Donated Chemical Probe

Dual ADAMTS7/ADAMTS12 Inhibitor – in vitro & in vivo Probe BAY-9835

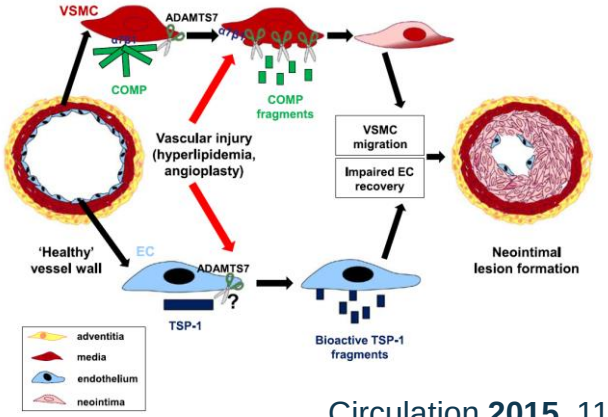
June 5th, 2024

Presenter:
Daniel Meibom
On behalf of the team

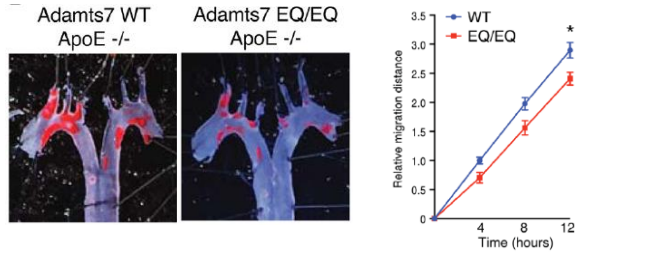
Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Rationale for inhibition of ADAMTS7 in heart and vascular diseases

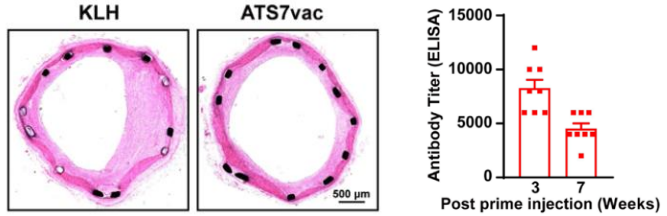
- ADAMTS7 is a secreted zinc metalloprotease with proteolytic activity against extracellular matrix proteins (e.g. COMP, TSP1, TIMP1, LTBP4, EFEMP1)
- ADAMTS7 has been identified by multiple independent genome wide association studies as an enzyme contributing to coronary artery disease development
- The catalytic function of ADAMTS7 is mediating plaque formation in CAD
- An ADAMTS7 mutant with reduced catalytic activity impairs VSMC migration in a wound healing assay
- Vaccination-induced ADAMTS7 antibodies reduced neointima formation in stented coronary arteries in swine



Circulation **2015**, 1156



Circ. Res. **2021**, 458



Circulation **2023**, 728

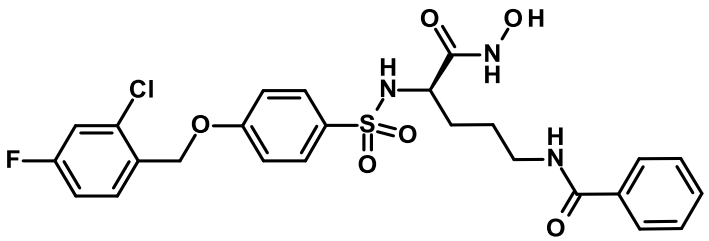
An ADAMTS7 inhibitor might lead to reduced plaque formation in coronary artery disease and might reduce restenosis after stent placement in CAD or PAD

VSMC: Vascular Smooth Muscle Cells, CAD: Coronary Artery Disease, PAD: Peripheral Arterial Disease

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Published reference compounds

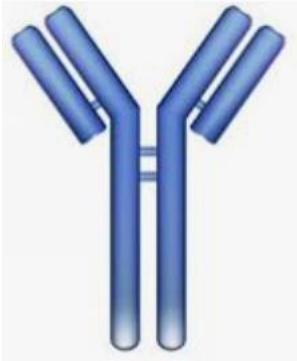
EDV33, J. Enzyme Inhib. Med. Chem. 2021 (2160-2169), Preclinical, University College London

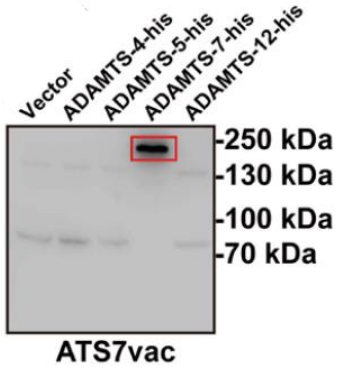


ADAMTS7-K_i	68 nM
ADAMTS4	0.5x
ADAMTS5	0.1x
MMP2	0.1x
MMP12	<0.1x
ADAM17	0.1x

x = selectivity factor

Antibody, Circulation 2023 (728-742), Preclinical, Peking University

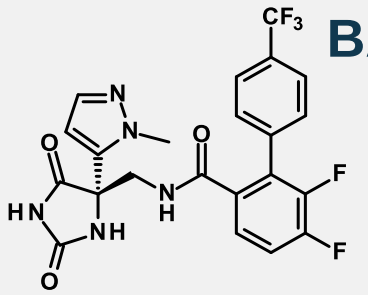




- Published small molecule is unselective while antibody requires parenteral administration
- There is currently no reasonably selective inhibitor available to investigate ADAMTS7 mediated pharmacology after oral administration

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Technical in vitro profile



BAY-9835

Structure confirmed by X-ray

Potency [nM]	
Biochem IC_{50} vs hADAMTS7	6
Biochem IC_{50} vs mADAMTS7	8
Biochem IC_{50} vs rADAMTS7	27
Biochem IC_{50} vs hADAMTS12	30
Full protease panel	next slide

Physchem	
LogD @ pH 7.5	2.3
BEI / LLE (based on hIC_{50})	19 / 5.9
Sol @ pH 7 [mg/L], cryst. material	135
MW / TPSA [g/mol / Å^2]	493 / 105
Stable as tablet blend for 13 weeks	yes

in vitro DMPK Properties						
Metabolic stability		CL [L/h/kg]		F_{max} [%]		
	mouse hepatocytes	0.32		94		
	rat hepatocytes	0.17		96		
	dog hepatocytes	0.06		97		
	human hepatocytes	10^{-4}		100%		
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	>20	>20	>20	>20 (no TDI)
CYP1A2 / 3A4 induction [µM]	> 61 / ≥ 18					

Selectivity	
In-house kinase panel (14 assays), IC_{50} [µM]	>20 µM
Panlabs @ 10 µM Protease Panel	See next slide

Safety	
Ames, Micronucleus test	negative
hERG, $hNa_V1.5$, $hCa_V1.2$ IC_{50} [µM]	>10

h = human, r = rat, m = mouse

- BAY-9835 is a potent ADAMTS7 inhibitor with activity against ADAMTS12
- BAY-9835 does not hit other targets besides ADAMTS7 and ADAMTS12 at low concentrations

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Selectivity profile in more detail (off-targets & proteases)

Assay Name	Conc.	% Inh.
Aldose Reductase	10 µM	-4
ATPase, Na^+/K^+, Heart, Pig	10 µM	-5
Carbonic Anhydrase II	10 µM	-2
Cholinesterase, Acetyl, ACES	10 µM	3
Cyclooxygenase COX-1	10 µM	-5
Cyclooxygenase COX-2	10 µM	11
HMG-CoA Reductase	10 µM	-5
Leukotriene LTC_4 Synthase	10 µM	-14
Lipoxygenase 15-LO	10 µM	-6
Monoamine Oxidase MAO-A	10 µM	0
Monoamine Oxidase MAO-B	10 µM	10
Nitric Oxide Synthase, Neuronal (nNOS)	10 µM	0
Nitric Oxide Synthetase, Inducible (iNOS)	10 µM	0
Peptidase, Angiotensin Converting Enzyme	10 µM	3
Phosphodiesterase PDE3	10 µM	11
Phosphodiesterase PDE4D2	10 µM	3
Phosphodiesterase PDE5	10 µM	-5
Thromboxane Synthase	10 µM	11
Adenosine A_1	10 µM	-4
Adenosine A_{2A}	10 µM	3
Adenosine A_3	10 µM	0
Adrenergic α_{1A}	10 µM	-14
Adrenergic α_{2A}	10 µM	-3
Adrenergic α_{2B}	10 µM	-3
Adrenergic α_{2C}	10 µM	-1
Adrenergic β_1	10 µM	-8
Adrenergic β_2	10 µM	-9
Adrenergic β_3	10 µM	-3
Androgen (Testosterone)	10 µM	3

Assay Name	Conc.	% Inh.
Angiotensin AT_1	10 µM	-1
Angiotensin AT_2	10 µM	0
Bradykinin B_1	10 µM	1
Bradykinin B_2	10 µM	-6
Cannabinoid CB_1	10 µM	-3
Cannabinoid CB_2	10 µM	-1
Dopamine D_1	10 µM	-1
Dopamine D_{2L}	10 µM	2
Dopamine D_{2S}	10 µM	0
Dopamine D_3	10 µM	2
Endothelin ET_A	10 µM	2
Endothelin ET_B	10 µM	-17
Estrogen ERα	10 µM	13
$GABA_A$, Chloride Channel, TBOB	10 µM	0
$GABA_A$, Flunitrazepam, Central	10 µM	-6
$GABA_B$, Non-Selective	10 µM	3
Glucocorticoid	10 µM	3
Glutamate, AMPA	10 µM	-11
Glutamate, Kainate	10 µM	12
Glutamate, NMDA, Agonism	10 µM	1
Glutamate, NMDA, Glycine	10 µM	-4
Growth Hormone Secretagogue (GHS, Ghrelin)	10 µM	6
Histamine H_1	10 µM	-1
Histamine H_2	10 µM	16
Histamine H_3	10 µM	6
Insulin	10 µM	-8
Motilin	10 µM	3
Muscarinic M_1	10 µM	-6

Assay Name	Conc.	% Inh.
Muscarinic M_2	10 µM	1
Muscarinic M_3	10 µM	9
Muscarinic M_4	10 µM	-6
Nicotinic Acetylcholine α3β4	10 µM	2
Opiate δ_1 (OP1, DOP)	10 µM	18
Opiate κ (OP2, KOP)	10 µM	-4
Opiate µ (OP3, MOP)	10 µM	2
Progesterone PR-B	10 µM	-13
Purinergic P2X	10 µM	0
Purinergic P2Y	10 µM	-4
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	10 µM	-3
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	10 µM	-7
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	10 µM	-1
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	10 µM	-1
Transporter, Adenosine	10 µM	11
Transporter, Dopamine (DAT)	10 µM	7
Transporter, GABA	10 µM	7
Transporter, Norepinephrine (NET)	10 µM	6
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	10 µM	10
Vasopressin V_{1A}	10 µM	7

Protease (biochem)	IC_{50} [nM]	Selectivity factor vs hADAMTS7 IC_{50}
hADAMTS4	6726	1121x
hADAMTS5	9924	1654x
hADAMTS12	30	5x
hADAM8	2250	375x
hADAM10	32802	5467x
hADAM17	5772	962x
hMMP2	>100000	>17300x
hMMP12	5376	896x
hMMP14	>100000	>16667x
hMMP15	78474	13079x
Calpain 1	>100000	>16667x
Caspase 3	>100000	>16667x
Cathepsin B	>100000	>16667x
Cathepsin S	>100000	>16667x
MALT1	>100000	>16667x

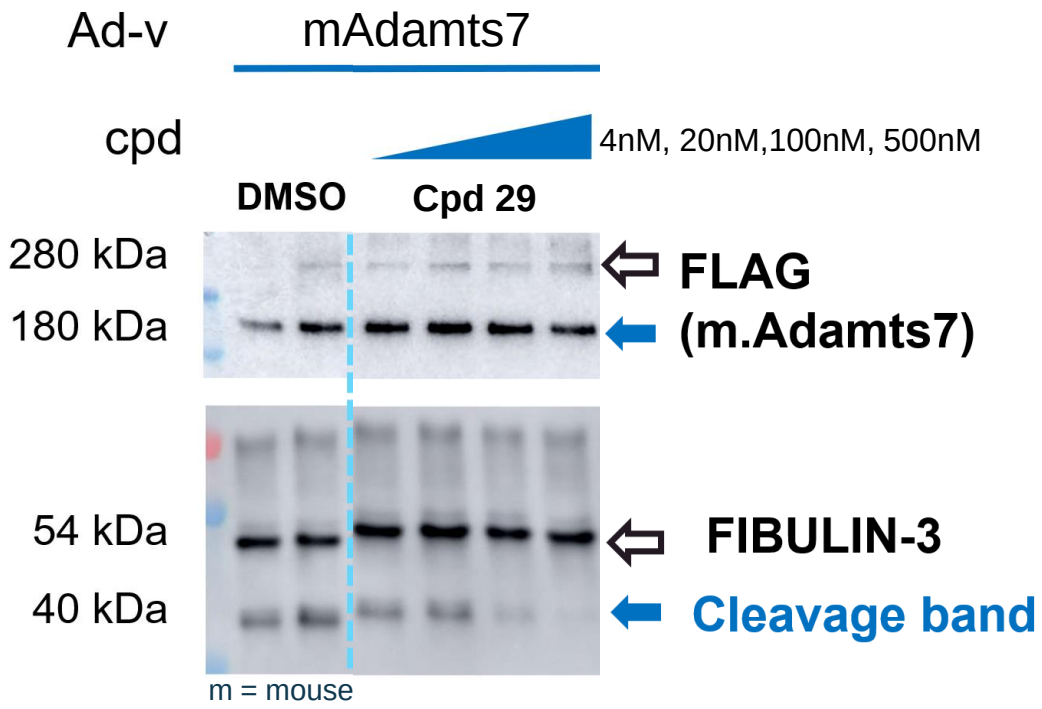
h = human

BAY-9835 shows good selectivity vs 77 off-targets
Meets criteria >30x in target family, except for ADAMTS12

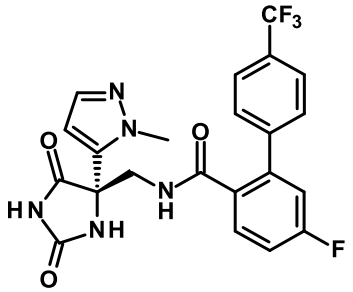
Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Cell-based potency of close analogue Cpd 29 (J. Med. Chem. **2024**, 2907)

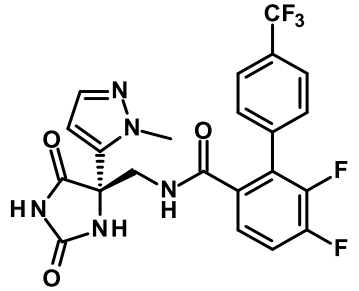
- Fibulin-3 was identified as endogenous ADAMTS7 substrate by TAILS proteomics (Mol. Cell. Proteomics, **2022**, 21(4),100223).
- Fibulin-3 is dose-dependently cleaved by Adamts7 expressed by an adenoviral vector in HUVEC cells. Cleavage is detected by western blot from concentrated conditioned media (*data not presented*).
- **Cleavage of Fibulin-3 from concentrated conditioned media can be dose-dependently inhibited by Cpd 29** (*see below*).



m = mouse



Cpd 29
hADAMTS7-IC_{50} 19 nM
rADAMTS7-IC_{50} 85 nM



BAY-9835
hADAMTS7-IC_{50} 6 nM
rADAMTS7-IC_{50} 27 nM

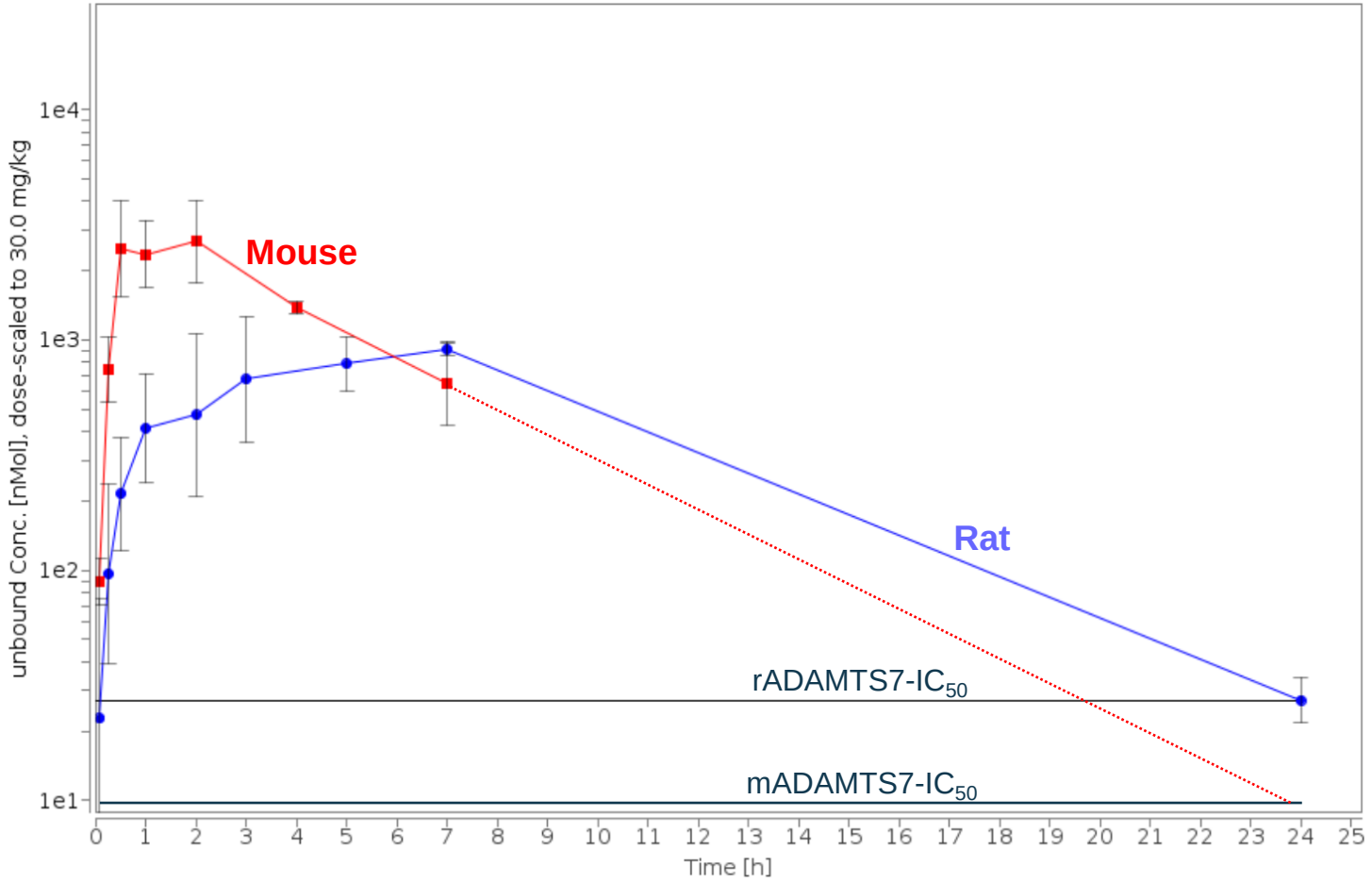
Cpd 29 significantly inhibits mADAMTS7 at 100 nM in Fibulin-3 cellular cleavage assay
A concentration 100 nM of BAY-9835 is advised for cell-based assays

FLAG = protein tag

Data generated and provided by Broad Institute / Alessandro Arduini

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

In vivo po PK data (scaled from 1 mg/kg to 30 mg/kg)



Mouse PK data:

$AUC_{n,\ po}$	2.0 l/h/kg
F	complete
MRT_{po}	4.2 h
f_u	11%

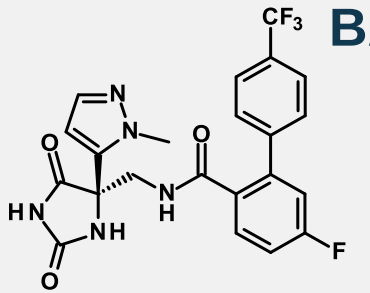
Rat PK data:

$AUC_{n,\ po}$	3.0 l/h/kg
F	96%
MRT_{po}	8.0 h
f_u	5%

An oral dose of 30 mg/kg BAY-9835 once daily to mice or rats might cover respective IC_{50}'s for 24 h

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Technical in vitro profile of negative control



BAY-1880*

Potency [nM]	
Biochem IC_{50} vs hADAMTS7	23400
Biochem IC_{50} vs mADAMTS7	n.d.
Biochem IC_{50} vs hADAMTS4	>50000
Biochem IC_{50} vs hADAMTS12	n.d.
Full protease panel	n.d.

Physchem	
LogD @ pH 7.5	2.2
BEI / LLE (based on hIC_{50})	11 / 2.4
Sol @ pH 7 [mg/L], amorphous	>500
MW / TPSA [g/mol / Å^2]	475 / 105
Stable in solution (pH1/7/10, 24 h)	yes

in vitro DMPK Properties						
Metabolic stability		CL [L/h/kg]		F_{max} [%]		
	mouse hepatocytes	10^{-4}		100		
	rat hepatocytes	0.03		99		
	dog hepatocytes	n.d.		n.d.		
	human hepatocytes	10^{-4}		100		
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	>20	>20	>20	>20
CYP1A2 / 3A4 induction [µM]	> 63 / ≥ 3.7					

Selectivity	
In-house kinase panel (14 assays), IC_{50} [µM]	>20 µM
Panlabs @ 10 µM Protease Panel	n.d.

Safety	
Ames, Micronucleus test	n.d.
hERG, $hNa_V1.5$, $hCa_V1.2$ IC_{50} [µM]	>10

n.d. = not determined
h = human, r = rat, m = mouse

Negative control BAY-1880 is >3500x less active on human ADAMTS7 than the probe BAY-9835

* Distomer of Cpd 29 from J. Med. Chem. **2024**, 2907

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Summary / Conclusion

Probe criteria	
Inhibitor potency: goal is < 100 nM (IC_{50})	**Meets criteria** hADAMTS7-IC_{50} in biochemical assay: 6 nM
Selectivity within target family: goal is > 30-fold	**Meets criteria for majority of tested metalloproteases** Selectivity factors for 9 metalloproteases >374x Selectivity factor for hADAMTS12 is low: 5x
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Meets criteria** Clean in a panel of 77 off-targets at 10 µM (Panlabs) and 14 kinases at 20 µM Top hits from inhouse 3NN target prediction[#] experimentally devalidated
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Not applicable** Secreted target
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**Meets criteria** Cell-based mADAMTS7 inhibition of close congener Cpd 29[*] at 100 nM Recommended concentration for use in cellular assays: 100 nM Recommended concentration for use in biochemical assays: 10-50 nM
Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Meets criteria** Negative control >3500 times less active than probe (based on biochemical assay)

We ask for acceptance of ADAMTS7 inhibitor BAY-9835 as chemical probe, accompanied by BAY-1880 as negative control

[#] nearest neighbour search in internal and external bioactivity databases

[*] J. Med. Chem. **2024**, 2907

h = human

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Project team / Acknowledgement

Journal of Medicinal Chemistry

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Article

BAY-9835: Discovery of the First Orally Bioavailable ADAMTS7 Inhibitor

Daniel Meibom,* Pierre Wasnaire, Kristin Beyer, Andreas Broehl, Yolanda Cancho-Grande, Nadine Elowe, Kerstin Henninger, Sarah Johannes, Natalia Jungmann, Tanja Krainz, Niels Lindner, Stefanie Maassen, Bryan MacDonald, Denis Menshykau, Joachim Mittendorf, Guzman Sanchez, Martina Schaefer, Eric Stefan, Afra Torge, Yi Xing, and Dmitry Zubov

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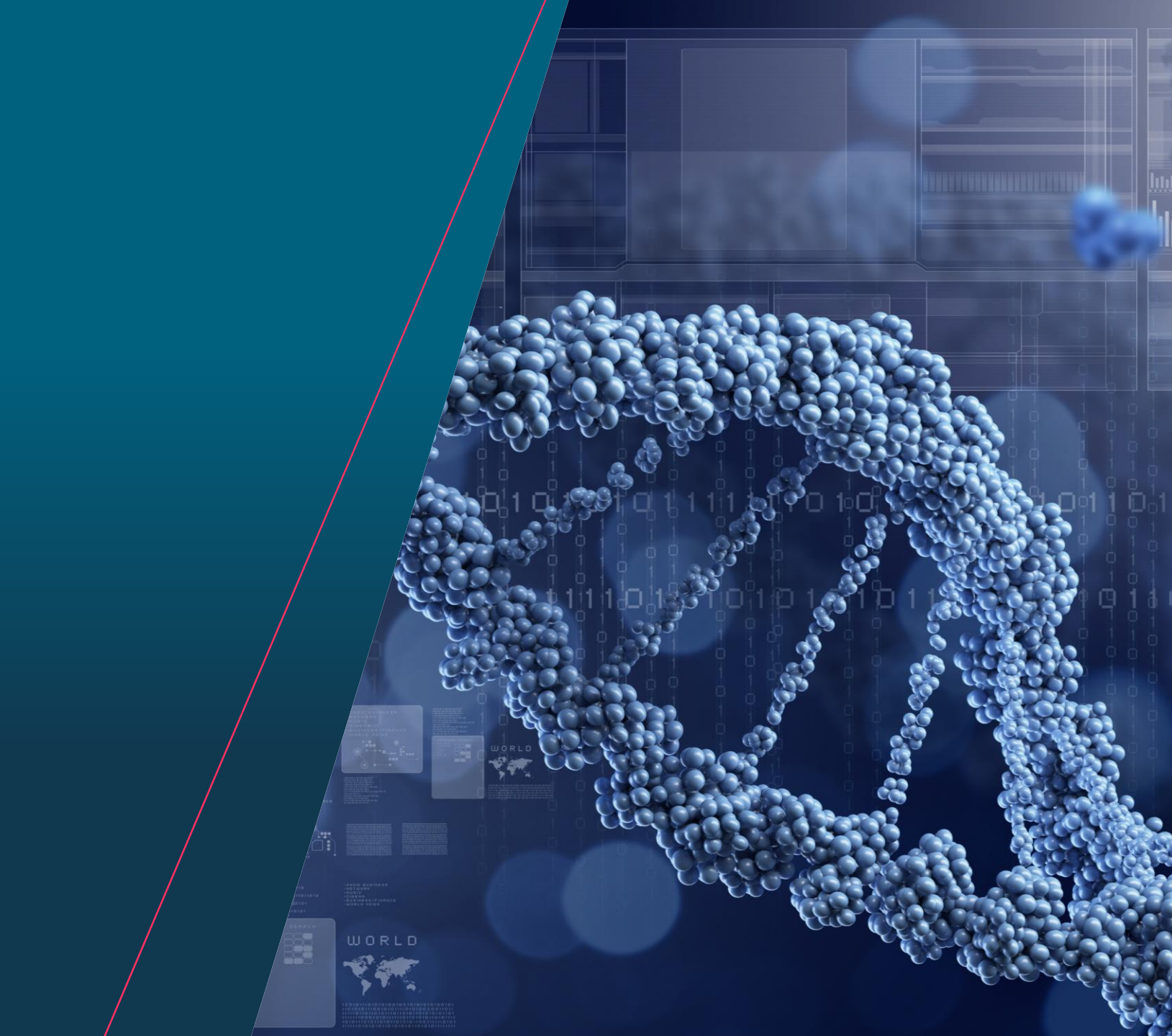
https://doi.org/10.1021/acs.jmedchem.3c02036

Thank You



Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Assays in more detail

Metalloprotease panel
IC_{50} measurements vs ADAMTS4, ADAMTS5, ADAMTS7 with human catalytic domain, mouse ADAMTS7, rat ADAMTS7, ADAMTS12, ADAM17, MMP2, MMP12 and MMP15 were performed as described in WO2021/094434 and WO2021/094436.

Assay for ADAMTS-7 enzymatic activity and testing of inhibitory compounds

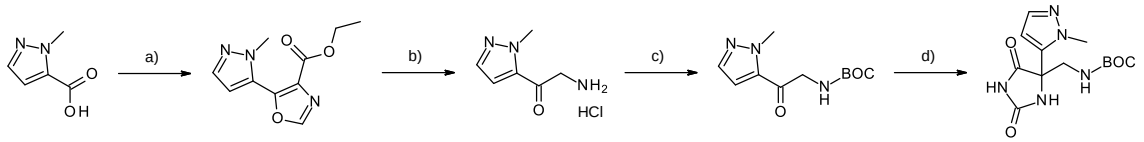
Purified recombinant ADAMTS-7 was diluted in reaction buffer (20 mM HEPES pH 8.0, 150 mM NaCl, 5 mM $CaCl_2$, 0,004 % Brij, 10 µM $ZnCl_2$) for a concentration of approximately 20 nM. 25 µl of the solution were transferred into each well of a 384-well white microtiter plate (Greiner Bio-One 781075) and 1 µl test compound solution (modulator/inhibitor dissolved in DMSO, at the corresponding concentration) or pure DMSO as a control were added per well. The enzymatic reaction was initiated by addition of 25 µl of a 1 µM solution of the FRET substrate, HiLyteFluor-488 DELSSMVLELRGLRT-K(QXL520)-E-NH2; (custom synthesis by Anaspec) in the reaction buffer. Amino acids DELSSMVLELRGLRT are derived from Thrombospondin-1 sequence (275-289). An additional carboxyl glutamic acid was added after the QXL520 quencher to increase substrate solubility. The microtiter plate was incubated for 120 min at the temperature of 32°C. The increase of fluorescence intensity was measured in appropriate fluorescence plate reader (e.g. TECAN Ultra) using excitation wavelength of 485 nm and emission wavelength of 520 nm. IC_{50} values were calculated from percentage of inhibition of ADAMTS-7 activity as a function of test compound concentration.

MMP14, ADAM8 and ADAM10 enzymatic assays:
Recombinant MMP14 (R&D Systems # 918-MP-010) was diluted in the reaction buffer (50 mM TRIS pH 7,5; 150 mM NaCl; 10 mM $CaCl_2$; 0,05% Brij; 0,1% SmartBlock (Candor Bioscience #113125)) to a concentration of approximately 2 nM and 25 µl was transferred into each single well of a 384-well white microtiter plate (Greiner Bio One 781075). 1 µl of the inhibitor compound solution (dissolved in DMSO, at the corresponding concentration) or pure DMSO as a control was added to the same wells. The enzymatic reaction was initiated by addition of 25 µl of a 20 µM solution of the FRET substrate Mca-KPLGL-Dpa-AR-NH_2 (R&D Systems # ES010) to the reaction buffer. The microtiter plate was incubated for 60 min at a temperature of 30°C. The increase of fluorescence intensity was measured in an appropriate fluorescence plate reader (e.g. TECAN Ultra) using excitation wavelength of 320 nm and emission wavelength of 420 nm. IC_{50} values were calculated from percentage of inhibition of MMP14 activity as a function of test compound concentration.

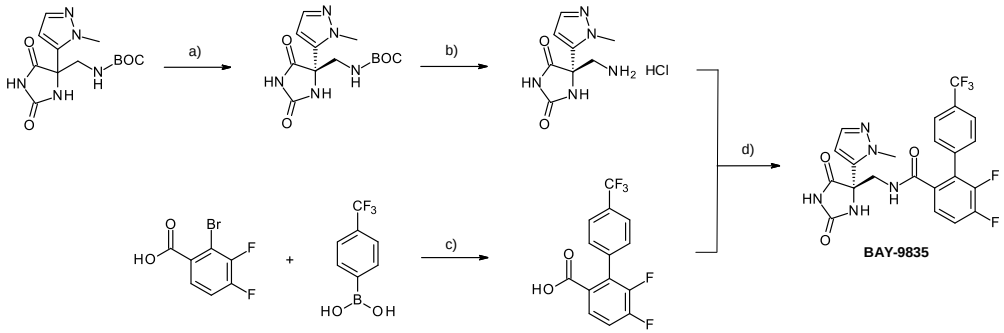
Assays for ADAM8 and ADAM10 were performed using the same scheme as for MMP14 with the following exceptions: Recombinant ADAM10 (R&D Systems # 936-AD-020) was diluted to 8 nM in the ADAM10 reaction buffer (25 mM TRIS pH 9,0; 2 mM $ZnCl_2$; 0,01% Brij; 0,01% BSA) and substrate (R&D Systems # ES010) was diluted to 20 µM in the ADAM10 reaction buffer. Thermolysin activated ADAM8 (R&D Systems # 1031-AD-020, as described by the manufacturer) was diluted to 100 nM in the ADAM8 reaction buffer (50mM TRIS pH 7.5, 150mM $CaCl_2$, 150mM NaCl, 0,01% Brij) and FRET substrate Dabcyl-SSNQLQRR-Glu(EDANS)-NH2 (Custom synthesis by JPT, Berlin) was diluted to 100 µM in the ADAM8 reaction buffer. Enzymatic reaction was incubated for 180 min at 30°C. The increase of fluorescence intensity was measured using excitation wavelength of 338 nm and emission wavelength of 510 nm.

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Synthesis of BAY-9835



Reagents and conditions: a) CDI, ethyl isocyanoacetate, LiHMDS, THF, 0 °C -> r.t., 4 h, 69%; b) 6N HCl aq., 100 °C, 2 h, 86%; c) $(BOC)_2O$, TEA, DCM, r.t., 1.5 h, 88%; d) KCN, $(NH4)_2CO_3$, MeOH, 80 °C, 2 d, 81%. aq. = aqueous, $(BOC)_2O$ = Di(tert-butyl)carbonate, CDI = carbonyl diimidazole, d = days, DCM = dichloromethane, h = hour(s), LiHMDS = Lithium bis(trimethylsilyl)amide, MeOH = methanol, N = normal, r.t. = room temperature, TEA = triethylamine, THF = tetrahydrofuran.

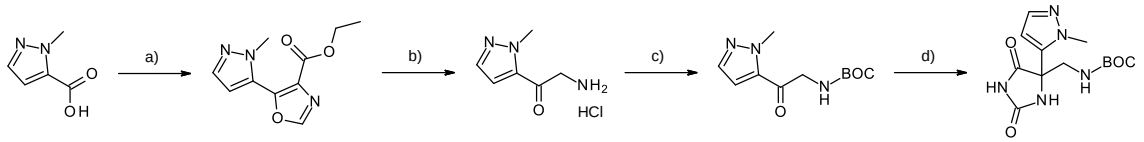


Reagents and conditions: a) chiral separation, CO_2, MeOH, 40 °C, 99.5% ee, 38%; b) HCl in dioxane 1 M, DCM, r.t., 3 h, quant.; c) Dichlorobis(triphenylphosphin)palladium (II), XPhos, K_3PO_4, dioxane, H_2O, 80 °C, 3 h, 84%; d) T3P, DIPEA, ACN, r.t., overnight, 45%. ACN = acetonitrile, DCM = dichloromethane, DIPEA = diisopropylethylamine, ee = enantiomeric excess, MeOH = methanol, quant. = quantitative conversion, r.t. = room temperature, T3P = Propanephosphonic acid anhydride, XPhos = Dicyclohexyl[2',4',6'-tris(propan-2-yl)[1,1'-biphenyl]-2-yl]phosphane.

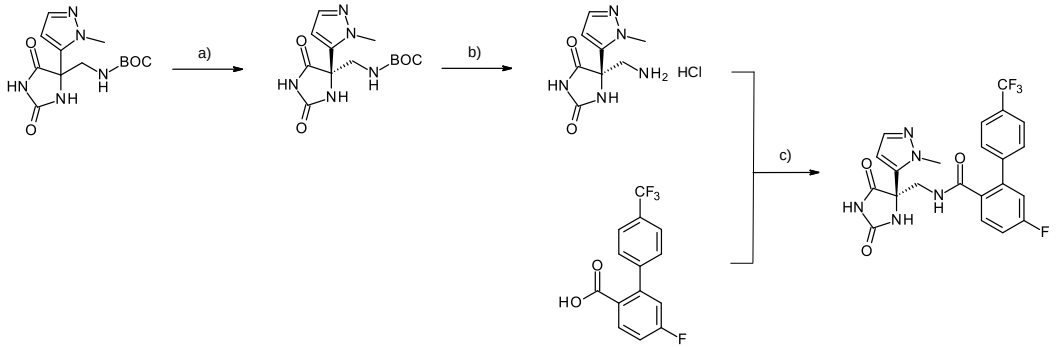
BAY-9835 was synthesized in a convergent sequence of overall 8 steps

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

Synthesis of negative control BAY-1880*



Reagents and conditions: a) CDI, ethyl isocyanoacetate, LiHMDS, THF, 0 °C -> r.t., 4 h, 69%; b) 6N HCl aq., 100 °C, 2 h, 86%; c) $(BOC)_2O$, TEA, DCM, r.t., 1.5 h, 88%; d) KCN, $(NH4)_2CO_3$, MeOH, 80 °C, 2 d, 81%. aq. = aqueous, $(BOC)_2O$ = Di(tert-butyl)carbonate, CDI = carbonyl diimidazole, d = days, DCM = dichloromethane, h = hour(s), LiHMDS = Lithium bis(trimethylsilyl)amide, MeOH = methanol, N = normal, r.t. = room temperature, TEA = triethylamine, THF = tetrahydrofuran.



Reagents and conditions: a) chiral separation, CO_2, MeOH, 40 °C, 99.9% ee, 34%; b) HCl in dioxane 1 M, DCM, r.t., 3 h, quant.; c) EDC*HCl, HOBt*H_2O, DIPEA, DMF, r.t., overnight, 33%. DCM = dichloromethane, DIPEA = diisopropylethylamine, DMF = N,N-Dimethylformamide, EDC*HCl = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, ee = enantiomeric excess, HOBt*H2O = 1-hydroxybenzotriazole hydrate, MeOH = methanol, quant. = quantitative conversion, r.t. = room temperature.

BAY-1880 was synthesized in a convergent sequence of overall 7 steps

* Distomer of Cpd 29 from J. Med. Chem. **2024**, 2907

Dual ADAMTS7/ADAMTS12 Inhibitor Probe BAY-9835

SMOL X-ray BAY-9835

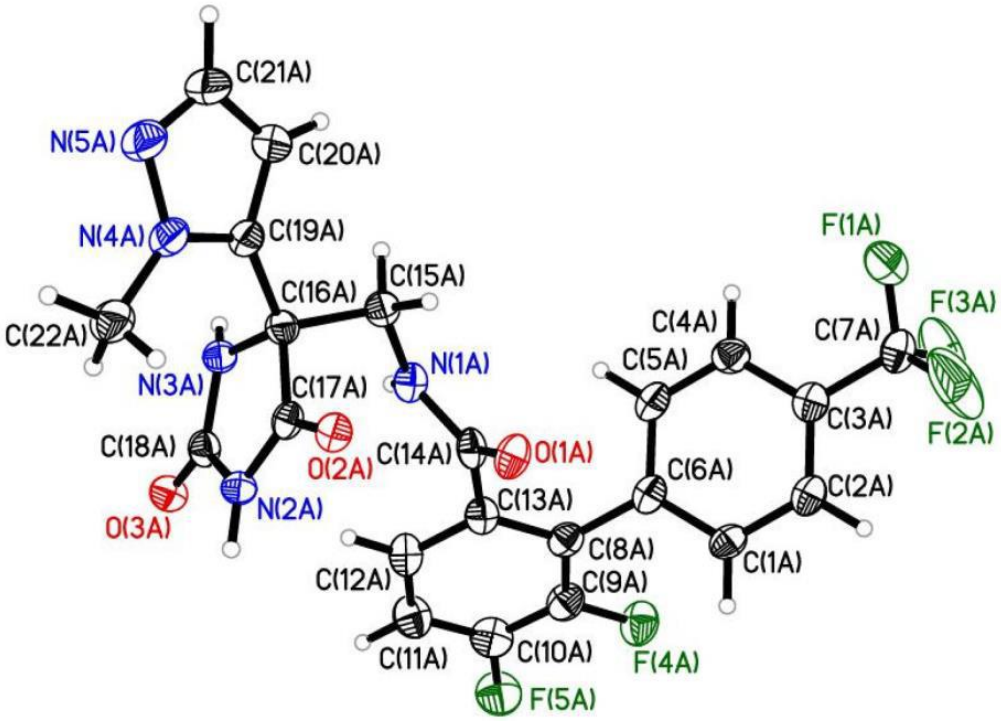


Table S4. Crystal data and structure refinement

Temperature	110 K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 9.6124(10) Å	a= 90°.
	b = 16.8528(19) Å	b= 105.405(6)°.
	c = 14.5638(14) Å	g = 90°.
Volume	2274.5(4) Å3	
Z	2	
Density (calculated)	1.508 Mg/m3	
Absorption coefficient	1.143 mm-1	
F(000)	1060	
Crystal size	0.14 x 0.04 x 0.03 mm3	
Theta range for data collection	5.249 to 65.308°.	
Index ranges	-11 ≤ h ≤ 10, -19 ≤ k ≤ 19, -17 ≤ l ≤ 17	
Reflections collected	19075	
Independent reflections	7427 [R(int) = 0.0281]	
Completeness to theta = 65.308°	98.2 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.94 and 0.85	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	7427 / 1 / 670	
Goodness-of-fit on F2	1.068	
Final R indices	[I>2sigma(I)] R1 = 0.0408, wR2 = 0.1110	
R indices (all data)	R1 = 0.0509, wR2 = 0.1209	
Absolute structure parameter	0.05(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.688 and -0.319 e.Å-3	

Stereocenter in BAY-9835 is (*S*)-configured