for soaking**



IC50 (human) = 5.1 nM

IC50 (bact.) = 192 nM

→ **Sufficiently potent for soaking**

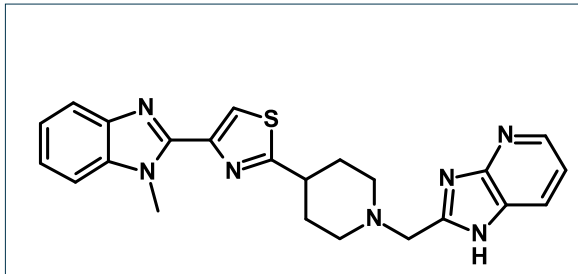
- In collaboration with Prof. Leonid Sazanov, BAY-179 and related compounds were tested for activity on Complex 1 from *Th. Thermophilus* in a biochemical activity assay*.
- Some compounds showed very similar IC50 values in human vs. bact. assay, others showed differences (more often weaker on bact. than human enzyme).
- Probe BAY-179 itself showed poor potency, but chemically related compound-1 showed acceptable potency in bacterial surrogate system and was successfully soaked into crystals of bacterial Complex 1.

Crystal structure shows density for ligand in quinone-binding site (structure cannot yet be disclosed, manuscript in preparation).

* Bacterial activity assay: NADH:decylubiquinone assay; rate of NADH oxidation by complex I was followed at different compound concentrations which was subsequently used to plot a dose response curve

Complex 1 negative control BAY-070

Overall profile



BAY-070

Molecular Properties

MWcorr [g/mol]	429.5
i.s.o. PS (0-10)	2
TPSA [Å2]	76
clogP / cLogD@pH7.5	2.6 / 2.3
Rotatable bonds	4

PhysChem

Sw solid @pH 6.5 [mg/L]	
Log D (pH 7.5)	2.1
Stability (plasma h,r,m)	
Stability (pH 1,7,10) [% remaining after (1h)	

Pharmacology

IC_{50} H1299 HTS ATP (human)	>30 µM
IC_{50} H1299 CTG ATP (human)	
IC_{50} 4T1 CTG ATP (mouse)	

Kinase Panel

Bayer Kinase Panel 34 Kinases Tested	All > 20 µM

In vitro PK

		CL_B [L/h/kg]	Fmax [%]
LM	Human		
	Mouse (f)		
	Rat		
Hep	Rat		
	Dog		
	Human		

CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
	127	245	1.9

Cyp Inh profile [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc

Cyp Ind [µM]	PXR EC_{50}	PXR Rating	CYP Ind NOEL

Complex 1 probe BAY-179

Summary / conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC_{50}, Kd)	not applicable – see **on target cell activity**
Selectivity within target family: goal is >30-fold	**Surpasses criteria**; compound class shows selectivity against other complexes of the respiratory chain
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Surpasses criteria**; clean LeadProfilingScreen; selectivity > 500 fold against numerous kinases tested
On target cell activity for cell-based targets: goal is < 1 micromolar IC50/EC50	**Surpasses criteria**; Active in cellular mechanistic assay demonstrating cellular target engagement cellular proliferation assays; (IC_{50}= 79nM)
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**Surpasses criteria**; suitable pharmacokinetic profile for in vivo studies
Neg ctrl: in vitro potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Surpasses criteria**; BAY-070 inactive (IC_{50} >20µM) in cellular assay

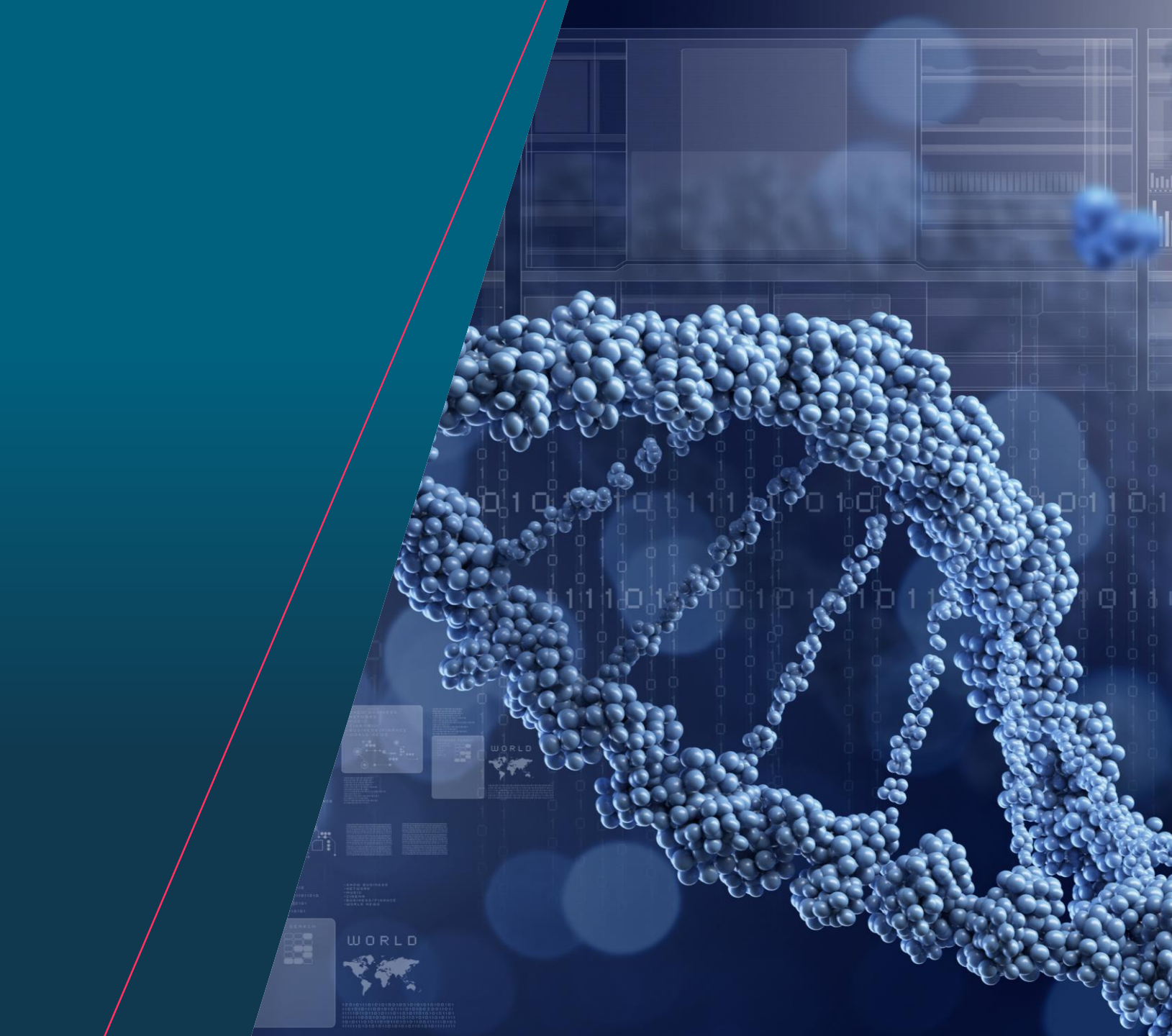
We ask for acceptance of Complex 1 inhibitor BAY-179 as chemical probe, accompanied by BAY-070 as negative control

Complex 1 probe BAY-179

Acknowledgement

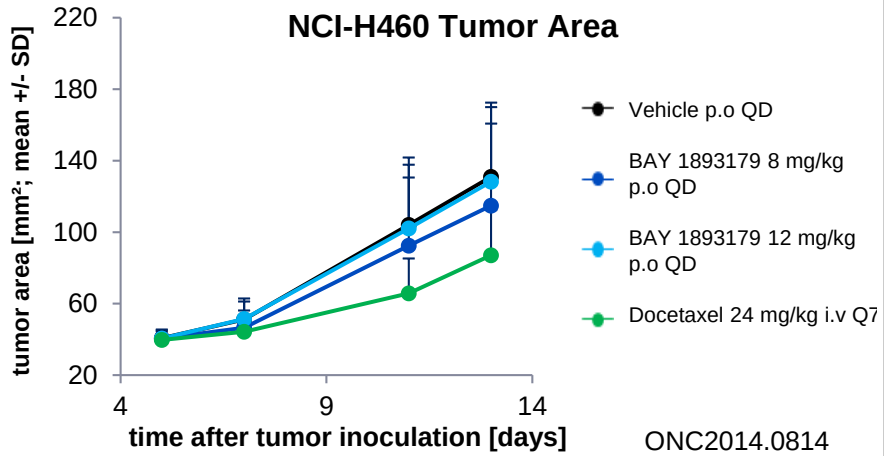
MedChem Berlin	Hue-Quan Lao Andrea Koehlke-Nork Michael Tietz Anni Knitter Lisa Schälicke Martin Schötz Karl Sauvageot-Witzku
MedChem Wuppertal	Carl Nising Nils Griebenow
MedChem external	Peakdale Support
TRG Oncology	Laura Schöckel Melanie Appel Maria Spelling Seren Nesan Michael Reinhardt Elisabeth Krahl Ulrike Lenhard
LD	Nina Scheweling Patrick Steigemann Roman Hillig
DMPK	Michaela Bairlein Karsten Denner Roland Neuhaus Michael Niehues
BIP	Wolfgang Halfbrodt Stephan Völkening Jochen Kossmann
FTO Analysis	Peter Spreyer
Toxicology	Anna-Lena Frisk Ute Bach
Exp. Med.	Eleni Lagkadinou
ER moderators	Andrea Haegebarth Marcus Bauser [Holger Hess-Stumpp]
Structural Biology	Leonid Sazanov, IST Vienna, Austria

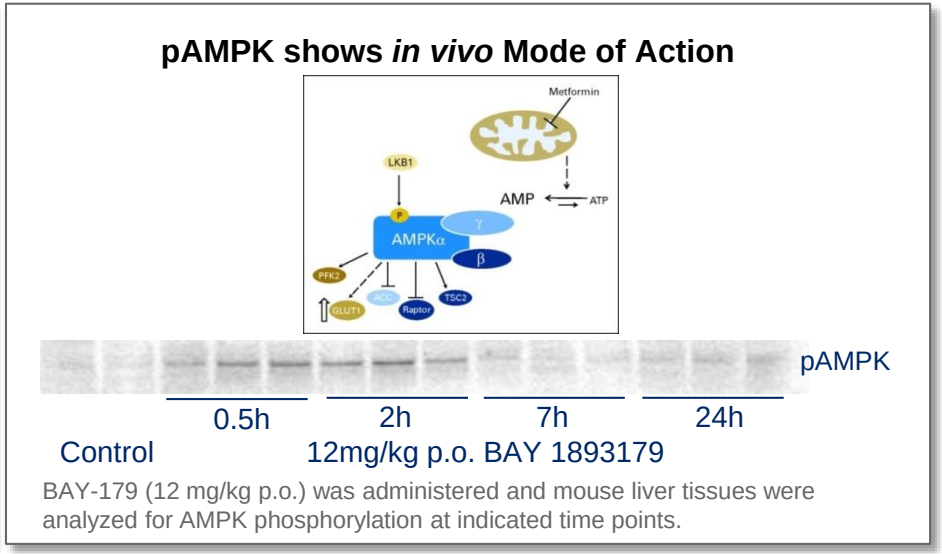
Thank You



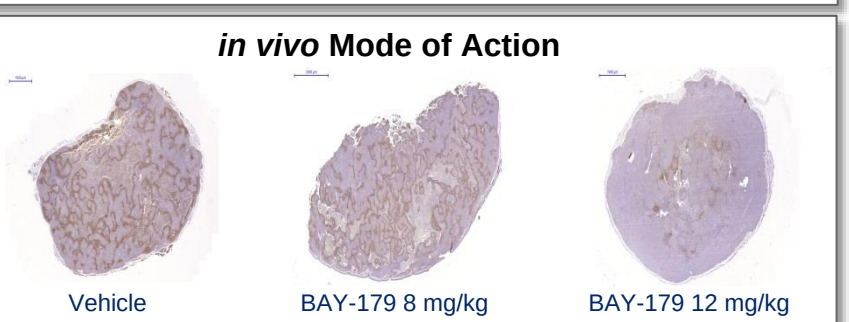
Complex 1 probe BAY-179

In vivo studies in LKB-/- tumor model NCI-H460





BAY-179 (12 mg/kg p.o.) was administered and mouse liver tissues were analyzed for AMPK phosphorylation at indicated time points.

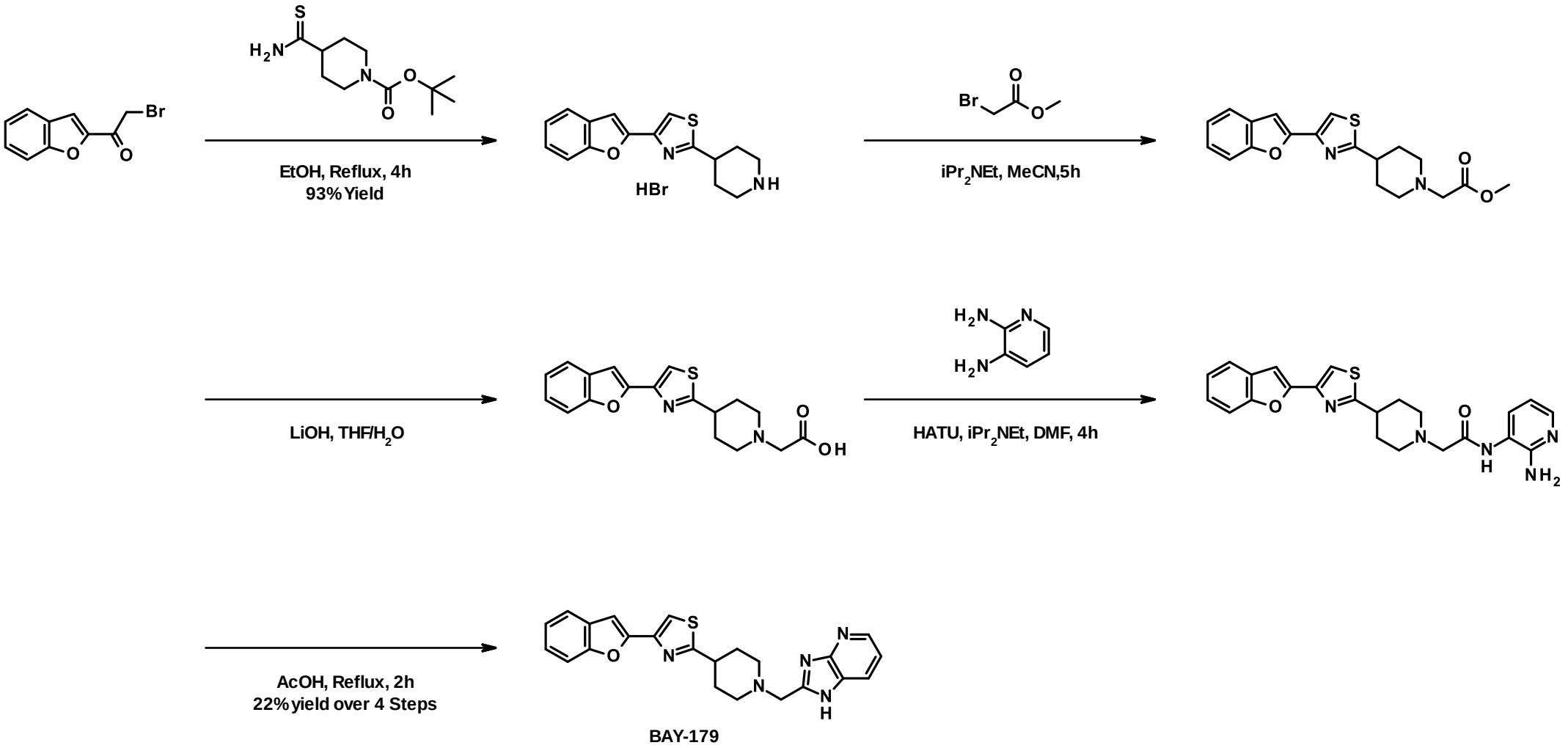


Mice were treated p.o QD with BAY-179. Prior to sacrifice, animals were injected with hypoxyprobe and later NCI-H460 tumors were stained for hypoxic areas. BAY-179, at 12 mg/kg, effectively reduced hypoxic areas for up to 7 h. At 24 h post cmdt administration, hypoxic areas reappeared. Images shown are time = 2 h post cmd admin.

Unfortunately, BAY-179 shows no in vivo efficacy was reported in LKB1-/- tumor model NCI-H460

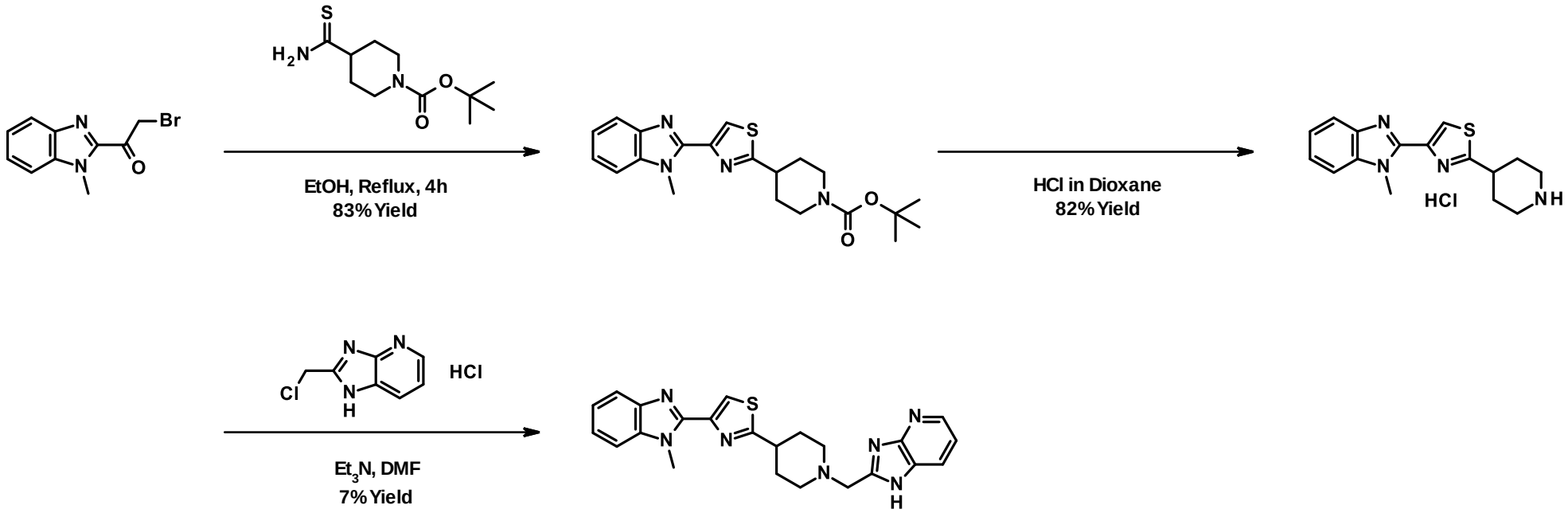
Complex 1 probe BAY-179

Synthesis scheme



Complex 1 negative control BAY-070

Synthesis scheme



Complex 1 probe BAY-179

Selectivity against other Complexes

Compound		Related Analog to BAY-179	Rotenone
Cellular ATP assay*	Complex I	25 nM	10 nM
Bio-chemical bovine OxPhos activity assay**	Complex I	47 nM	9,2 nM
	Complex II	> 30 µM	> 30 µM
	Complex III	> 30 µM	> 30 µM
	Complex IV	> 30 µM	> 30 µM
	Complex V	> 30 µM	> 30 µM

Analog of BAY-179 displays target specificity in biochemical assays

* Measured by cell based primary HTS assay (human cell line H1299 with ter luc ATP sensor +/- succinate)
** Measured by Abcam MitoTox complete oxphos activity assay kit using bovine isolated mitochondria

Complex 1 probe BAY-179

Complex 1 cellular activity assay description

Cellular Complex I activity

For the measurement of cellular Complex I activity, cellular ATP levels in absence or presence of the Complex II substrate succinate are measured. Therefore, H1299 cells (ATCC CRL-5803) were stably co-transfected with a pcDNA3 vector (purchased from Invitrogen™) encoding for ATP dependent Pyrearinus termitilluminans larval click 20 beetle luciferase under control of a CMV promoter (H1299 tluc cell). In brief, H1299tluc cells (4000/well) are seeded into white 384 well plates. After one day in culturing in DMEM (2% FCS) without glucose, but supplemented with 11 mM galactose, 10 µL of a luciferin/test compound mixture (150 µM D-luciferin, 0.4% DMSO final concentration in 2 mM Ca2+ Tyrode +0.01% BSA) is added to each well and incubated for 1 h at 37° C, 25 5% CO2 and luminescence measurement is performed. After this measurement 20 µL succinate (0.67 M, pH 5.3 in Tyrode, final concentration 25 mM) is added. The plate is then incubated for another 1 h at room temperature before the second measurement is performed.

The succinate dependency of cellular ATP level obtained in the second measurement gives information on Complex I specificity of compound action (i. e. Complex I inhibitors will lose their effect).

Measurements were always performed in quadruple. For determination of inhibition constants IC_{50} and the Hill slope, the normalized luminescence intensities (DMSO control = 100%) were plotted as a function of the concentration of test compound, and data points were fitted by nonlinear regression with the equation

$$Y=Bottom + (Top-Bottom)/(1+10^{((logIC50-X)*HillSlope)})$$

where Y is the measured luminescence normalized to DMSO control (=100%), X is the log of compound concentration and Top/Bottom are the plateaus of the curve (normalized luminescence units).