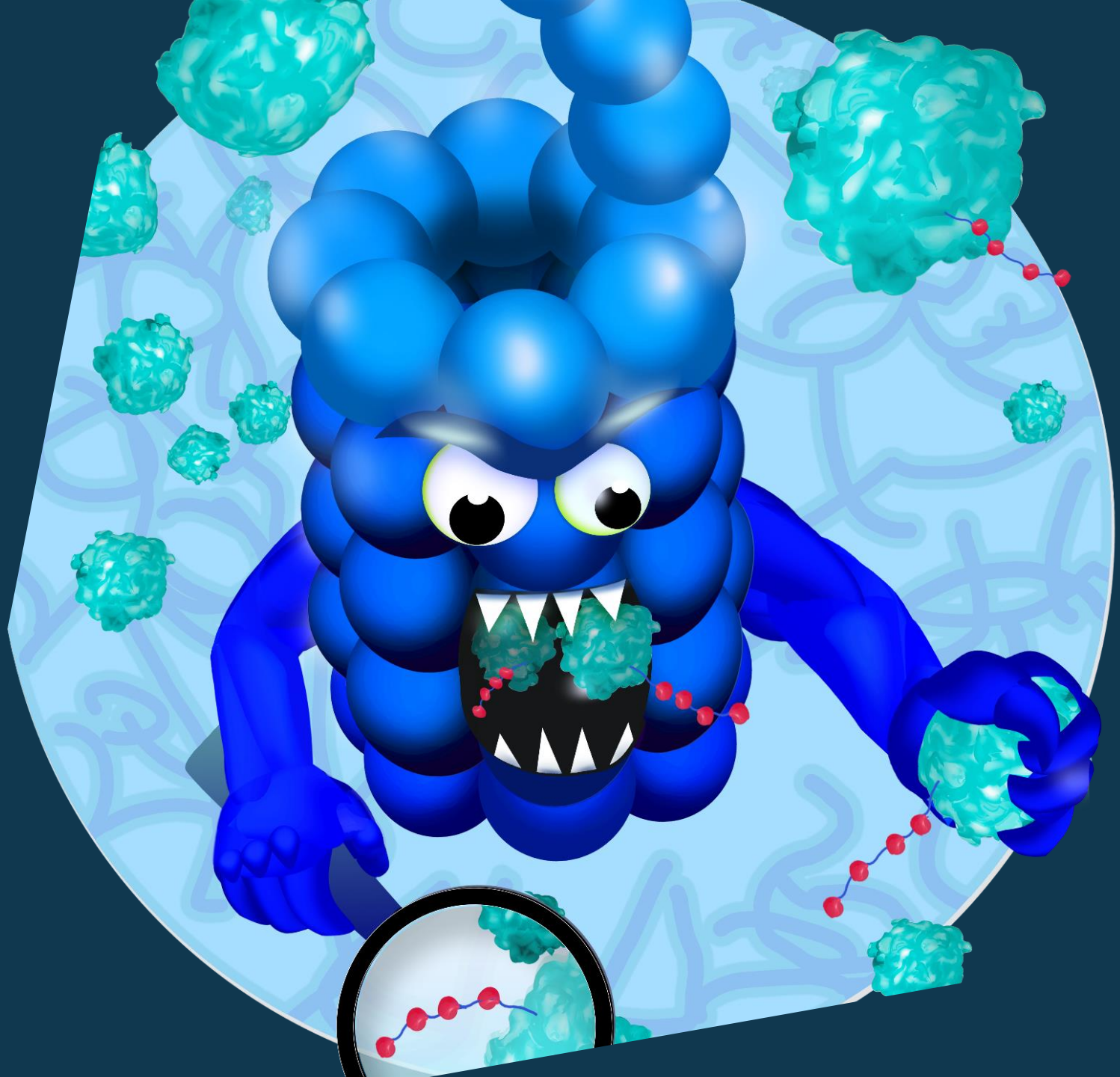
Donated Chemical Probe

MetAP2 Degrader Probe BAY-277

December 2023

Presenters:
Philipp Cromm
On behalf of the team



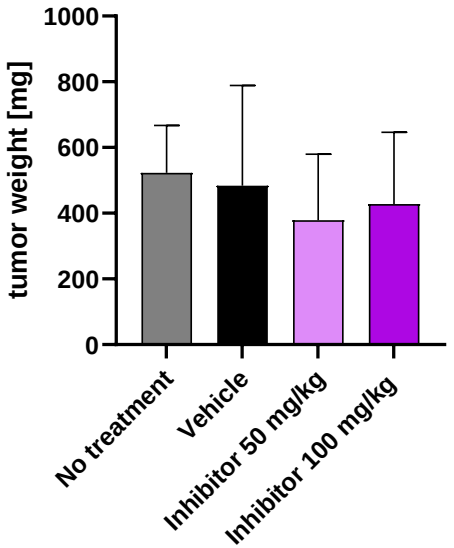
MetAP2 Probe BAY-277

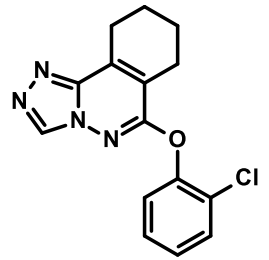
Target Background & Hypothesis

Methionine aminopeptidase: N-terminal methionine excision (NME) of proteins

- MetAP2 (methionine aminopeptidase 2) overexpressed across various types of cancer
- KO/KD results in anti-angiogenesis & direct tumor growth inhibition
  - Intensively researched target in cancer, obesity and autoimmunity with multiple clinical trials over the last decades → no clinical success so far
- Biochemically potent internal inhibitors (e.g. **BAY-896**) available which proved not to show desired effects in efficacy models

Potency IC_{50} [nM] BAY-896	
Biochemical IC_{50} (human)	39
2D HUVEC proli IC_{50}	390





BAY-896
MetAP2 IC_{50} : 39 nM

Hypothesis: A degrader can reproduce the KO results, help to understand the lack of efficacy of the inhibitor and show *in vivo* efficacy

MetAP2 Biology: Essential role in angiogenesis

MetAP2 has an enzymatic and non-enzymatic function

Enzymatic Function

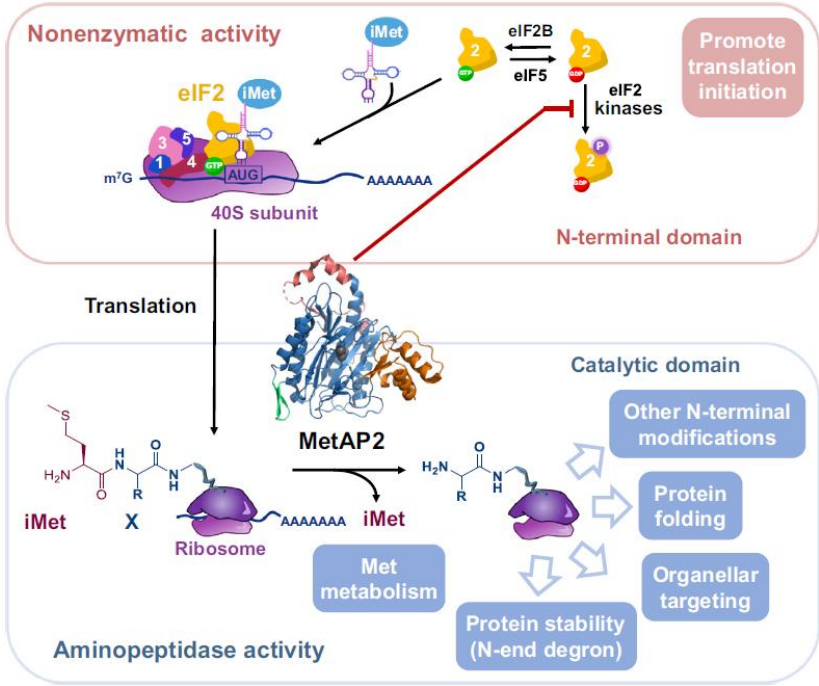
- Catalyzes hydrolytic cleavage of initiator methionine from newly synthesized proteins
- Critical role in protein maturation during protein synthesis
  - Removal of the initiator methionine is important for the activity, stability, or compartmentalization of many proteins

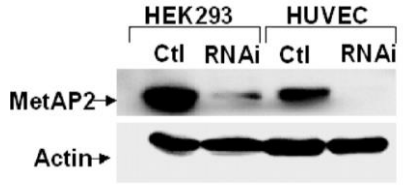
Non-enzymatic Function

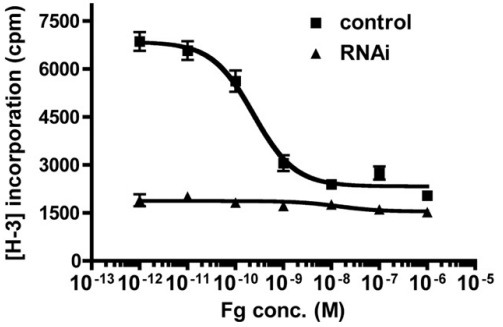
- Binds to eIF2a (eukaryotic factor 2a)
- Protects it from inhibitory phosphorylation and promotes general protein synthesis

MetAP2 as a cancer target

- MetAP2 has been identified as the target of the anti-angiogenic agent fumagillin (Fg) and synthetic derivative TNP-470
- Fg and analogues inhibit endothelial cell growth in a p53-dependent manner
- siRNA knockdown of MetAP2 results in decreased endothelial proliferation



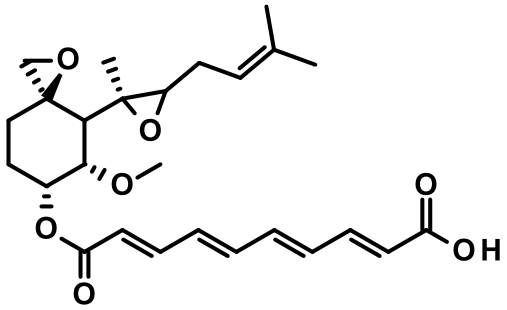




A. Goya Grocin, W. W. Kallemeijn, E. W. Tate, *Trends Pharmacol. Sci.* **2021**, *42*, **870**. Jing-Ruey J *et al. PNAS*, **2006**, *103* (27), **10379-10384**

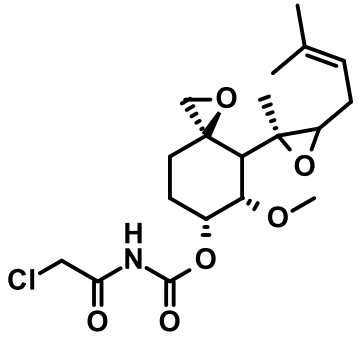
MetAP2 Probe BAY-277

Reference compounds



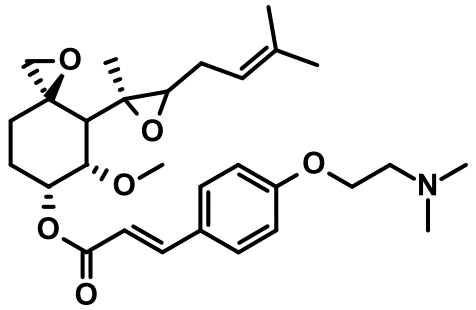
Fumagillin
CAS 23110-15-8

- Covalent MetAP2 inhibitor
- Biochem IC_{50}: 1.5 nM
- Off-target liability
- Terminated after clinical investigation[1]



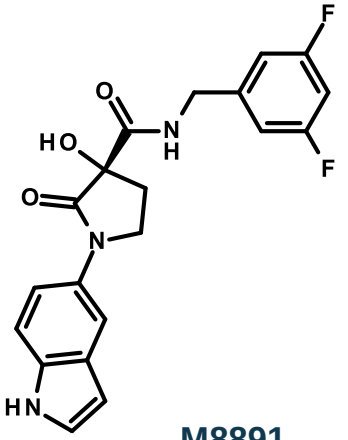
TNP-470
CAS 129298-91-5

- Covalent MetAP2 inhibitor
- Biochem IC_{50}: 7.5 nM
- Abandoned after strong neurotoxic adverse effects in Phase III [1]



Beloranib ZGN-433
CAS 251111-30-5

- Covalent MetAP2 inhibitor
- Abandoned after multiple fatal events of pulmonary embolism and deep-vein thrombosis in Phase III [1]



M8891
CAS 1464842-09-8

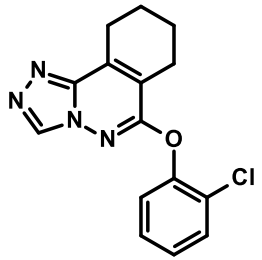
- Reversible MetAP2 inhibitor
- Biochem IC_{50}: 54 nM [2]
- 2D HUVEC proli IC_{50}: 20 nM [2]
  After Phase I trial out licensed from Merck to Cureteq
- „manageable safety profile“; „no objective responses observed “[3]

Multiple compounds have been evaluated in the clinic for cancer, obesity and auto immune diseases without success

[1] A. Goya Grocin, W. W. Kallemeijn, E. W. Tate, *Trends Pharmacol. Sci.* **2021**, *42*, **870**. [2] T. Heinrich, J. Seenisamy, F. Becker, B. Blume, J. Bomke, M. Dietz, U. Eckert, M. Friese-Hamim, J. Gunera, K. Hansen, B. Leuthner, D. Musil, J. Pfalzgraf, F. Rohdich, C. Siegl, D. Spuck, A. Wegener, F. T. Zenke, *J. Med. Chem.* **2019**, *62*, 11119. [3] M. A. Carducci, D. Wang, C. Habermehl, M. Bödding, F. Rohdich, F. Lignet, K. Duecker, O. Karpenko, L. Pudelko, C. Gimmi, P. LoRusso, *Cancer Research Communications* **2023**, *3*, 1638.

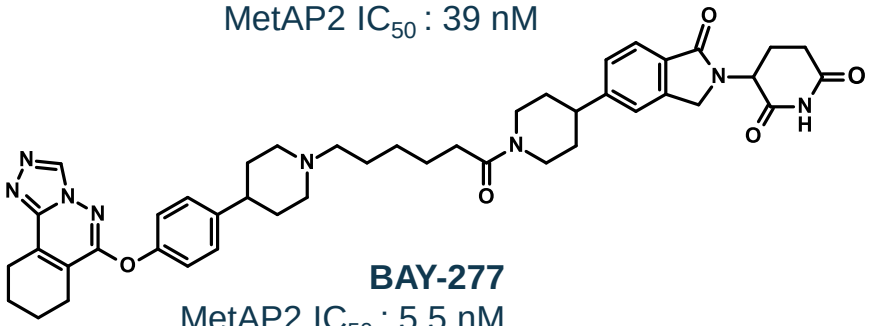
MetAP2 Probe BAY-277

MetAP2 degrader design & identification of linker attachment points



BAY-896

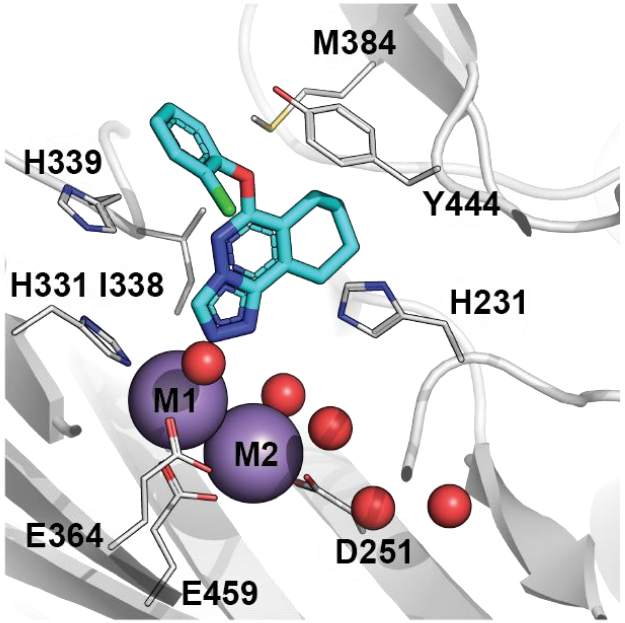
MetAP2 IC_{50} : 39 nM

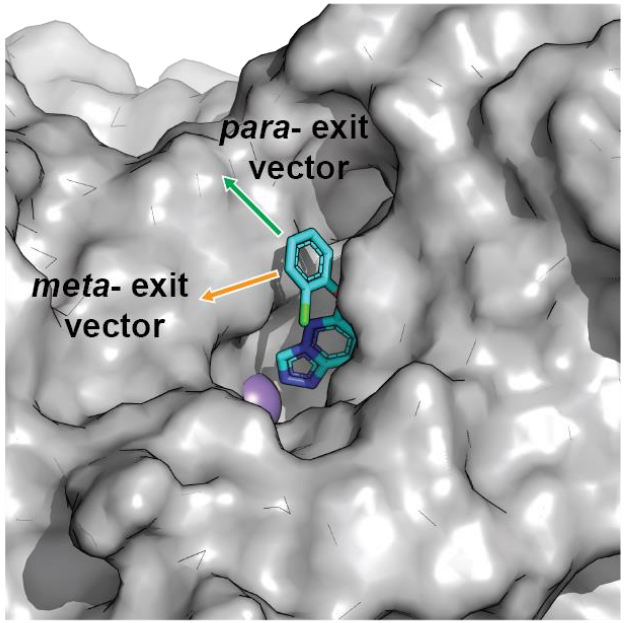


BAY-277

MetAP2 IC_{50} : 5.5 nM

MetAP2 DC_{50}: 8.9 nM D_{max}: 89%

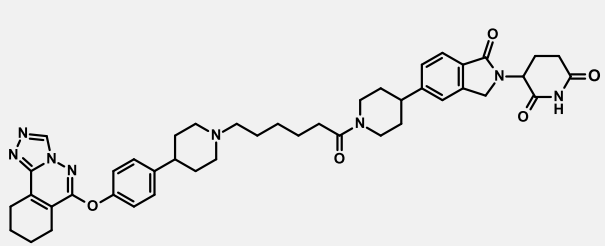




Docking of MetAP2 inhibitor **BAY-896** identified two potential linker attachment points from which MetAP2 degrader **BAY-277** was derived

MetAP2 Probe BAY-277

Technical in vitro profile



BAY-277

Potency (IC_{50} [nM])	
Biochemical IC_{50} (h / m)	5.8 / 5.9
CRBN IC_{50} (Biochem / live)	30 / 26
HT1080 (CE) DC_{50} / D_{max}	8.9 / 89%
HUVEC (WB) DC_{50} / D_{max}	0.2 / 94%
2D HUVEC proli IC_{50}	12

Properties & Physchem	
LogD @ pH 7.5	2.1
BEI / LLE (based on DC_{50} HT1080)	10 / 5.95
Sw @ pH 2 / 4 / 6.5 [mg/L]	344 / 253 / 175
MW / TPSA [g*mol / $Å^2$]	773 / 142
Stability (r /h plasma, 4h) [%]	56 / 64

***in vitro* DMPK Properties**

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	10.2		233		22.8	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		3.9 / 1.3 / 0.47 / 0.5		28 / 69 / 77 / 62	
	Hepatocytes (m / r / h)		2.1 / 0.92 / 0.38		61 / 78 / 71	
Protein binding	$F_{unbound}$ (Serum / m / h) [%]		23 / 3.1/ 6.1			
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	>20	>20	>20	>20
CYP3A4 induction [µM]	@ 1.4					

Selectivity

Panlabs @10 µM	High selectivity see next slide
Eurofins @ 1 µM (kinase panel)	No kinase binding See backup
Proteomics @ 0.1 µM	Only MetAP2

SAFETY

hERG IC_{50} [µM]	8

- **BAY-277** is a potent and selective MetAP2 degrader

MetAP2 Probe BAY-277

Panlabs Safety Screen

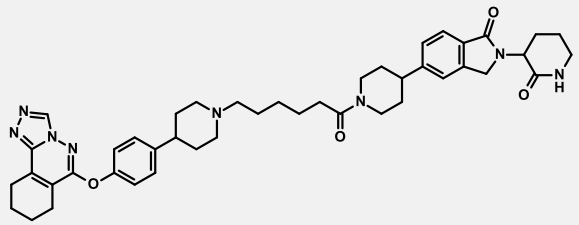
Assay Name	Conc.	% Inh.
Aldose Reductase	10 µM	32
ATPase, Na^+/K^+, Heart, Pig	10 µM	-7
Carbonic Anhydrase II	10 µM	-8
Cholinesterase, Acetyl, ACES	10 µM	45
Cyclooxygenase COX-1	10 µM	16
Cyclooxygenase COX-2	10 µM	-6
HMG-CoA Reductase	10 µM	12
Leukotriene LTC_4 Synthase	10 µM	2
Lipoxygenase 15-LOX-2	10 µM	-1
Monoamine Oxidase MAO-A	10 µM	16
Monoamine Oxidase MAO-B	10 µM	34
Nitric Oxide Synthase, Neuronal (nNOS)	10 µM	9
Nitric Oxide Synthetase, Inducible (iNOS)	10 µM	0
Peptidase, Angiotensin Converting Enzyme	10 µM	-25
Phosphodiesterase PDE3A	10 µM	6
Phosphodiesterase PDE4D2	10 µM	10
Phosphodiesterase PDE5A	10 µM	25
Thromboxane Synthase	10 µM	10
Adenosine A_1	10 µM	7
Adenosine A_{2A}	10 µM	6
Adenosine A_3	10 µM	16
Adrenergic α_{1A}	10 µM	32
Adrenergic α_{2A}	10 µM	28
Adrenergic α_{2B}	10 µM	19
Adrenergic α_{2C}	10 µM	79
Adrenergic β_1	10 µM	0
Adrenergic β_2	10 µM	-4
Adrenergic β_3	10 µM	16
Androgen (Testosterone)	10 µM	-6
Angiotensin AT_1	10 µM	-4
Angiotensin AT_2	10 µM	13
Bradykinin B_1	10 µM	1
Bradykinin B_2	10 µM	10

Assay Name	Conc.	% Inh.
Cannabinoid CB_1	10 µM	1
Cannabinoid CB_2	10 µM	14
Dopamine D_1	10 µM	16
Dopamine D_{2L}	10 µM	22
Dopamine D_{2S}	10 µM	-8
Dopamine D_3	10 µM	90
Endothelin ET_A	10 µM	-1
Endothelin ET_B	10 µM	2
Estrogen ERα	10 µM	17
$GABA_A$, Chloride Channel, TBOB	10 µM	-6
$GABA_A$, Flunitrazepam, Central	10 µM	10
$GABA_B$, Non-Selective	10 µM	15
Glucocorticoid	10 µM	13
Glutamate, AMPA	10 µM	0
Glutamate, Kainate	10 µM	-2
Glutamate, NMDA, Agonism	10 µM	21
Glutamate, NMDA, Glycine	10 µM	-16
Growth Hormone Secretagogue (GHS, Ghrelin)	10 µM	2
Histamine H_1	10 µM	23
Histamine H_2	10 µM	32
Histamine H_3	10 µM	81
Insulin	10 µM	-7
Motilin	10 µM	6
Muscarinic M_1	10 µM	53
Muscarinic M_2	10 µM	53
Muscarinic M_3	10 µM	26
Muscarinic M_4	10 µM	70
Nicotinic Acetylcholine α3β4	10 µM	24
Opiate δ_1 (OP1, DOP)	10 µM	0
Opiate κ (OP2, KOP)	10 µM	18
Opiate µ (OP3, MOP)	10 µM	19
Progesterone PR-B	10 µM	24
Purinergic P2X	10 µM	-19
Purinergic P2Y, Non-Selective	10 µM	4

Assay Name	Conc.	% Inh.
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	10 µM	25
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	10 µM	35
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	10 µM	41
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	10 µM	14
Transporter, Adenosine	10 µM	40
Transporter, Dopamine (DAT)	10 µM	38
Transporter, GABA	10 µM	5
Transporter, Norepinephrine (NET)	10 µM	28
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	10 µM	32
Vasopressin V_{1A}	10 µM	-8

MetAP2 Negative Control BAY-8805

Technical in vitro profile



BAY-8805

Potentcy (IC_{50} [nM])	
Biochemical IC_{50} (h / m)	5.7 / n.d.
CRBN IC_{50} (Biochem / live)	> 10.000
HT1080 (CE) DC_{50} / D_{max}	> 1.000
HUVEC (WB) DC_{50} / D_{max}	> 100
2D HUVEC proli IC_{50}	450

Properties & Physchem	
LogD @ pH 7.5	2.1
BEI / LLE (based on DC_{50} HT1080)	n.d.
Sw @ pH 2 / 4 / 6.5 [mg/L]	466 / 592 / 516
MW / TPSA [g*mol / $Å^2$]	759 / 125
Stability (r /h plasma, 4h) [%]	100 / 100

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	1.1		61.2		54	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		1.5 / 2.3 / 0.6		72 / 44 / 55	
	Hepatocytes (m / r / h)		4.3 / 2.4 / 0.01		21 / 42 / 100	
Protein binding	$F_{unbound}$ (m) [%]		2.2			
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	>20	>20	>20	>20
CYP3A4 induction [µM]	@ 7.1					

Selectivity	
Panlabs @10 µM	High selectivity see backup
Eurofins @ 1 µM (kinase panel)	No kinase binding see backup
Proteomics @ 0.01 µM	No degradation

SAFETY	
hERG IC_{50} [µM]	n.d.

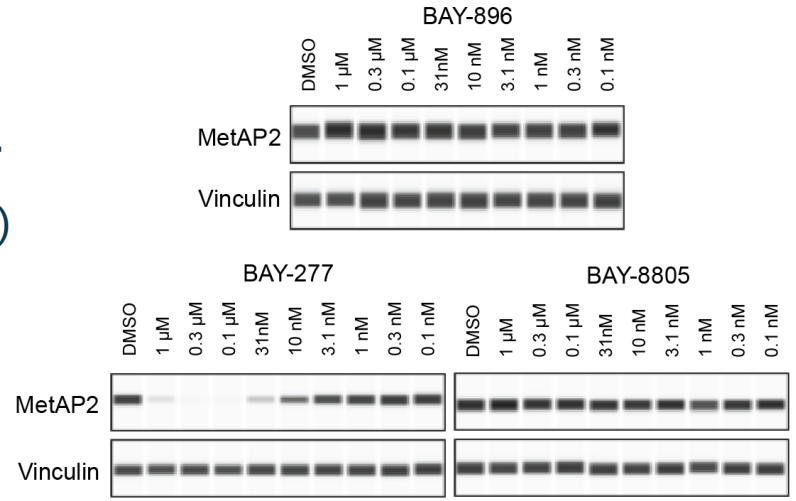
- **BAY-8805** is an E3-blocked MetAP2 bifunctional (negative control)

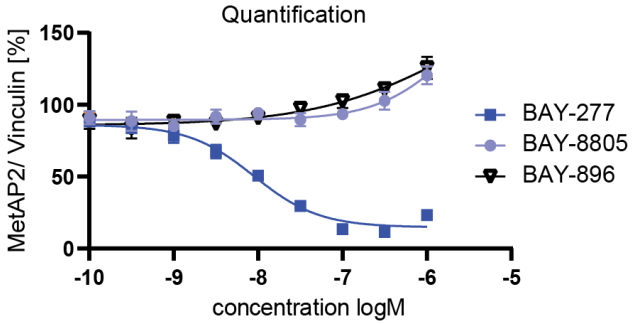
n.d. : not determined

BAY-277 induces degradation of MetAP2 in HT1080 cells

Capillary electrophpresis (HT1080)

- Dose dependent degradation of **BAY-277** in HT1080 cells (capillary electrophoresis)
  - corresponding E3 blocked control (**BAY-8805**) and the SMOL inhibitor (**BAY-896**) have no effect on MetAP2 levels
- Potent and deep degradation detected (Dmax >85%)





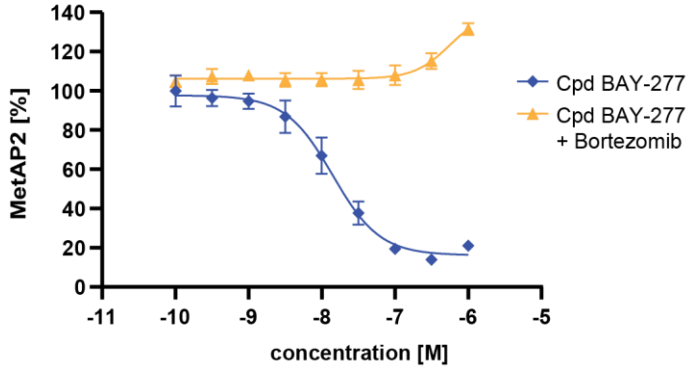
Cpd	BC-Assay IC50 [mol/L]	DC50 [M]	Dmax [%]
BAY-896	3.91E-08	no degr	-
BAY-277	5.52E-09	8.93E-09	88.5
BAY-8805	5.65E-09	no degr	-

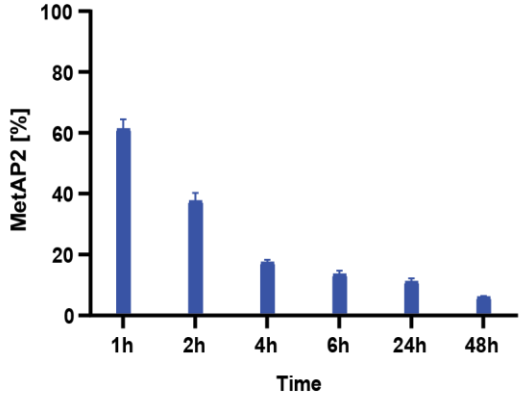
MetAP2 degraders can be validated in a tag free system and shows nM potencies while E3 blocked controls do not induce MetAP2 degradation

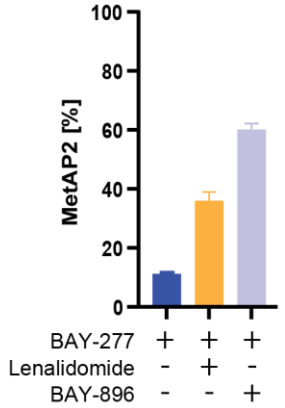
MoA analysis of BAY-277

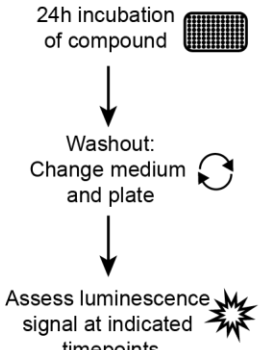
MoA analysis, HT1080-HiBit

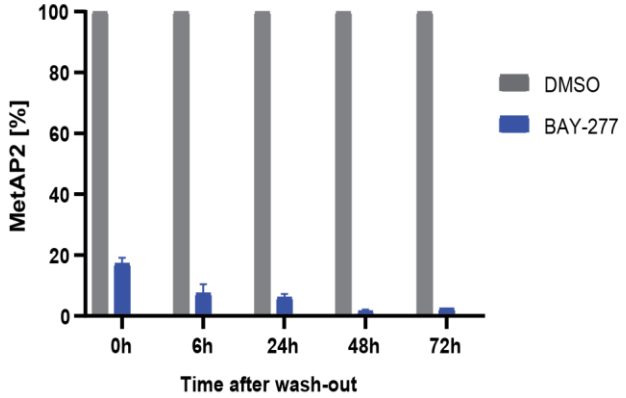
- Protasome dependency shown by degradation assay +/- bortezomib
- Kinetics reveal a quick onset of degradation >80% after 6h
- Competition with MetAP2 inhibitor or CRBN binder shows expected heterobifunctional MoA via CRBN engagement
- Wash out experiments reveals long lasting effect, no recovery of MetAP2 levels detected after 72h after wash out











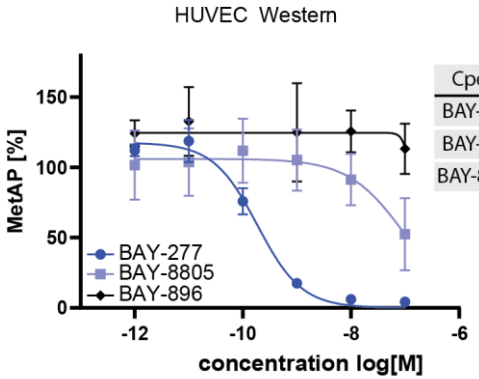
Degradation mode of action validated. Quick onset of degradation and long-lasting effects detectable

BAY-277: *In vitro* pharmacologic effect

HUVEC cells

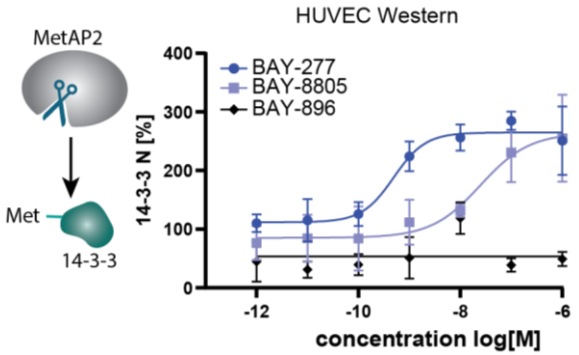
- Degradation validated in HUVEC cells
  - High potency pM range and deep degradation (>90%)
- Target of MetAP2 14-3-3γ monitoring of N-terminal Methionine presence reveals MetAP2 degradation results in strong inhibition of peptidase activity
- Proliferation of HUVEC cells is inhibited upon MetAP2 degradation, only weak effect with inhibitor and E3 blocked control

Degradation



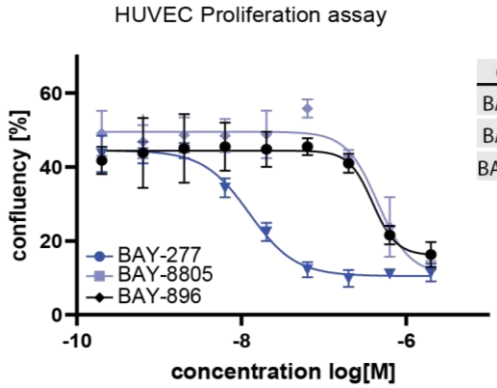
Cpd	DC_{50} [mol/L]	D_{max} [%]
BAY-896	-	
BAY-277	1.8E-10	94.6
BAY-8805	~1E-07	

Downstream effect



Cpd	IC_{50} [mol/L]
BAY-896	-
BAY-277	4.9E-10
BAY-8805	2.5E-8

Proliferation



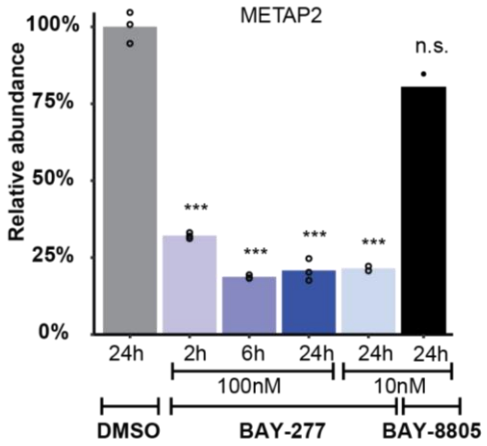
Cpd	IC_{50} [mol/L]
BAY-896	3.9E-7
BAY-277	1.2E-8
BAY-8805	4.5E-07

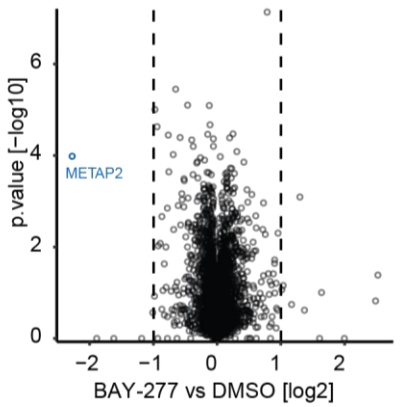
Active degrader **BAY-277** outperforms inhibitor (**BAY-896**), as well as E3 blocked control (**BAY-8805**) with respect to proliferation and cellular efficacy in a 14-3-3 methionine peptidase assay

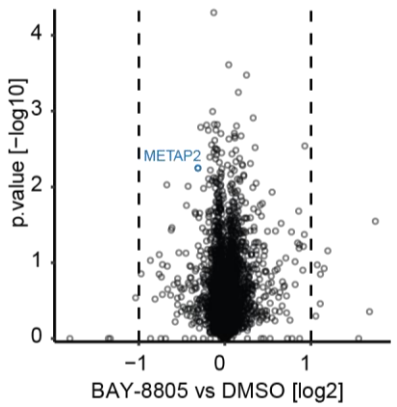
BAY-277: Selectivity of degradation: global proteomics

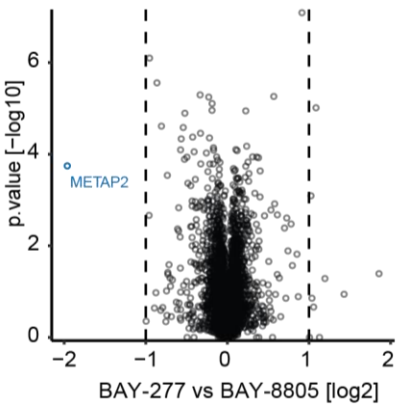
HUVEC cells

- MetAP2 is the only protein among ~5000 detected proteins in HUVEC cells which is highly and significantly reduced by **BAY-277** when compared to DMSO and a E3 blocked reference (**BAY-8805**)
- No degradation with E3 blocked control **BAY-8805**
- Strong degradation already after 2h of incubation, full effect in HUVEC cells after 6h









BAY-277 is a highly selective MetAP2 degrader.

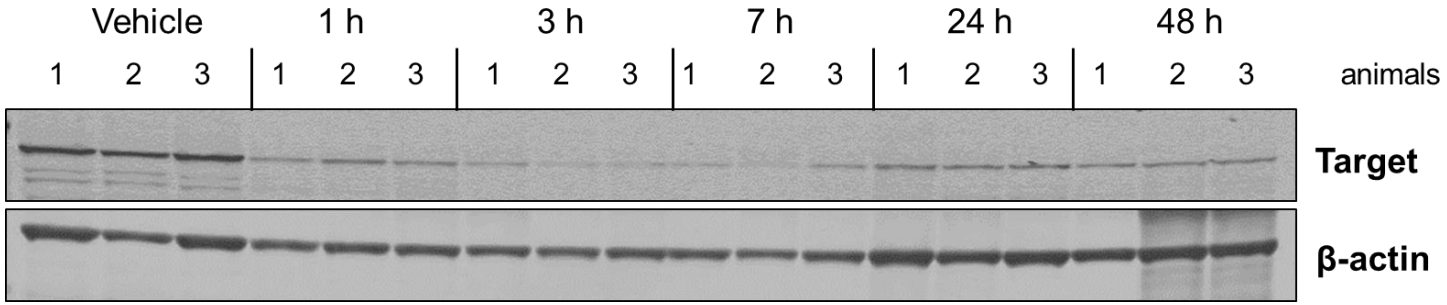
BAY-277: *In vivo* degradation

In vivo target degradation - lung

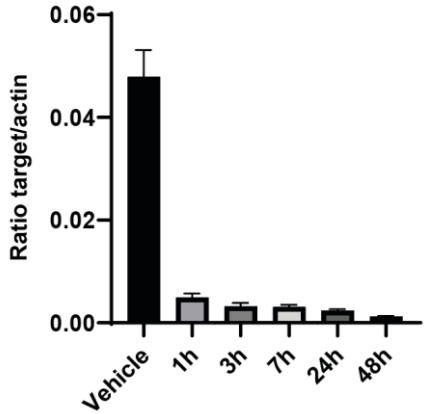
Kinetic assessment of *in vivo* degradation

- Fast i.v. study of **BAY-277** in female nude mice, single dose, lung, 5 mg/kg
- Different timepoints assessed after administration

Lung



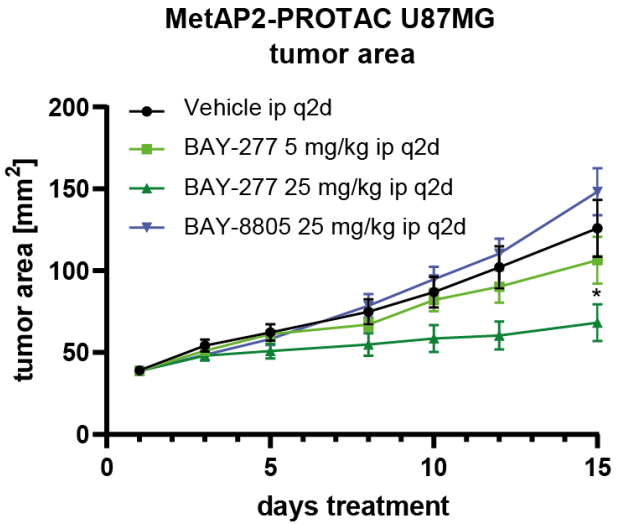
Treatment	Dmax [%]
vehicle	0.0
1h	89.7
3h	93.3
7h	93.5
24h	95.1
48h	97.4

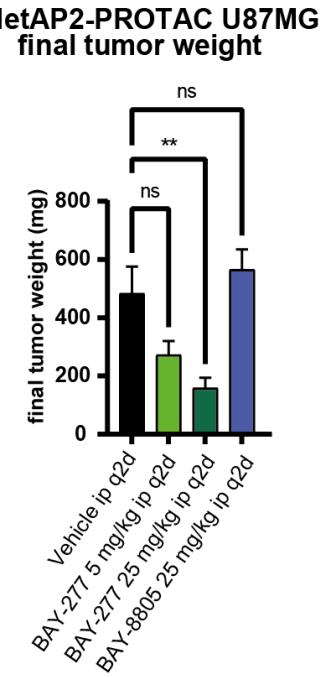


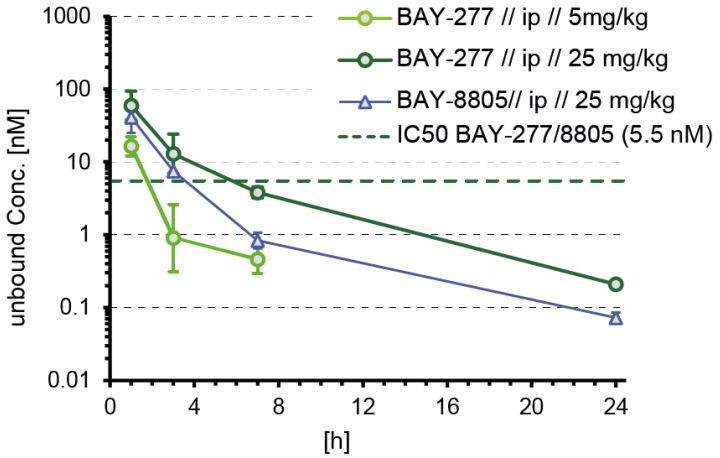
BAY-277 shows fast and potent degradation in lung tissue @ 5mg/kg up until at least 48 h post injection
→ Xenograft studies dosing every other day

BAY-277 shows efficacy in two different xenograft models

In vivo efficacy U87MG xenograft







// Animals were treated ip for 14 days q2d

// Overall good tolerability (body weight)

// No effect of E3 blocked control (**BAY-8805**)

// Dose dependent TGI for **BAY-277**

// Significant TGI at 25 mg/kg

BAY-277 inhibits tumor growth in a U87MG xenograft model in a dose dependent manner

BAY-277 shows efficacy in two different xenograft models

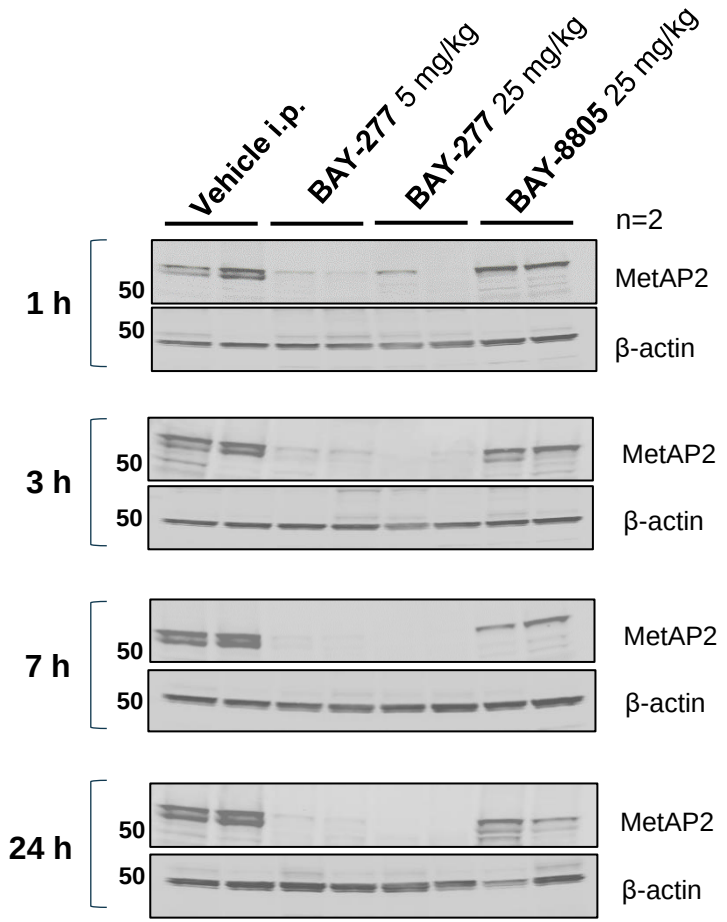
MoA confirmation U87MG xenograft

***Ex vivo* Western Blot analysis of U87MG tumor tissue**

// **BAY-277** potently degrades MetAP2 at 5 mg/kg and 25 mg/kg for at least 24 h

// E3 blocked control **BAY-8805** does not reduce MetAP2 levels at 25 mg/kg

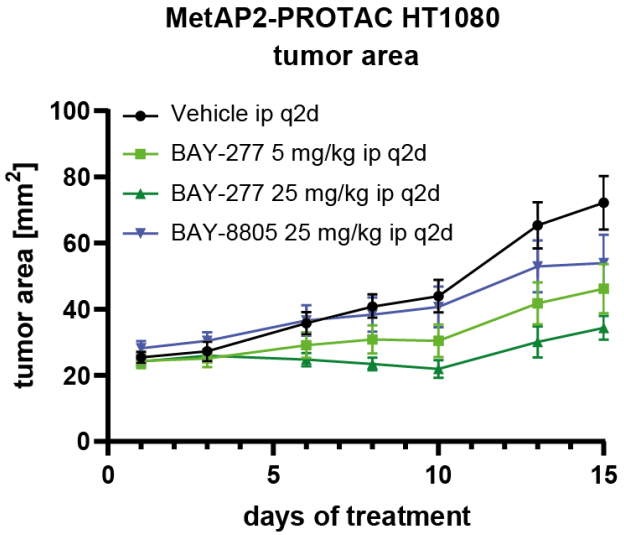
In vivo validation of **BAY-277** mode of action

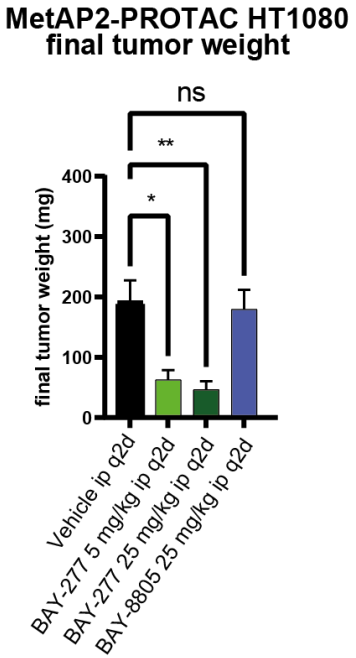


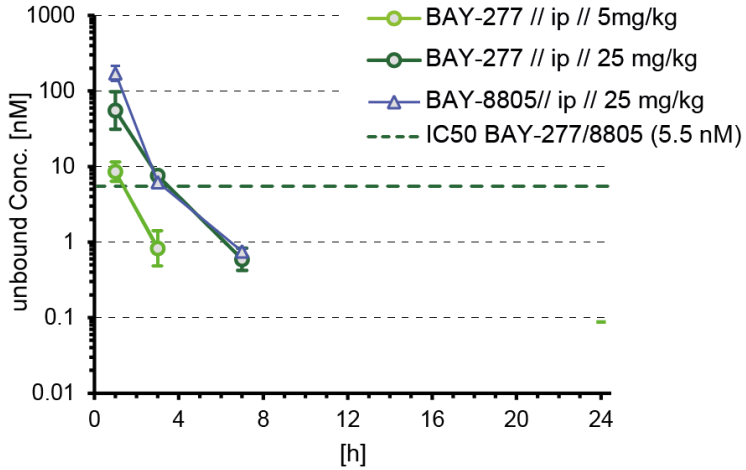
BAY-277 shows fast and potent MetAP2 degradation U87MG in tumor tissue

BAY-277 shows efficacy in two different xenograft models

In vivo efficacy HT1080 xenograft







// Animals were treated ip for 14 days q2d

// Overall good tolerability (body weight)

// No effect of E3 blocked control (**BAY-8805**)

// Dose dependent TGI for **BAY-277**

// Significant TGI at 5 mg/kg and 25 mg/kg

BAY-277 inhibits tumor growth in two different xenograft models in a dose dependent manner

MetAP2 Probe BAY-277

Summary / Conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC_{50}, Kd)	IC_{50}: **5.8 nM**; DC_{50} : **8.9 nM**; **D_{max}: 89%**
Activity: Evidence of MoA	Proteasome-dependent degradation; rescue experiments with CRBN binder, target binder and proteasome inhibitor
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	Proteomics: **selective MetAP2 degradation** Panlabs: **High selectivity**; Kinase panel: **no kinases engaged**
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	DC_{50} : **8.9 nM**; IC_{50} (HUVEC): **12 nM**
On target cell activity	MoA and **downstream target accumulation** confirmed (14-3-3)
In vivo activity	Dose-dependent **tumor growth inhibition** in two different **xenograft models**
Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Negative control: no degradation**, **>30-fold reduced IC_{50} HUVEC** negative control: E3 blocked compound, MetAP2 binder

We ask for acceptance of MetAP2 degrader **BAY-277** as chemical probe, accompanied by **BAY-8805** and **BAY-896** as negative control

Acknowledgement

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Franziska Witte

Back up

MetAP2 Probe BAY-277

Eurofins, Kinases inhibition above 10%

Compound No	Kinase	ATP Conc. [mol/L]	Concentration [mol/L]	Inhibition [%]
BAY-277	Haspin(h)	1.0E-05	1.0E-05	28.62
BAY-277	Syk(h)	1.0E-05	1.0E-05	24.98
BAY-277	Met(h)	1.0E-05	1.0E-05	22.72
BAY-277	JAK2(h)	1.0E-05	1.0E-05	21.83
BAY-277	MATK(h)	1.0E-05	1.0E-05	19.66
BAY-277	MSK1(h)	1.0E-05	1.0E-05	19.36
BAY-277	GCN2(h)	1.0E-05	1.0E-05	17.16
BAY-277	SGK2(h)	1.0E-05	1.0E-05	16.49
BAY-277	CaMKIgamma(h)	1.0E-05	1.0E-05	15.74
BAY-277	Pim-2(h)	1.0E-05	1.0E-05	15.45
BAY-277	Lck(h)	1.0E-05	1.0E-05	14.33
BAY-277	Blk(h)	1.0E-05	1.0E-05	13.93
BAY-277	CLK1(h)	1.0E-05	1.0E-05	13.86
BAY-277	Hck(h)	1.0E-05	1.0E-05	12.78
BAY-277	TGFBR2(h)	1.0E-05	1.0E-05	12.73
BAY-277	Flt4(h)	1.0E-05	1.0E-05	12.49
BAY-277	TLK1(h)	1.0E-05	1.0E-05	12.3
BAY-277	GRK2(h)	1.0E-05	1.0E-05	11.91
BAY-277	ZIPK(h)	1.0E-05	1.0E-05	11.72
BAY-277	ALK1(h)	1.0E-05	1.0E-05	11.47
BAY-277	CaMKIdelta(h)	1.0E-05	1.0E-05	11.41
BAY-277	GCK (h)	1.0E-05	1.0E-05	11.33
BAY-277	Yes(h)	1.0E-05	1.0E-05	10.87
BAY-277	Pim-3(h)	1.0E-05	1.0E-05	10.33
BAY-277	Wee1B(h)	1.0E-05	1.0E-05	10.28

MetAP2 Negative Control BAY-8805

Panlabs Safety Screen

Assay Name	Conc.	% Inh.
Aldose Reductase	10 µM	29
ATPase, Na^+/K^+, Heart, Pig	10 µM	1
Carbonic Anhydrase II	10 µM	-8
Cholinesterase, Acetyl, ACES	10 µM	38
Cyclooxygenase COX-1	10 µM	21
Cyclooxygenase COX-2	10 µM	-7
HMG-CoA Reductase	10 µM	-18
Leukotriene LTC_4 Synthase	10 µM	15
Lipoxygenase 15-LOX-2	10 µM	0
Monoamine Oxidase MAO-A	10 µM	18
Monoamine Oxidase MAO-B	10 µM	30
Nitric Oxide Synthase, Neuronal (nNOS)	10 µM	-4
Nitric Oxide Synthetase, Inducible (iNOS)	10 µM	-3
Peptidase, Angiotensin Converting Enzym	10 µM	-30
Phosphodiesterase PDE3A	10 µM	6
Phosphodiesterase PDE4D2	10 µM	18
Phosphodiesterase PDE5A	10 µM	10
Thromboxane Synthase	10 µM	23
Adenosine A_1	10 µM	3
Adenosine A_{2A}	10 µM	6
Adenosine A_3	10 µM	21
Adrenergic α_{1A}	10 µM	21
Adrenergic α_{2A}	10 µM	32
Adrenergic α_{2B}	10 µM	8
Adrenergic α_{2C}	10 µM	62
Adrenergic β_1	10 µM	10
Adrenergic β_2	10 µM	13
Adrenergic β_3	10 µM	13
Androgen (Testosterone)	10 µM	-2
Angiotensin AT_1	10 µM	2
Angiotensin AT_2	10 µM	17
Bradykinin B_1	10 µM	7
Bradykinin B_2	10 µM	13

Assay Name	Conc.	% Inh.
Cannabinoid CB_1	10 µM	-14
Cannabinoid CB_2	10 µM	12
Dopamine D_1	10 µM	20
Dopamine D_{2L}	10 µM	22
Dopamine D_{2S}	10 µM	13
Dopamine D_3	10 µM	76
Endothelin ET_A	10 µM	3
Endothelin ET_B	10 µM	-5
Estrogen ERα	10 µM	22
$GABA_A$, Chloride Channel, TBOB	10 µM	-5
$GABA_A$, Flunitrazepam, Central	10 µM	15
$GABA_B$, Non-Selective	10 µM	22
Glucocorticoid	10 µM	20
Glutamate, AMPA	10 µM	3
Glutamate, Kainate	10 µM	1
Glutamate, NMDA, Agonism	10 µM	0
Glutamate, NMDA, Glycine	10 µM	-6
Growth Hormone Secretagogue (GHS Ghrelin)	10 µM	5
Histamine H_1	10 µM	22
Histamine H_2	10 µM	36
Histamine H_3	10 µM	85
Insulin	10 µM	-5
Motilin	10 µM	10
Muscarinic M_1	10 µM	53
Muscarinic M_2	10 µM	47
Muscarinic M_3	10 µM	29
Muscarinic M_4	10 µM	57
Nicotinic Acetylcholine α3β4	10 µM	6
Opiate δ_1 (OP1, DOP)	10 µM	7
Opiate κ (OP2, KOP)	10 µM	35
Opiate μ (OP3, MOP)	10 µM	16
Progesterone PR-B	10 µM	19
Purinergic P2X	10 µM	-11
Purinergic P2Y, Non-Selective	10 µM	12

Assay Name	Conc.	% Inh.
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	10 µM	6
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	10 µM	39
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	10 µM	10
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	10 µM	2
Transporter, Adenosine	10 µM	44
Transporter, Dopamine (DAT)	10 µM	39
Transporter, GABA	10 µM	8
Transporter, Norepinephrine (NET)	10 µM	15
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	10 µM	22
Vasopressin V_{1A}	10 µM	-3

MetAP2 Negative Control BAY-8805

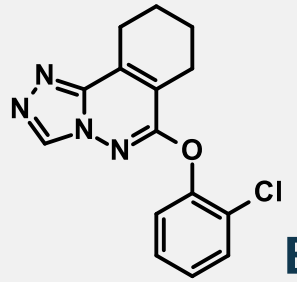
Eurofins, Kinases inhibition above 10%

Compound No	Kinase	ATP Conc. [mol/L]	Concentration [mol/L]	Inhibition [%]
BAY-8805	Blk(h)	1.0E-05	1.0E-05	26.11
BAY-8805	Haspin(h)	1.0E-05	1.0E-05	26.02
BAY-8805	PASK(h)	1.0E-05	1.0E-05	23.94
BAY-8805	Syk(h)	1.0E-05	1.0E-05	20.44
BAY-8805	PDHK2(h)	1.0E-05	1.0E-05	18.65
BAY-8805	Hck(h)	1.0E-05	1.0E-05	17.95
BAY-8805	Mer(h)	1.0E-05	1.0E-05	17.94
BAY-8805	CDKL2(h)	1.0E-05	1.0E-05	17.56
BAY-8805	NEK5(h)	1.0E-05	1.0E-05	17.52
BAY-8805	Met(h)	1.0E-05	1.0E-05	16.94
BAY-8805	CLK1(h)	1.0E-05	1.0E-05	16.74
BAY-8805	MAPKAP-K2(h)	1.0E-05	1.0E-05	16.62
BAY-8805	ZIPK(h)	1.0E-05	1.0E-05	15.92
BAY-8805	GRK2(h)	1.0E-05	1.0E-05	15.06
BAY-8805	NDR2(h)	1.0E-05	1.0E-05	14.38
BAY-8805	PKBbeta(h)	1.0E-05	1.0E-05	14.07
BAY-8805	SGK3(h)	1.0E-05	1.0E-05	13.44
BAY-8805	Lck(h) activated	1.0E-05	1.0E-05	13.33
BAY-8805	PKCalpha(h)	1.0E-05	1.0E-05	12.76
BAY-8805	TNIK(h)	1.0E-05	1.0E-05	12.48
BAY-8805	MSSK1(h)	1.0E-05	1.0E-05	12.12
BAY-8805	DCAMKL2(h)	1.0E-05	1.0E-05	12.03

Compound No	Kinase	ATP Conc. [mol/L]	Concentration [mol/L]	Inhibition [%]
BAY-8805	Lck(h)	1.0E-05	1.0E-05	11.89
BAY-8805	PI3 Kinase (p110d/p85a)(h)	1.0E-05	1.0E-05	11.6
BAY-8805	STK39(h)	1.0E-05	1.0E-05	11.48
BAY-8805	NEK1(h)	1.0E-05	1.0E-05	11.41
BAY-8805	MAPKAP-K3(h)	1.0E-05	1.0E-05	11.36
BAY-8805	TGFBR2(h)	1.0E-05	1.0E-05	11.26
BAY-8805	NDR1(h)	1.0E-05	1.0E-05	11.2
BAY-8805	PKCgamma(h)	1.0E-05	1.0E-05	10.92
BAY-8805	CK2alpha2(h)	1.0E-05	1.0E-05	10.88
BAY-8805	TRB2(h)	1.0E-05	1.0E-05	10.87
BAY-8805	SGK(h)	1.0E-05	1.0E-05	10.81
BAY-8805	TSSK4(h)	1.0E-05	1.0E-05	10.81
BAY-8805	CLK4(h)	1.0E-05	1.0E-05	10.8
BAY-8805	MLK4(h)	1.0E-05	1.0E-05	10.67
BAY-8805	SNRK(h)	1.0E-05	1.0E-05	10.63
BAY-8805	Pim-2(h)	1.0E-05	1.0E-05	10.55
BAY-8805	DCAMKL3(h)	1.0E-05	1.0E-05	10.47
BAY-8805	PKR(h)	1.0E-05	1.0E-05	10.3
BAY-8805	p70S6K(h)	1.0E-05	1.0E-05	10.21
BAY-8805	ICK(h)	1.0E-05	1.0E-05	10.1
BAY-8805	Fgr(h)	1.0E-05	1.0E-05	10.08
BAY-8805	MST4(h)	1.0E-05	1.0E-05	10.07

MetAP2 Reference Control BAY-896

Technical in vitro profile



BAY-896

Potentcy (IC_{50} [nM])	
Biochemical IC_{50} (h / m)	39 / 16
CRBN IC_{50} (Biochem / live)	> 10.000
HT1080 (CE) DC_{50} / D_{max}	> 1.000
HUVEC (WB) DC_{50} / D_{max}	> 100
2D HUVEC proli IC_{50}	390

Properties & Physchem	
LogD @ pH 7.5	2.6
BEI / LLE (based on DC_{50} HT1080)	n.d.
Sw @ pH 2 / 4 / 6.5 [mg/L]	n.d. / n.d. / 30
MW / TPSA [g*mol / $Å^2$]	301 / 52
Stability (r /h plasma, 4h) [%]	100 / 100

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	222		146		0.7	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		4.4 / 2.7 / 1.1		18 / 35/ 13	
	Hepatocytes (r)		2.4		42	
Protein binding	$F_{unbound}$ (m) [%]		2.5			
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	2.7	7.5	4.8	>20	>20	>20
CYP3A4 induction [µM]	n.d.					

Selectivity	
Panlabs @10 µM	n.d.
Eurofins @ 1 µM (kinase panel)	n.d.
Proteomics @ 0.01 µM	n.d.

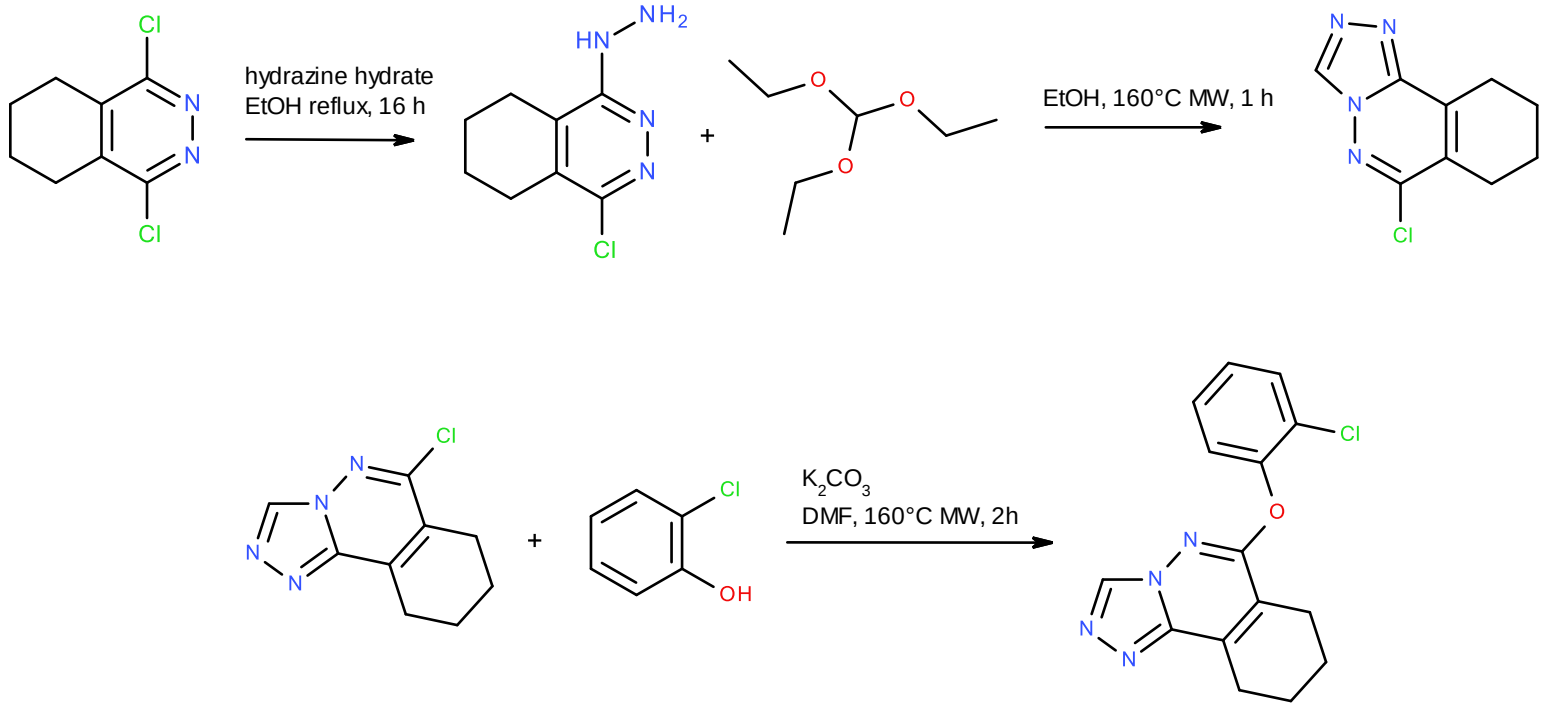
SAFETY	
hERG IC_{50} [µM]	n.d.

- **BAY-896** is a MetAP2 inhibitor (reference control)

n.d. : not determined

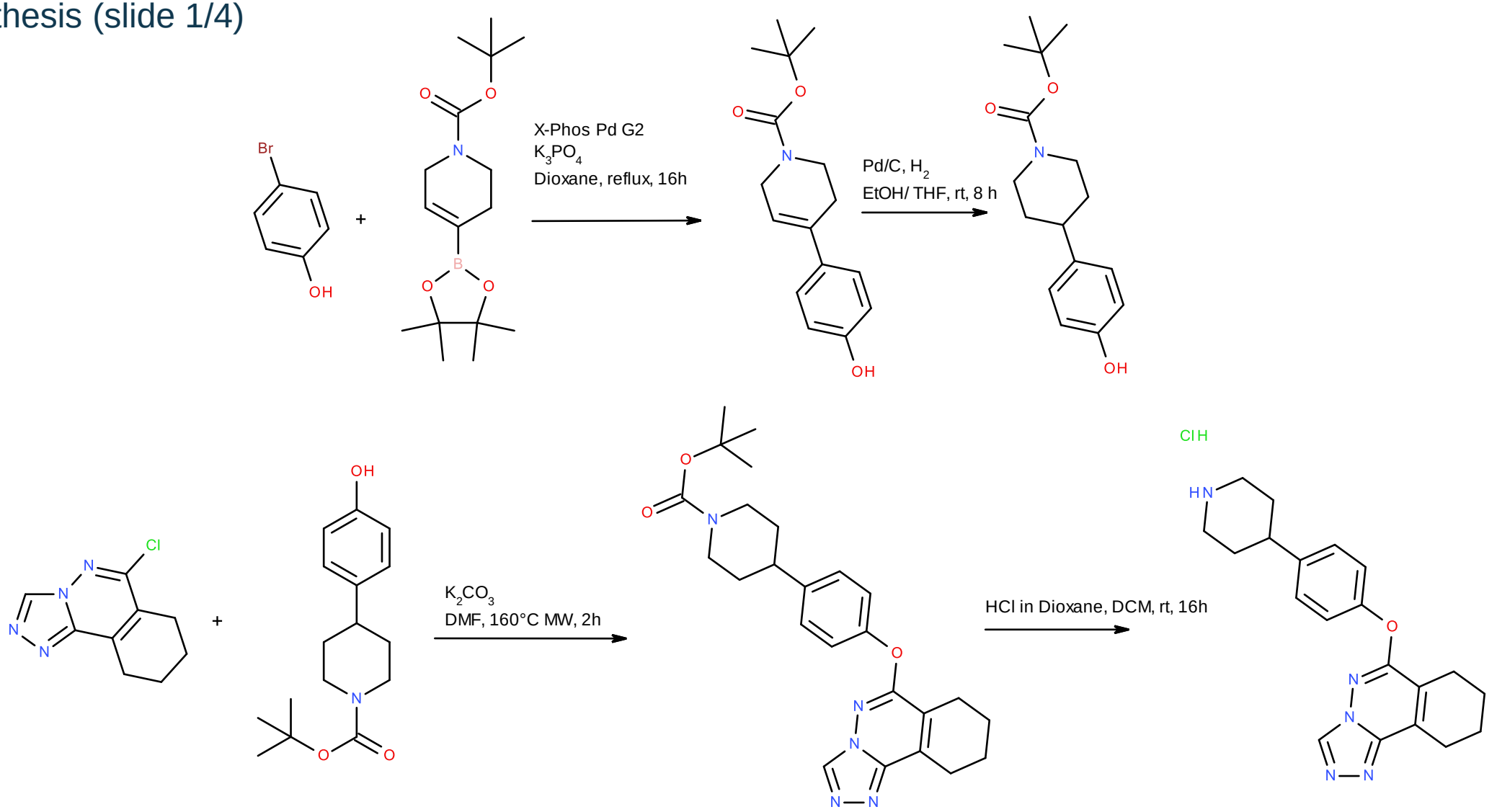
MetAP2 Reference Control BAY-896

Synthesis



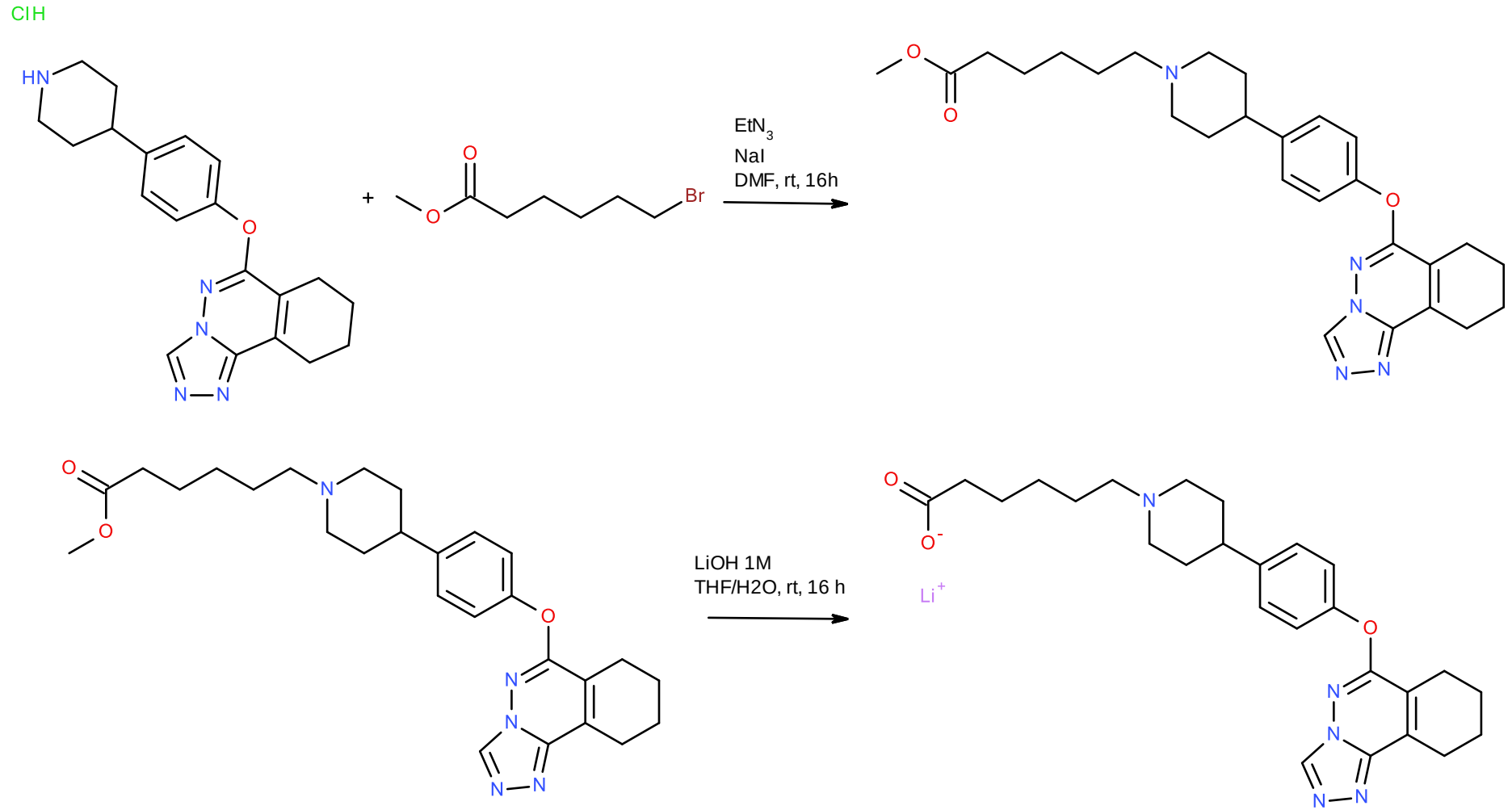
MetAP2 Probe BAY-277

Synthesis (slide 1/4)



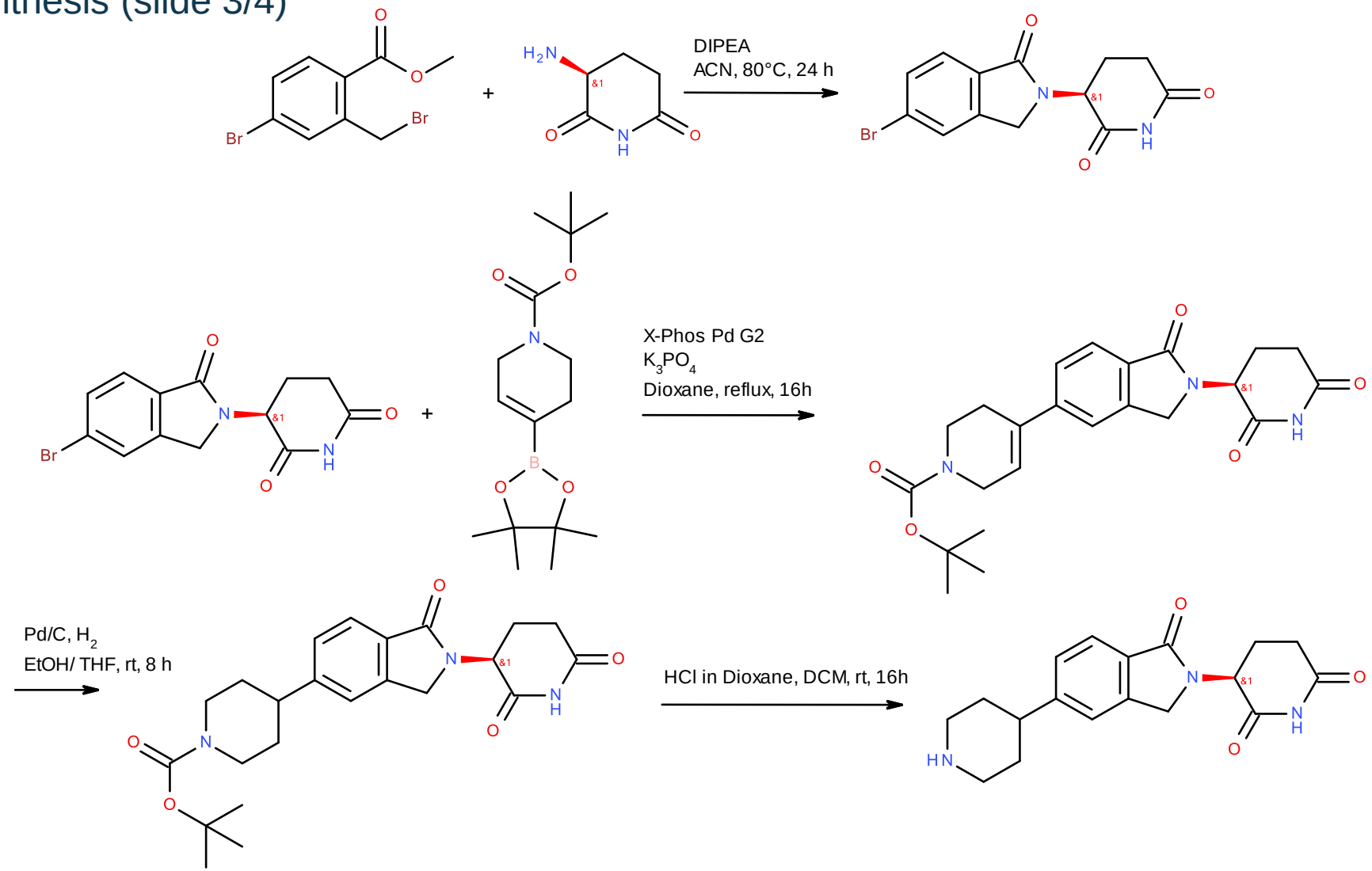
MetAP2 Probe BAY-277

Synthesis (slide 2/4)



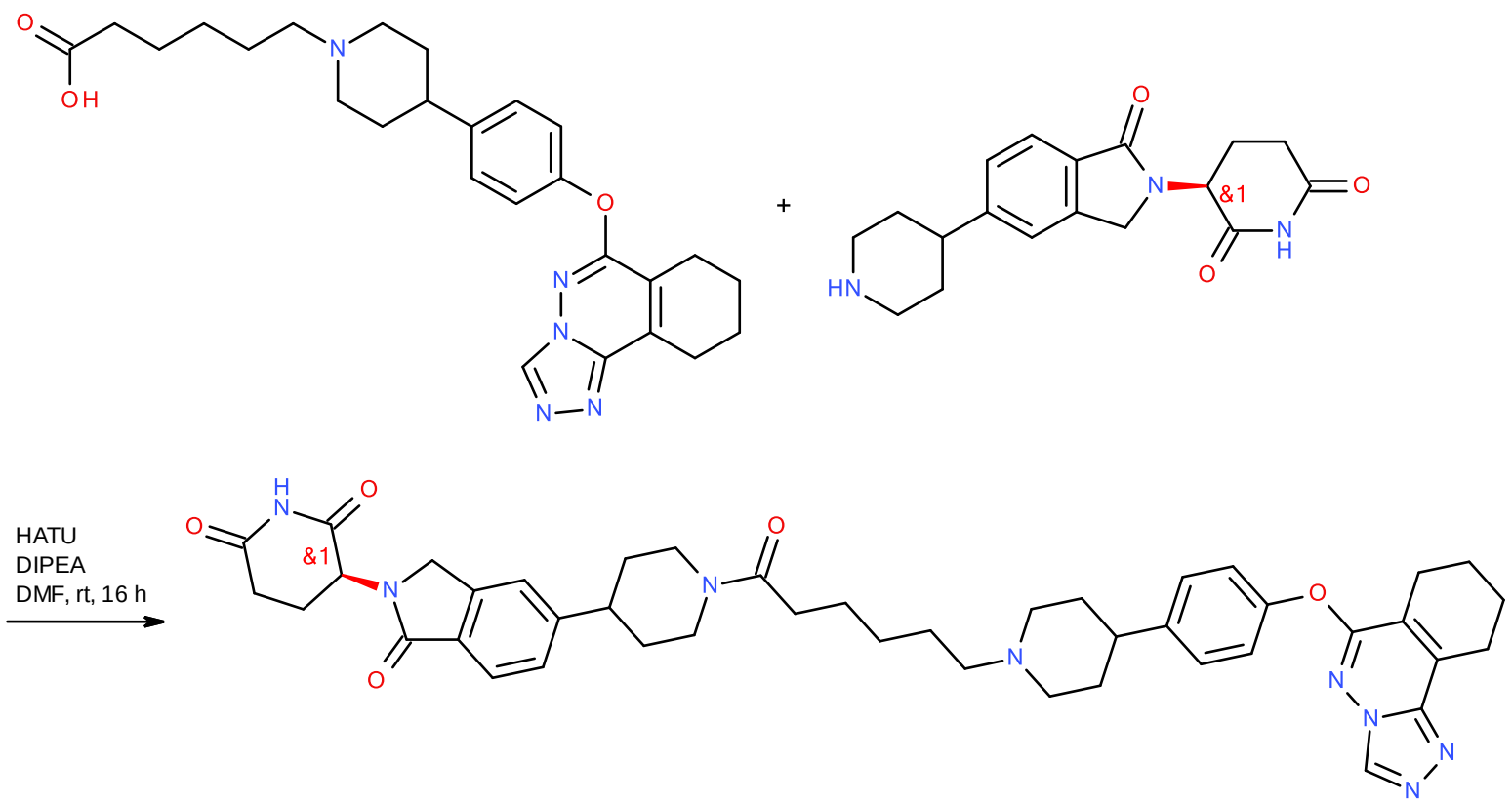
MetAP2 Probe BAY-277

Synthesis (slide 3/4)



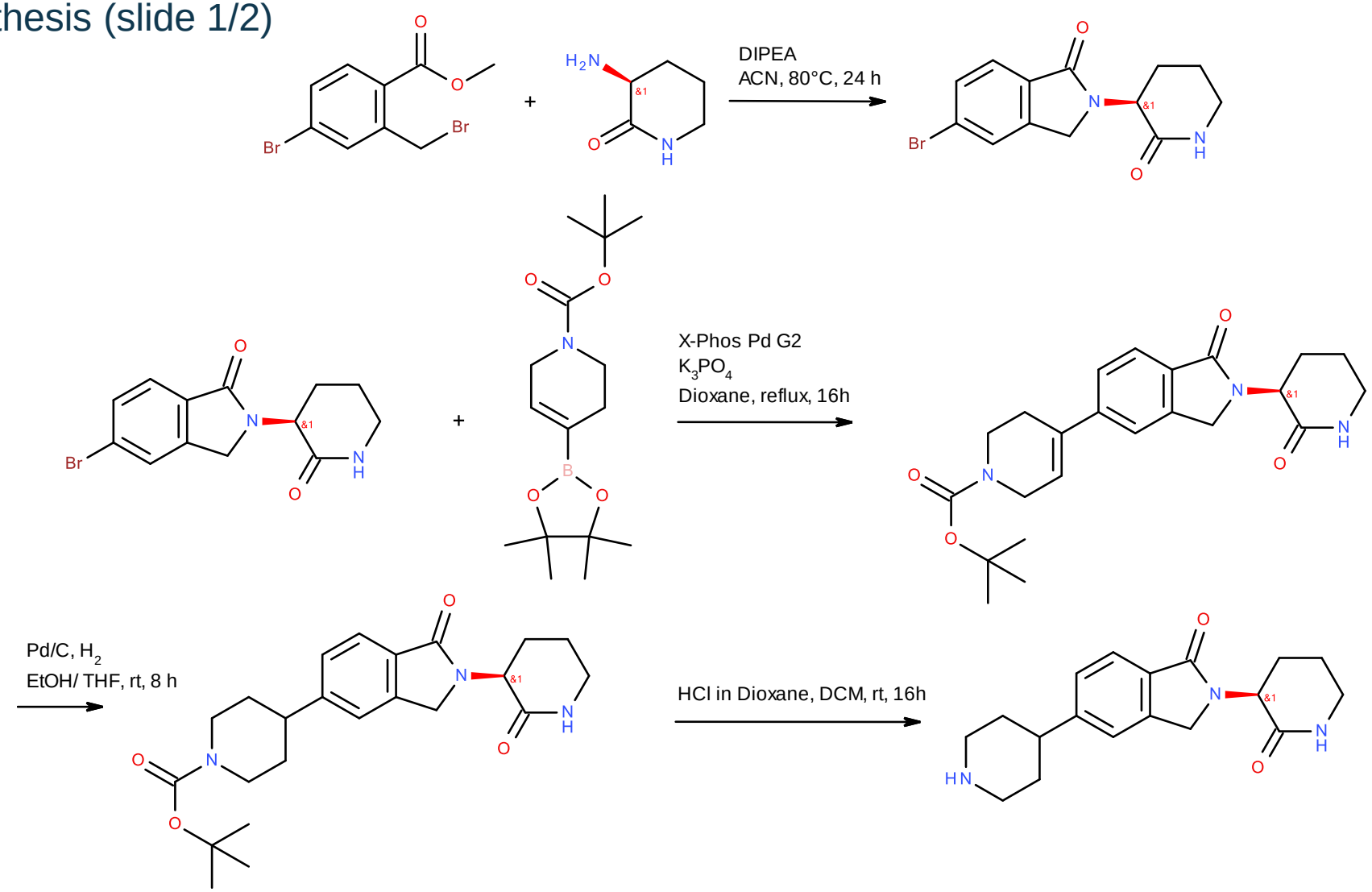
MetAP2 Probe BAY-277

Synthesis (slide 4/4)



MetAP2 Negative Control BAY-8805

Synthesis (slide 1/2)



MetAP2 Negative Control BAY-8805

Synthesis (slide 2/2)