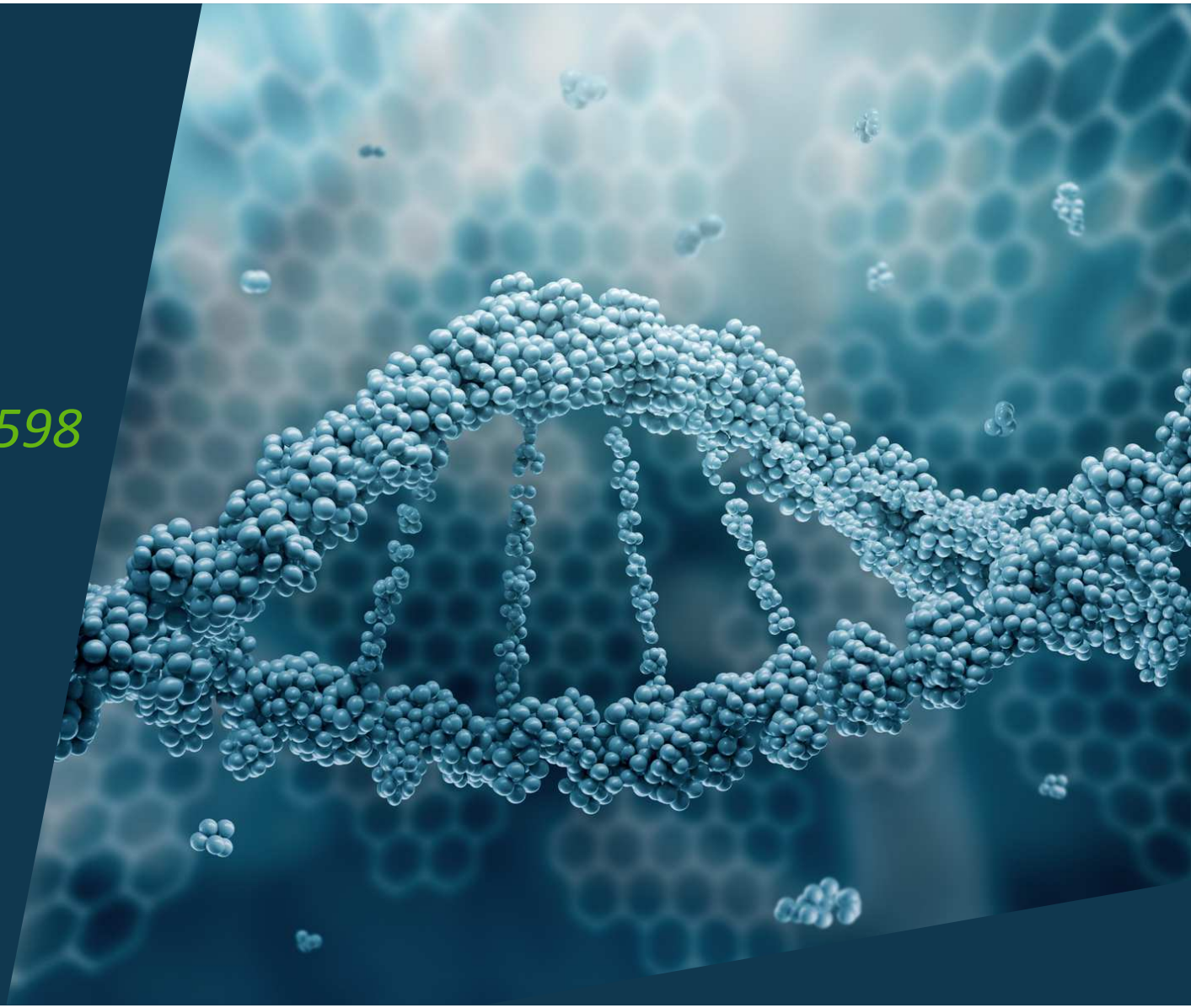
Donated Chemical Probe

Chemical Probe BAY-7598
MMP12 Inhibitor

March, 2018

Hartmut Beck, Volkhart Li



MMP12 probe BAY-7598:

Scientific rationale: anti-inflammation & anti-remodeling

Matrixmetallo Protease 12 (MMP12)

// degrades elastin and basement membrane components

// plays an important role in macrophage-mediated penetration and inflammation

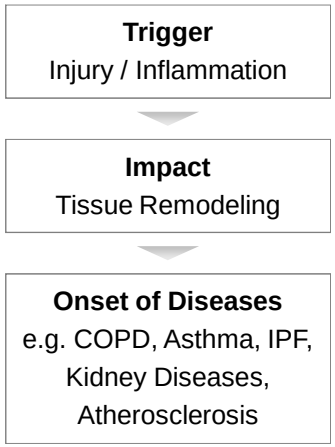
// activity is controlled by endogenous inhibitors (TIMPs)

Disease Hypothesis

// A pathophysiological protease/antiprotease imbalance in diseases leads to a

- sustained inflammation
- extracellular matrix degradation
- tissue remodeling

Thus, disease progression might be stopped or even reversed by inhibition of MMP12



Clinical Evidence

// genome-wide association studies: mmp12 SNPs linked to COPD, asthma & sclerosis

// elevated MMP12 in sputum of COPD & asthma patients

Experimental Evidence

// increased mmp12 expression & MMP12 concentration in disease models

// KO mice with ameliorated / resistant phenotype (lung emphysema & fibrosis)

// in vivo efficacy demonstrated with unselective (!) MMP12 inhibitors

MMP12 probe BAY-7598:

Challenges with regard to MMP12 target validation

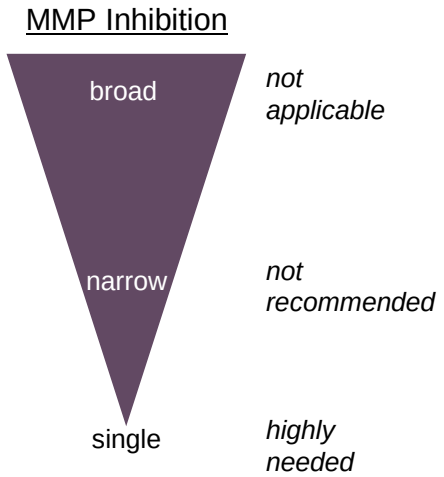
Challenging Target Validation: selectivity issue of MMP12 inhibitors

// most known MMP12 inhibitors reveal only a poor target selectivity (broad MMP inhibitors)

// more recent described MMP12 inhibitors with an improved selectivity (narrow MMP inhibitors) however turned out to be less selective versus the orthologous rodent MMPs, which impedes a proper interpretation of rodent *in vivo* efficacy data

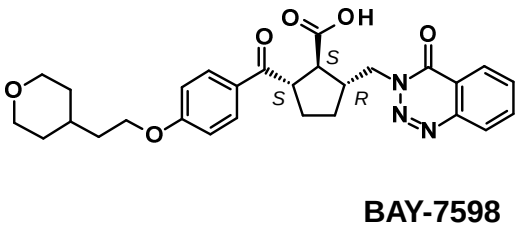
// the MMP12 selective inhibitor RXP470.1 (rodent MMP inhibition data not known) lacks drug-like features and hinders a broad *in vivo* efficacy assessment: RXP470.1 has to be administered via mini-pumps (requires surgery)

There is a high need for a cross-species selective MMP12 inhibitor with suitable physicochemical features. This will allows to serve as an appropriate tool compound in experimental pharmacology to better understand the role of MMP12 in disease biology.



MMP12 probe BAY-7598:

Overall profile



BAY-7598

▪ **Molecular Properties**

MW [g/mol]	506
MWcorr [g/mol]	506
TPSA [Å2]	118
Rotatable bonds	9

▪ **PhysChem**

Sw $^{pH\ 6.5}$ [mg/L]	338*
log D (pH 7.5)	2.0

*(thermodynamic, measured form DMSO solution)

▪ **Pharmacology**

MMP12 IC_{50}	0.085 nM

▪ **Safety**
(LeadProfilingScreen, total # of assay: 68)

BAY-7598 (10 µM)	No significant results noted

Absolute configuration was determined via VCD spectra of the methyl ester of the negative control

▪ **In vitro PK**

		Cl_{blood} [L/h/kg]			Fmax [%]		
Hep	Rat	0.87			79		
CaCo2		A-B [nm/s]		B-A [nm/s]		Ratio	
		169		288		1.7	
CYP Inhibition, IC_{50} [µM]		1A2	2C8	2C9	2D6	3A4	3A4 pre
		>20	>20	>20	>20	>20	>20
CYP Induction No effect level [µg/L]		CYP1A2			CYP3A4		
		10,000			3,333		

Overall good profile with remarkable potency and specificity

MMP12 probe BAY-7598:

Biochemical potency and selectivity

MMP12	Potency
IC_{50} (human)	0.085 nM
IC_{50} (murine)	0.67 nM
IC_{50} (rat)	1.1 nM

MMP	Potency / Selectivity vs MMP12 orthologs					
	Human		Murine		Rat	
	IC_{50} [nM]	fold	IC_{50} [nM]	fold	IC_{50} [nM]	fold
MMP1	>40,000	>470,588				
MMP2	44	518	45	67	45	41
MMP3	360	4,235	270	403		
MMP7	600	7,059	130	194		
MMP8	15	176	54	81	67	61
MMP9	460	5,412	210	313	1,000	909
MMP10	13	153				
MMP13	67	788				
MMP14	250	2,941				
MMP16	940	11,059				

BAY-7598 is a very potent inhibitor of human MMP12 with excellent selectivity cross species

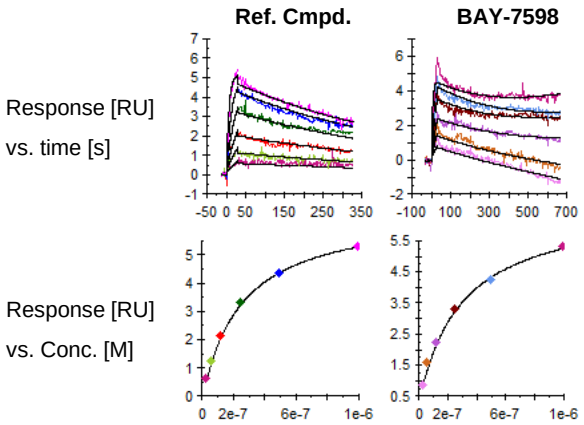
MMP12 probe BAY-7598:

Biophysical validation (SPR biosensor-based assay)

// MMP12 inhibition kinetics (Beactica AB, Sweden) applying ExtRA™ method for k_{off} determination

Interaction analysis using 6 different concentrations of compounds (0.031 – 1 µM)

Gossas et al. *Med. Chem. Comm*, 2013, **4**, 432-442



Compound	k_{on} *10^5 [1/Ms] N=2	k_{off} *10^{-3} [1/s] N=3	K_D [nM] (SD)	Residence time [h]
Ref. Cmpd. UK370106 (purch. from SantaCruz Biotech.)	1.6	3.7	24*	0.075
BAY-7598	5.1	0.02	0.004**	14

*/** compare with UK370106 IC_{50} 42 nM (Lit.) / BAY-7598 IC_{50} 0.085 nM (in house)

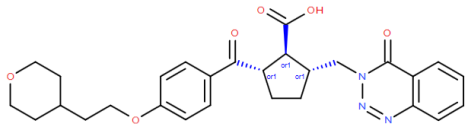
// A 1:1 interaction model described the interactions well (upper panels, black lines)

// Dose response plots (lower panels) show relationships between signal and concentration at the end of injections (ideally at equilibrium). Hyperbolic curves indicate that the interaction can be saturated, as expected for a specific interaction.

// BAY-7598 reveals a long residence time on target

MMP12 probe BAY-7598:

Co-crystal structure in MMP12 with BAY-7598



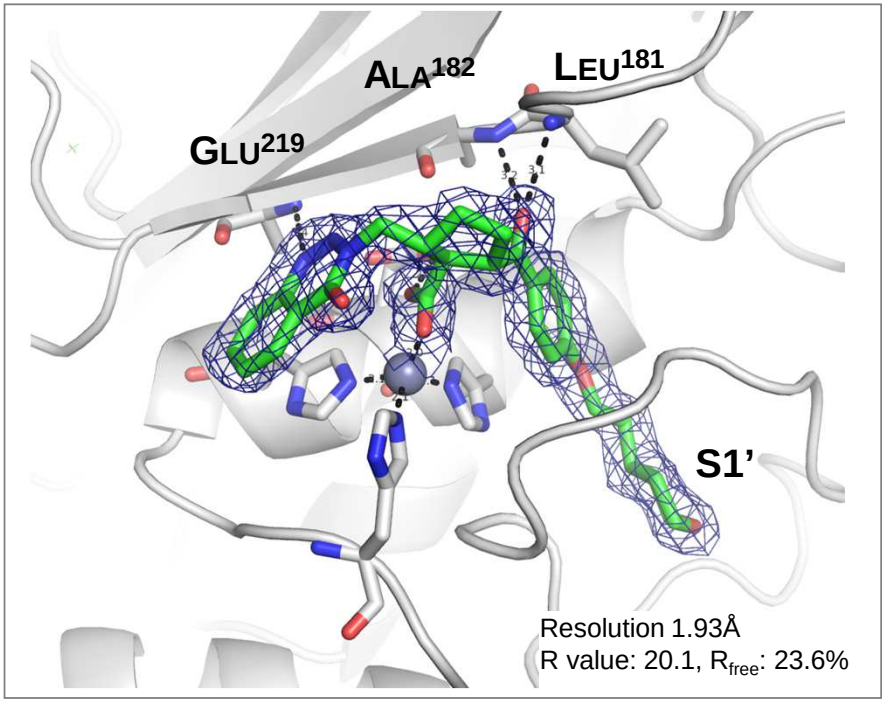
BAY-7598

- Four independent molecules of BAY-7598 in asymmetric unit

- Acid group coordinates Zn ion and interacts with side chain of GLU^{219}

- Hydrogen bonds of carbonyl group of BAY-7598 to main chain NH of ALA^{182} and LEU^{181}

- Phenoxyethyl-THP moiety penetrates deeply into hydrophobic S1' pocket



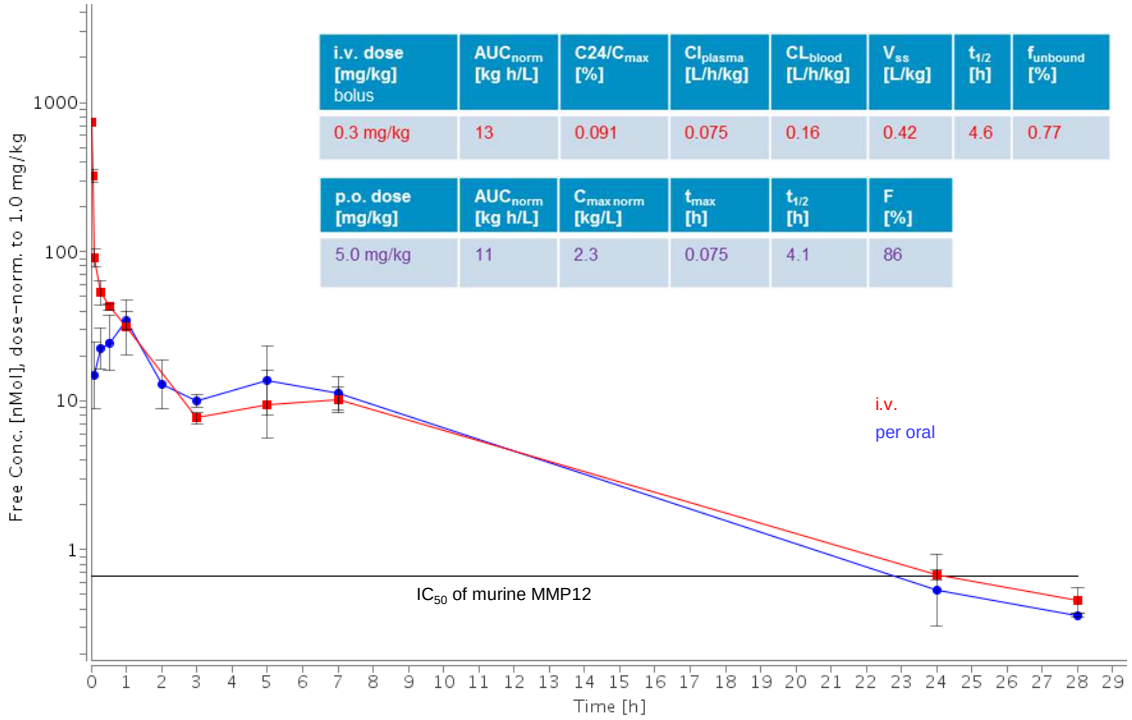
Residues: 106 - 263 of human MMP12 (P39900, F171D mutant)

MMP12 probe BAY-7598:

In vivo pharmacokinetics (mouse)

i.v. dose [mg/kg] bolus	AUC_{norm} [kg h/L]	$C24/C_{max}$ [%]	Cl_{plasma} [L/h/kg]	CL_{blood} [L/h/kg]	V_{ss} [L/kg]	$t_{1/2}$ [h]	$f_{unbound}$ [%]
0.3 mg/kg	13	0.091	0.075	0.16	0.42	4.6	0.77

p.o. dose [mg/kg]	AUC_{norm} [kg h/L]	$C_{max\,norm}$ [kg/L]	t_{max} [h]	$t_{1/2}$ [h]	F [%]
5.0 mg/kg	11	2.3	0.075	4.1	86



BAY-7598 reveals pharmacokinetic features which shall allow once daily oral administration

MMP12 probe BAY-7598:

Recommended concentration for in vitro cellular pharmacology

// **Assessment of protein binding in cell culture medium**

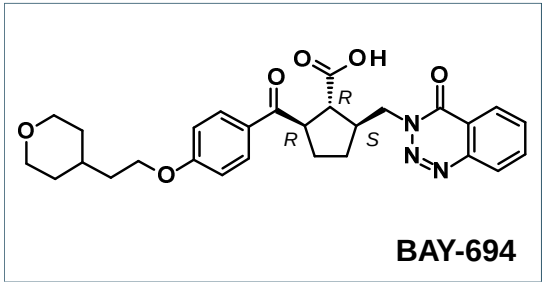
- // Fraction unbound (Williams E medium): 17 %

// **Recommended total compound concentration for application on cells**

- // Low single digit nanomolar concentration
- // Covers IC_{50} to IC_{90} range and maintains selectivity vs other MMPs*

MMP12 negative control BAY-694:

Profile of factor 1,000 less active enantiomer



BAY-694

▪ **Molecular Properties**

MW [g/mol]	506
MWcorr [g/mol]	506
TPSA [Å2]	118
Rotatable bonds	9

▪ **PhysChem**

$Sw^{pH\ 6.5}$ [mg/L]	355*
log D (pH 7.5)	2.0

*(thermodynamic, measured form DMSO solution)

▪ **Pharmacology**

MMP12 IC_{50}	80 nM**
MMP1 (Panlabs)	> 10,000 nM
MMP2 (Panlabs)	> 10,000 nM
MMP3 (Panlabs)	> 10,000 nM
MMP7 (Panlabs)	> 10,000 nM
MMP8 (Panlabs)	> 10,000 nM
MMP9 (Panlabs)	> 10,000 nM
MMP12 (Panlabs)	> 10,000 nM
MMP13 (Panlabs)	> 10,000 nM
MMP14 (Panlabs)	> 10,000 nM

▪ **In vitro PK**

		Cl_{blood} [L/h/kg]	Fmax [%]	
Hep	Rat	0.23	95	
CaCo2		A-B [nm/s]	B-A [nm/s]	Ratio
		170	543	3.2

** apparent potency is most probably due to the presence of very tiny amounts of the highly active enantiomer after the chiral chromatographic separation (<0.1 %); a different preparation revealed no activity vs MMP12 (s. MMP12 data in Panlabs panel)

Absolute configuration was determined via VCD spectra of the methyl ester of the negative control

MMP12 probe BAY-7598:

Summary / Conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC50, Kd)	**Surpasses criteria**; functional biochemical assay (MMP12) and biophysical binding assay with IC_{50} / K_D with picomolar potency / affinity
Selectivity within target family: goal is >30-fold	**Surpasses criteria;** > 100fold selectivity vs all other human MMPs tested (> 40fold selectivity of rodent ortholog MMP12 vs all rodent MMPs tested)
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Surpasses criteria**; No relevant activity in panel of 68 off-targets (@ 10µM compound conc.)
On target cell activity for cell-based targets: goal is < 1 micromolar IC50/EC50	**Not applicable**; extracellular function and localization of target, recommended low single digit nanomolar total concentration for application on cells
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	supporting mouse in vivo PK data (**oral bioavailability!**)
Neg ctrl: *in vitro* potency - > 100 times less; Cell activity - >100 times less potent than the probe	**Surpasses criteria;** >> 1,000 times less in functional biochemical assay (BAY-694, less active enantiomer)

// BAY-7598 is a very potent and selective MMP12 inhibitor with oral bioavailability, which will allow further pharmacological studies of MMP12 in vitro and in vivo to foster understanding of MMP12 disease biology

// We ask for acceptance of MMP12 inhibitor BAY-7598 as chemical probe, accompanied by BAY-694 as negative control

MMP12 probe BAY-7598:

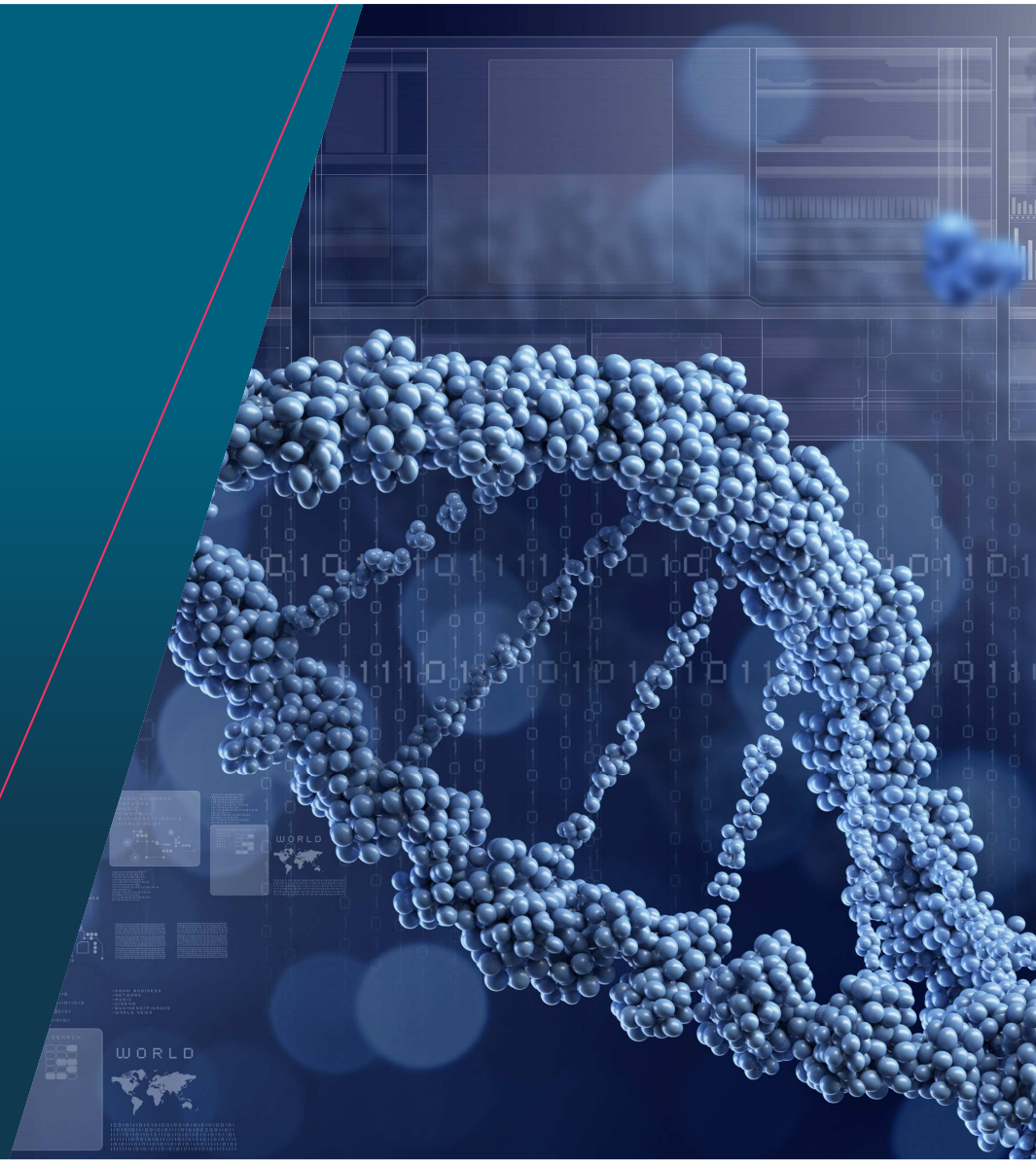
Acknowledgement

*Hartmut Beck**
*Pamela Bogner**
Dirk E. Brohm
*Yolanda Cancho Grande**
Karoline Dröbner
David Engel
Michael Gerisch
Hannah Jörißen
Dieter Lang
*Volkhart M.-J. Li**
Tibor Schomber
Christian Stegmann
*Andreas H. Timmermann**

Frank Eitner
Hubert Trübel
Thomas Krämer
Martin Bechem
Helmut Haning
Nils Burkhardt

Cora Scholten

Thank You



MMP12 probe BAY-7598:

LeadProfilingScreen, Eurofins (Panlabs) Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
Compound: CHH115-2013, PT #: 1175577							
200510	Adenosine A_1	341718	hum	2	10 µM	-2	
200610	Adenosine A_{2A}	341719	hum	2	10 µM	7	
200720	Adenosine A_3	341695	hum	2	10 µM	13	
203100	Adrenergic α_{1A}	341735	rat	2	10 µM	1	
203200	Adrenergic α_{1B}	341736	rat	2	10 µM	-1	
203400	Adrenergic α_{1D}	341737	hum	2	10 µM	-9	
203630	Adrenergic α_{2A}	341734	hum	2	10 µM	-16	
204010	Adrenergic β_1	341729	hum	2	10 µM	0	
204110	Adrenergic β_2	341730	hum	2	10 µM	4	
285010	Androgen (Testosterone) AR	341860	rat	2	10 µM	12	
212510	Bradykinin B_1	341798	hum	2	10 µM	6	
212620	Bradykinin B_2	341846	hum	2	10 µM	-10	
214510	Calcium Channel L-Type, Benzothiazepine	341851	rat	2	10 µM	-9	
214600	Calcium Channel L-Type, Dihydropyridine	341738	rat	2	10 µM	0	
216000	Calcium Channel N-Type	341852	rat	2	10 µM	6	
217030	Cannabinoid CB_1	341739	hum	2	10 µM	17	
219500	Dopamine D_1	341721	hum	2	10 µM	3	
219700	Dopamine D_{2S}	341722	hum	2	10 µM	2	
219800	Dopamine D_3	341899	hum	2	10 µM	12	
219900	Dopamine $D_{4.2}$	341689	hum	2	10 µM	-2	
224010	Endothelin ET_A	341809	hum	2	10 µM	7	
224110	Endothelin ET_B	341810	hum	2	10 µM	18	
225510	Epidermal Growth Factor (EGF)	341853	hum	2	10 µM	7	
226010	Estrogen ERα	341830	hum	2	10 µM	2	
226600	$GABA_A$, Flunitrazepam, Central	341728	rat	2	10 µM	-2	
226500	$GABA_A$, Muscimol, Central	341740	rat	2	10 µM	10	
228610	$GABA_{B1A}$	341801	hum	2	10 µM	0	
232030	Glucocorticoid	341850	hum	2	10 µM	12	
232700	Glutamate, Kainate	341835	rat	2	10 µM	-4	
232810	Glutamate, NMDA, Agonism	341834	rat	2	10 µM	-18	
232910	Glutamate, NMDA, Glycine	341814	rat	2	10 µM	9	
233000	Glutamate, NMDA, Phencyclidine	341747	rat	2	10 µM	4	
239610	Histamine H_1	341745	hum	2	10 µM	1	
239710	Histamine H_2	341854	hum	2	10 µM	-21	

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

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
239820	Histamine H_3	341855	hum	2	10 µM	-6	
241000	Imidazoline I_2, Central	341748	rat	2	10 µM	5	
243520	Interleukin IL-1	341805	mouse	2	10 µM	0	
250460	Leukotriene, Cysteinyl $CysLT_1$	341856	hum	2	10 µM	8	
251600	Melatonin MT_1	341879	hum	2	10 µM	2	
252610	Muscarinic M_1	341886	hum	2	10 µM	0	
252710	Muscarinic M_2	341723	hum	2	10 µM	0	
252810	Muscarinic M_3	341724	hum	2	10 µM	2	
257010	Neuropeptide Y Y_1	341848	hum	2	10 µM	1	
257110	Neuropeptide Y Y_2	341849	hum	2	10 µM	3	
258590	Nicotinic Acetylcholine	341725	hum	2	10 µM	-1	
258700	Nicotinic Acetylcholine α1, Bungarotoxin	341726	hum	2	10 µM	0	
260130	Opiate δ_1 (OP1, DOP)	341668	hum	2	10 µM	9	
260210	Opiate κ(OP2, KOP)	341669	hum	2	10 µM	7	
260410	Opiate μ(OP3, MOP)	341670	hum	2	10 µM	-9	
264500	Phorbol Ester	341741	mouse	2	10 µM	4	
265010	Platelet Activating Factor (PAF)	341823	hum	2	10 µM	6	
265600	Potassium Channel [K_{ATP}]	341746	ham	2	10 µM	9	
265900	Potassium Channel hERG	341744	hum	2	10 µM	7	
268420	Prostanoid EP_4	341742	hum	2	10 µM	19	
268700	Purinergic P2X	341694	rabbit	2	10 µM	-1	
268810	Purinergic P2Y	341857	rat	2	10 µM	-16	
270000	Rolipram	341733	rat	2	10 µM	10	
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	341858	hum	2	10 µM	-9	
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	341727	hum	2	10 µM	4	
271910	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_3$	341859	hum	2	10 µM	-7	
278110	Sigma σ_1	341732	hum	2	10 µM	15	
279510	Sodium Channel, Site 2	341731	rat	2	10 µM	-1	
255520	Tachykinin NK_1	341837	hum	2	10 µM	-4	
285900	Thyroid Hormone	341824	rat	2	10 µM	-16	
220320	Transporter, Dopamine (DAT)	341807	hum	2	10 µM	0	
226400	Transporter, GABA	341832	rat	2	10 µM	1	
204410	Transporter, Norepinephrine (NET)	341720	hum	2	10 µM	6	
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	341808	hum	2	10 µM	9	

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

MMP12 probe BAY-7598:

MMP Panel, Eurofins (Panlabs) Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Compound: CHH155-2017, PT #: 1211447						
114110	Peptidase, Matrix Metalloproteinase-1 (MMP-1)	408655	hum	2	10 µM	-17
114210	Peptidase, Matrix Metalloproteinase-2 (MMP-2)	408656	hum	2	10 µM	-13
114310	Peptidase, Matrix Metalloproteinase-3 (MMP-3)	408657	hum	2	10 µM	-18
114710	Peptidase, Matrix Metalloproteinase-7 (MMP-7)	408658	hum	2	10 µM	-17
114800	Peptidase, Matrix Metalloproteinase-8 (MMP-8)	408659	hum	2	10 µM	-12
114910	Peptidase, Matrix Metalloproteinase-9 (MMP-9)	408660	hum	2	10 µM	-29
115200	Peptidase, Matrix Metalloproteinase-12 (MMP-12)	408661	hum	2	10 µM	-8
115300	Peptidase, Matrix Metalloproteinase-13 (MMP-13)	408662	hum	2	10 µM	-9
115400	Peptidase, Matrix Metalloproteinase-14 (MMP-14)	408663	hum	2	10 µM	-19