Science For A Better Life

HNE chemical probe

BAY-678

F. von Nussbaum, V. Li

August 6th 2015

Human Neutrophil Elastase, HNE (EC 3.4.21.37)

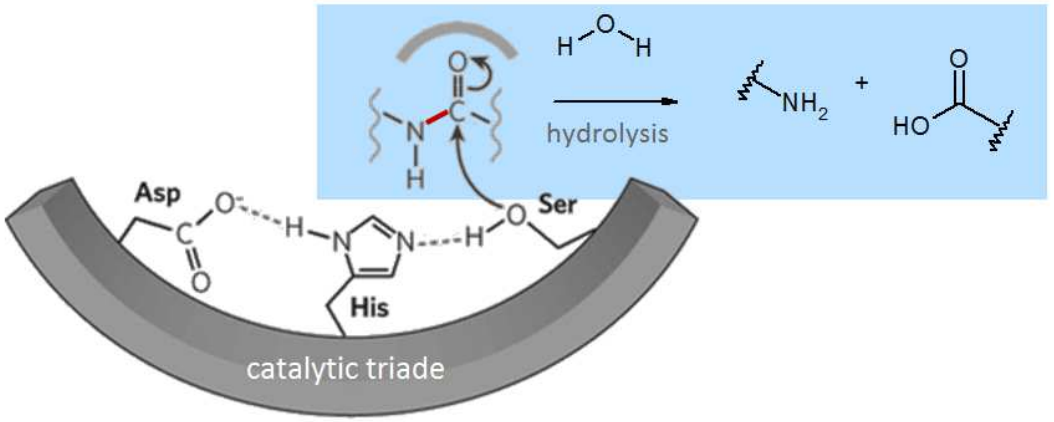
A Key Player in Inflammation (1/3)

Structure

- Ser protease (His-Asp-Ser)
- chymotrypsin family

Function

- broad substrate specificity
- Val-|-Xaa & Ala-|-Xaa



Classical Ser protease

Human Neutrophil Elastase, HNE (EC 3.4.21.37)

A Key Player in Inflammation (2/3)

Structure

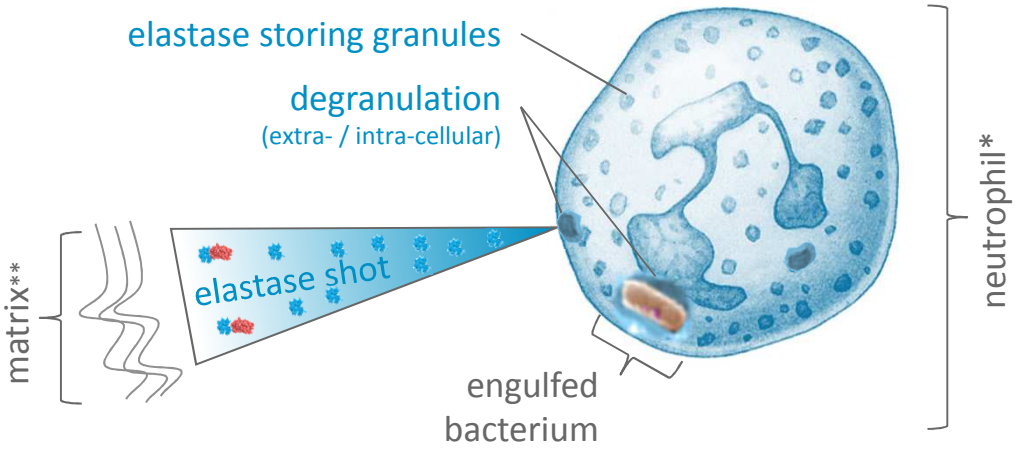
- Ser protease (His-Asp-Ser)
- chymotrypsin family

Function

- broad substrate specificity
- Val-|-Xaa & Ala-|-Xaa

Pharmacology

- inflammation (ECM**) & signaling
- host defense (bacteria)



Highly active enzyme …

*adapted from http://ckcsphysiology.wikispaces.com/

**extracellular matrix

Human Neutrophil Elastase, HNE (EC 3.4.21.37)

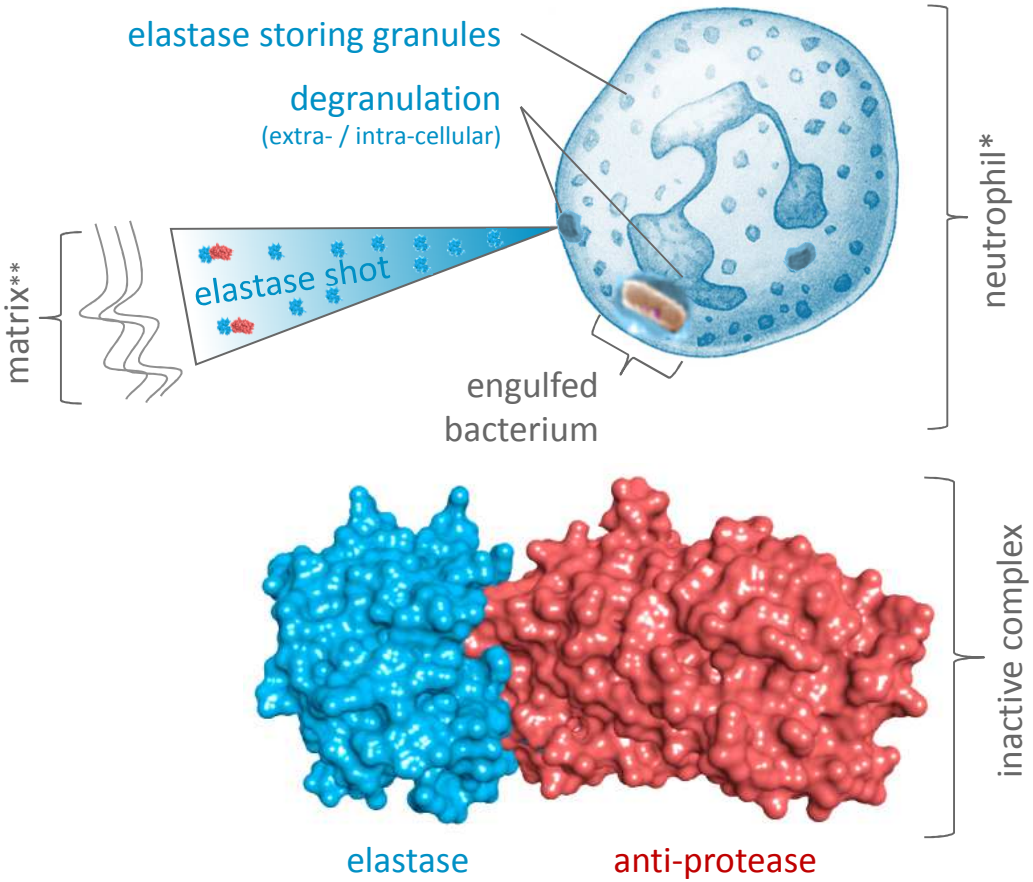
A Key Player in Inflammation (3/3)

Structure
- Ser protease (His-Asp-Ser)
- chymotrypsin family

Function
- broad substrate specificity
- Val-|-Xaa & Ala-|-Xaa

Pharmacology
- inflammation (ECM**) & signaling
- host defense (bacteria)

Regulation
- by compartimentation
- by anti-proteases (α1PI***)

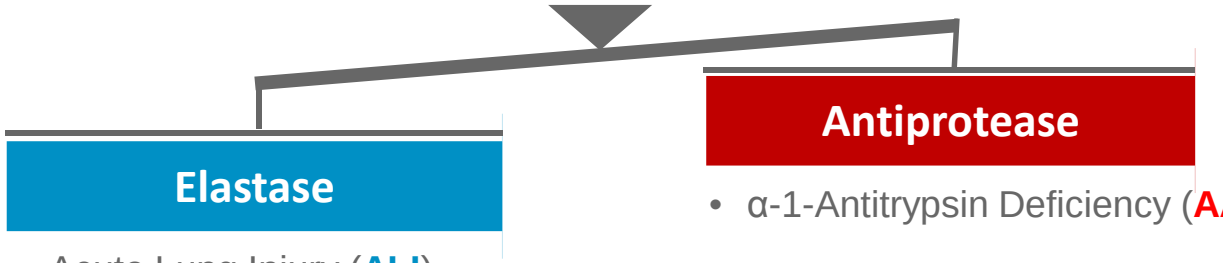


Highly active enzyme that has to be regulated strictly

*adapted from http://ckcsphysiology.wikispaces.com/
**extracellular matrix ***α-1-proteinase inhibitor

Elastase in Inflammation and Autoimmunity

The Elastase – Anti-Protease Balance is Disturbed



- α-1-Antitrypsin Deficiency (**AATD**)

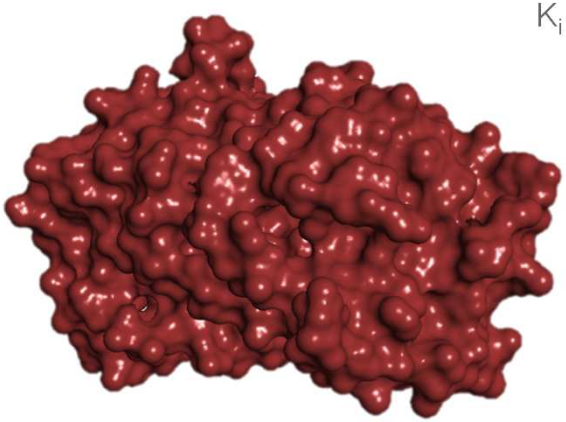
- Acute Lung Injury (**ALI**)
- Acute Respiratory Distress Syndrome (**ARDS**)
- Bronchiectasis (**BE**)
- Cystic Fibrosis (**CF**)
- Chronic Obstructive Pulmonary Disease (**COPD**)
- Non Small-Cell Lung Cancer (**NSCLC**)
- Pulmonary Hypertension (**PH**)
- Rheumatoid Arthritis (**RA**)
- Inflammatory Bowel Disease (**IBD**)
- Acute Myocardial Infarct (**AMI**)
- Graft versus Host Disease (**GvHD**)
- Systemic Inflammatory Response Syndrome (**SIRS**)
- Wound Healing disorders
- …

Exciting target for ULTRA-DD collaboration

History of HNE Inhibitors: Biologicals

Selection of Inhibitors

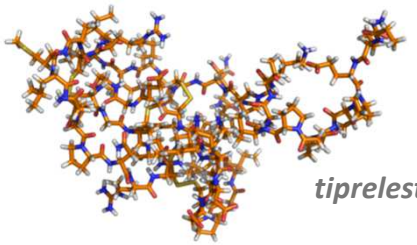
K_i < 0.1 nM
i.v.



Human alpha-1 protease inhibitor (AATD)

Prolastin®	**Zemaira®**	**Aralast®**
Talecris, 1987	CSL Behring, 2003	Baxter, 2003

K_i 0.08 nM
i.v.



Rec. human elafin (Ph 2 postoperative inflam. complication)

Elafin
Proteo Biotec, ongoing

➔ *Loss of activity under oxidative stress conditions*

➔ *Alpha-1 protease inhibitor: no inhibition of membrane bound HNE*

History of HNE Inhibitors: First SMOLs*

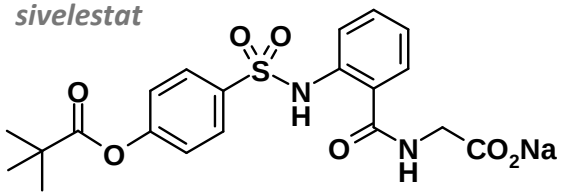
Suicide Inhibitors (a Selection of Inhibitors)

Mode-of-action: mechanism-based (covalent / reactive inhibitors)

multiple pharmacophores of electrophilic ketons, acylators, and transition state analogs

K_i 200 nM
i.v.

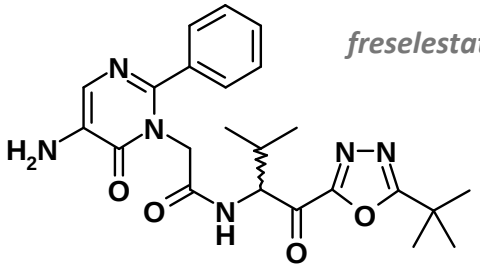
sivelestat



Elaspol® (ALI/SIRS, only Japan & South Korea)
Ono, 2002 & 2006

K_i 12 nM
p.o.

freselestat



ONO 6818 (Ph1/2 COPD)
Ono, discontinued 2002

➔ ***Metabolic liabilities***

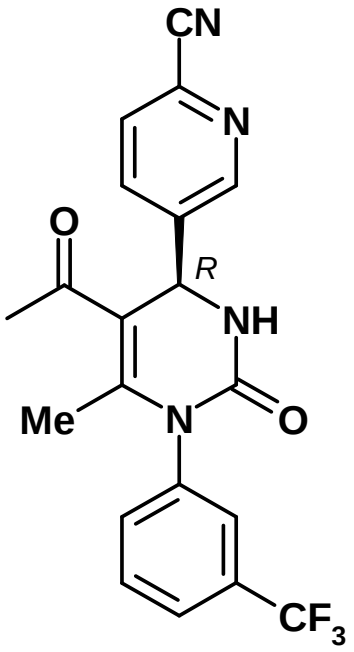
➔ ***Prone to adverse effects***

* small molecules

History of HNE Inhibitors: recent SMOLs

Reversible, Non-reactive Inhibitors (e.g. BAY-678)

Chemical Probe
BAY-678

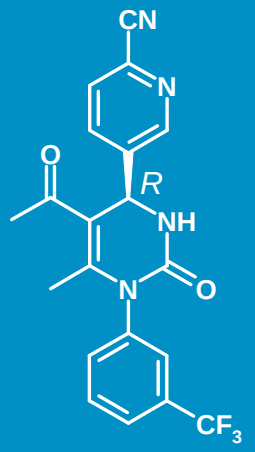


- ***Specific & selective***
- ***Oral bioavailable***
- ***Well tolerated & safe***

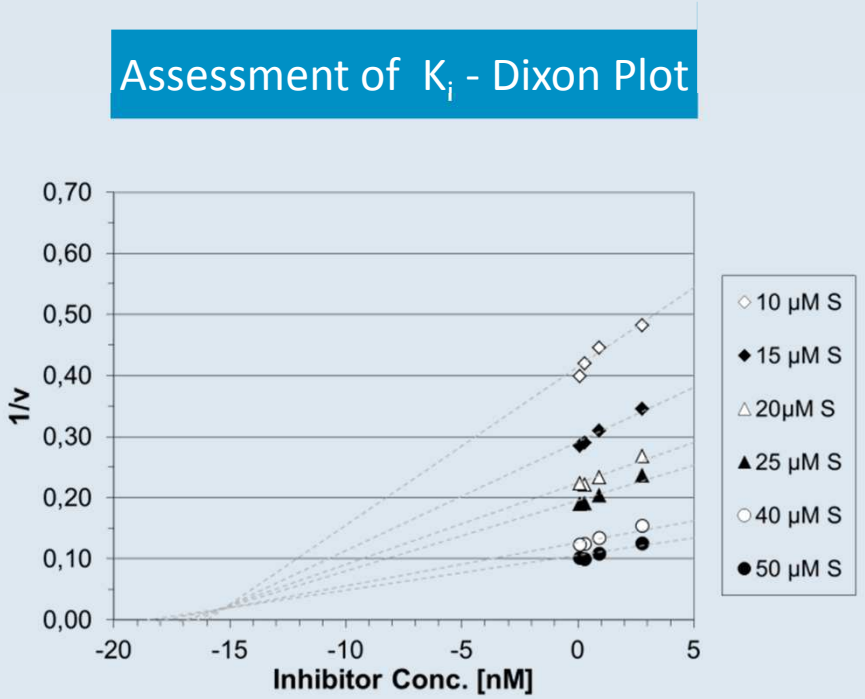
Characterization of Chemical Probe BAY-678

Potency

see also von Nussbaum F, Li V *et al. ChemMedChem* **2015**



	BAY-678
IC_{50} humanNE	20 nM (*R*) 60 nM (rac.); > 2,000 nM (*S*)
K_i humanNE	15 nM



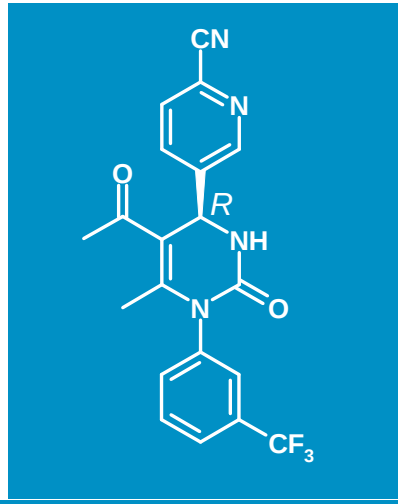
High potency for a non-reactive, reversible protease inhibitor

(S)-enantiomer of BAY-678 is inactive (negative control)

Characterization of Chemical Probe BAY-678

Selectivity

see also von Nussbaum F, Li V *et al. ChemMedChem* **2015**



	BAY-678
K_i humanNE (HNE)	15 nM
K_i ratNE (RNE)	600 nM
K_i murineNE (MNE)	700 nM

Serine Protease	IC_{50} [µM]	Serine Protease	IC_{50} [µM]
Pancreas elastase	> 30	Kallikrein-B1	> 30
Cathepsin G	> 30	Kallikrein-1	> 30
Chymotrypsin	> 30	Kallikrein-4	> 30
Trypsin	> 30	Kallikrein-5	> 30
Chymase	> 30	Kallikrein-7	> 30
DPPII	> 30	Kallikrein-12	> 30
DPPIV	> 30	FAP	> 30
Urokinase	> 30	FVIIa	> 30
Thrombin	> 30	FIXa	> 30
FXa	> 30	FXIa	> 30
Plasmin	> 30		

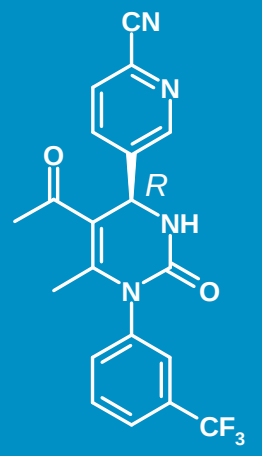
Very high selectivity (>2,000 fold) versus similar serine proteases

Lower potency versus rodent neutrophil elastases

Characterization of Chemical Probe BAY-678

Specificity (1/2) [See also Backup Slides]

see also von Nussbaum F, Li V *et al. ChemMedChem* **2015**

	BAY-678
	
K_i humanNE	15 nM

LeadProfilingScreen
@ MDS Pharma Services, Taiwan

Radioligand Binding Assays with 10 µM BAY-678:

Panel of **64 pharmacological relevant targets**

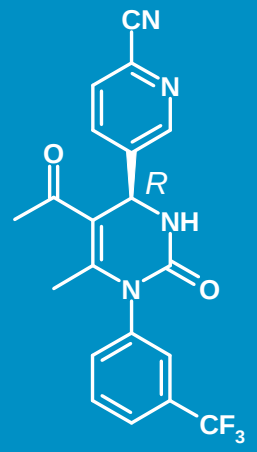
(e.g. receptors & transporter)

PASSED

No significant effects against numerous pharmaceutical relevant targets

Characterization of Chemical Probe BAY-678

Specificity (2/2)



	BAY-678
K_i humanNE	15 nM

Kinase Panel (in house)

Functional Kinase Activity with 20 µM* BAY-678:

Initial panel of **7 kinases**

(incl. 4 tyrosine & 3 serine/threonine kinases)

PASSED

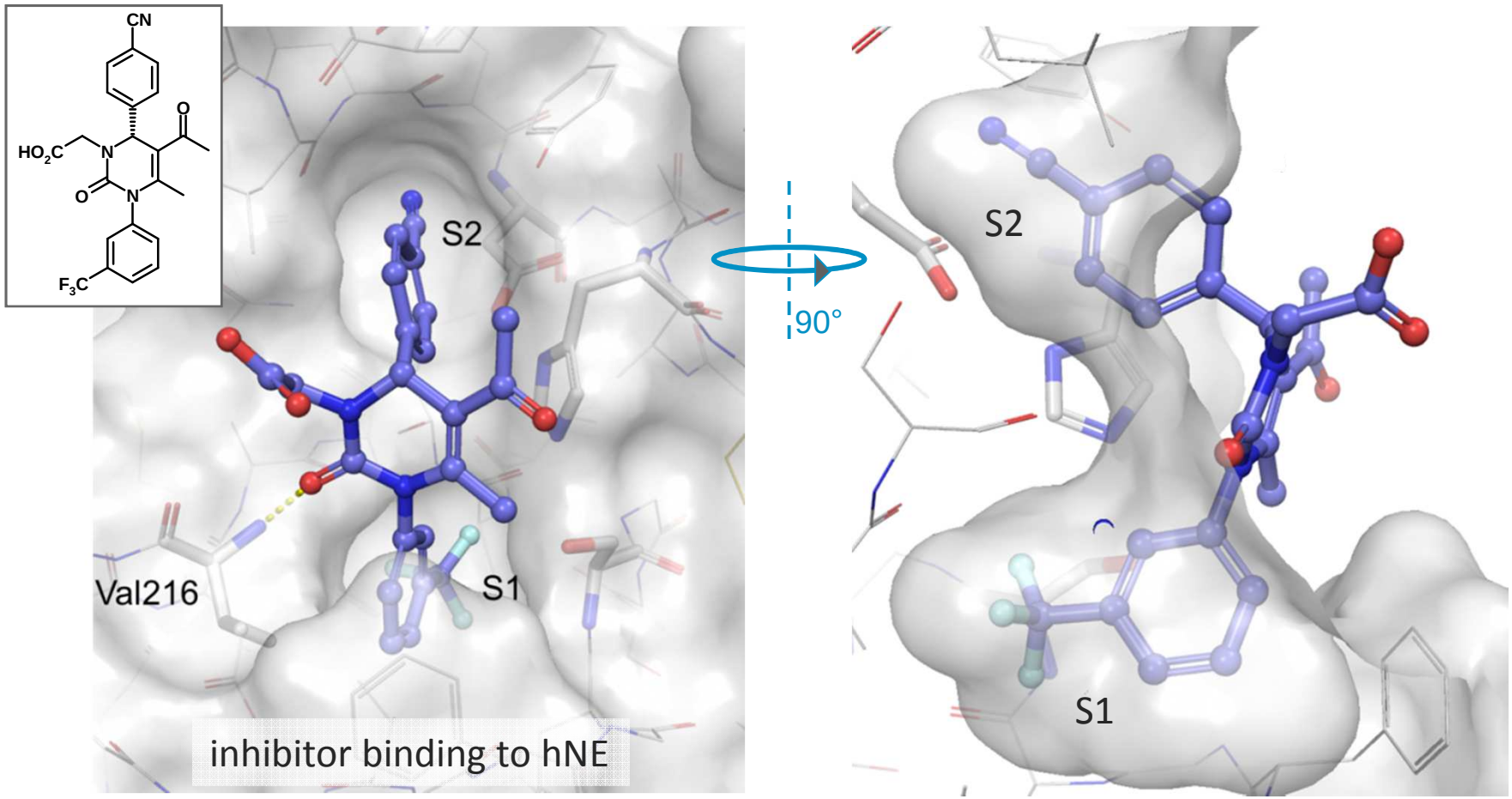
* dose response, highest concentration used: 20 µM

No significant effects against prominent kinase target class

Investigating the Binding Mode (X-Ray)

HNE Complex with Close Congener of BAY-678

see also Hansen *et al. J. Mol. Biol.* **2011**

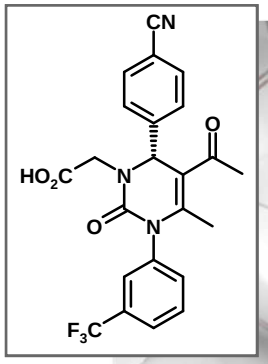


Elastase is clamped by combined S1/S2 pocket dive

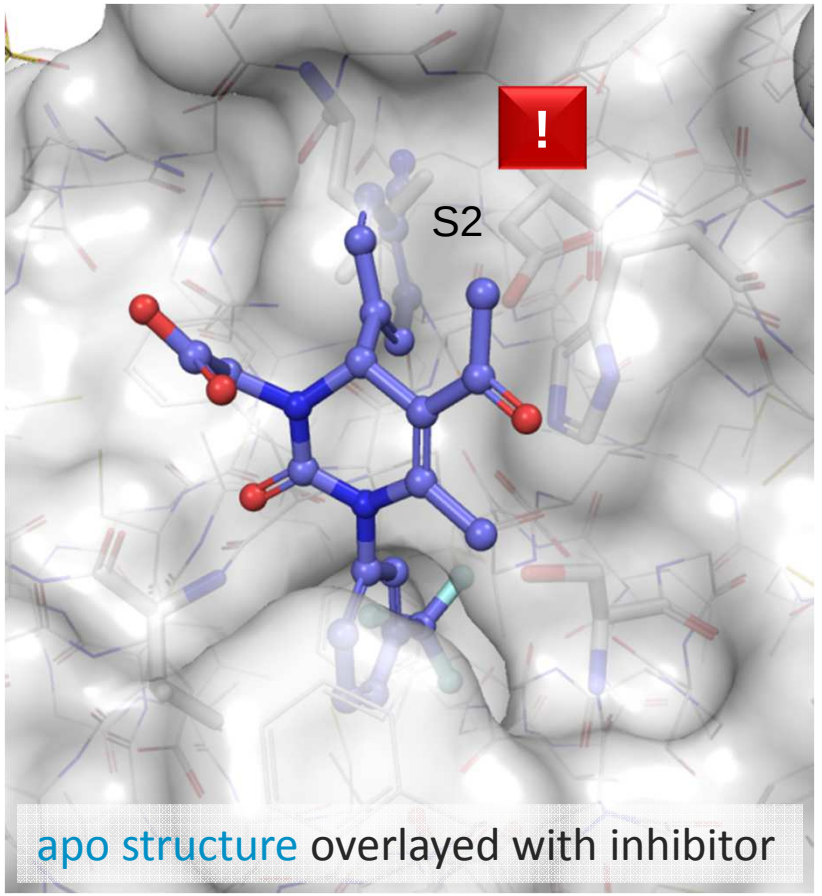
Induced-Fit Binding Mode (X-Ray)

S2 Pocket Widens for Cyanophenyl Residue

see also Hansen *et al. J. Mol. Biol.* **2011**



inhibitor binding to hNE



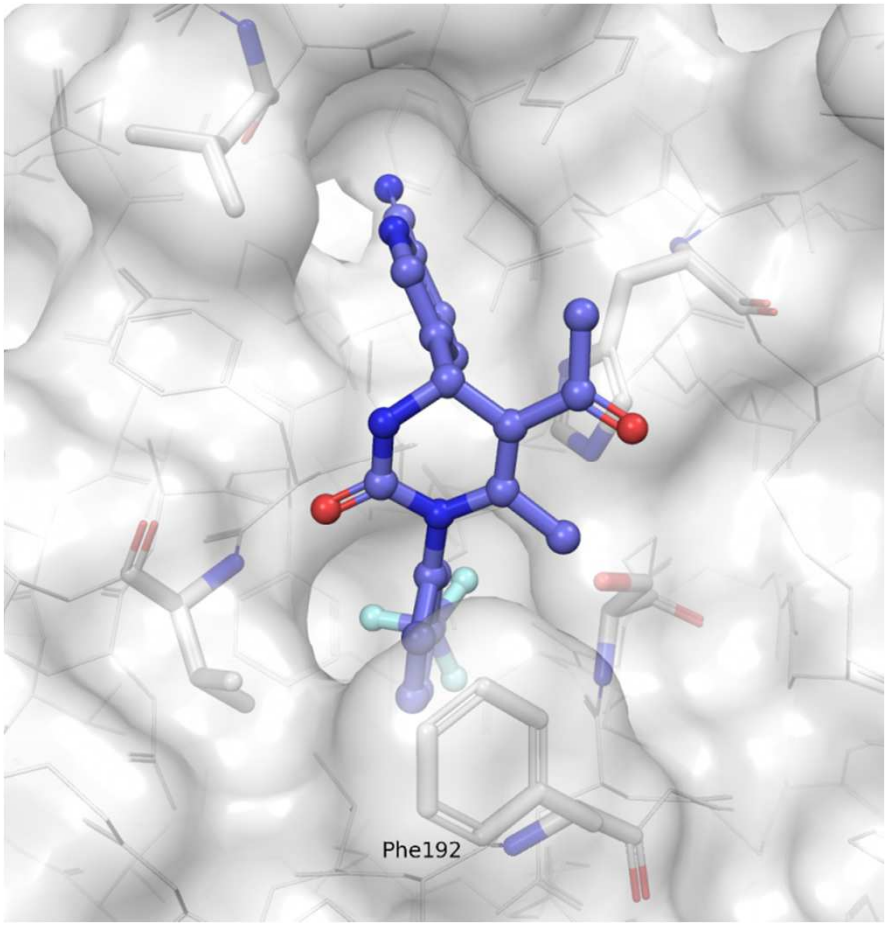
apo structure overlayed with inhibitor

Significantly smaller S2 pocket in apo structure

Induced-Fit Binding Mode (X-Ray)

BAY-678

see also von Nussbaum F, Li V *et al. ChemMedChem* **2015**



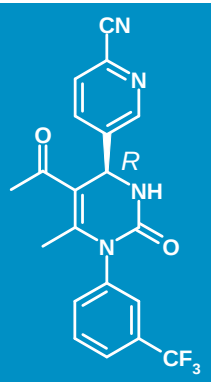
Crystal structure of HNE in complex with BAY-678: The protease is shown in a stick representation (white) with transparent Connolly-like surface; ligand 20 (purple) is shown as balls and sticks. Heteroatoms are colored as follows: oxygen, red; nitrogen, blue; fluorine, cyan.

RCSB Protein Data Bank (PDB) access code **5a0a**

Characterization of Chemical Probe BAY-678

Pharmacokinetics

see also von Nussbaum F, Li V *et al. ChemMedChem* **2015**

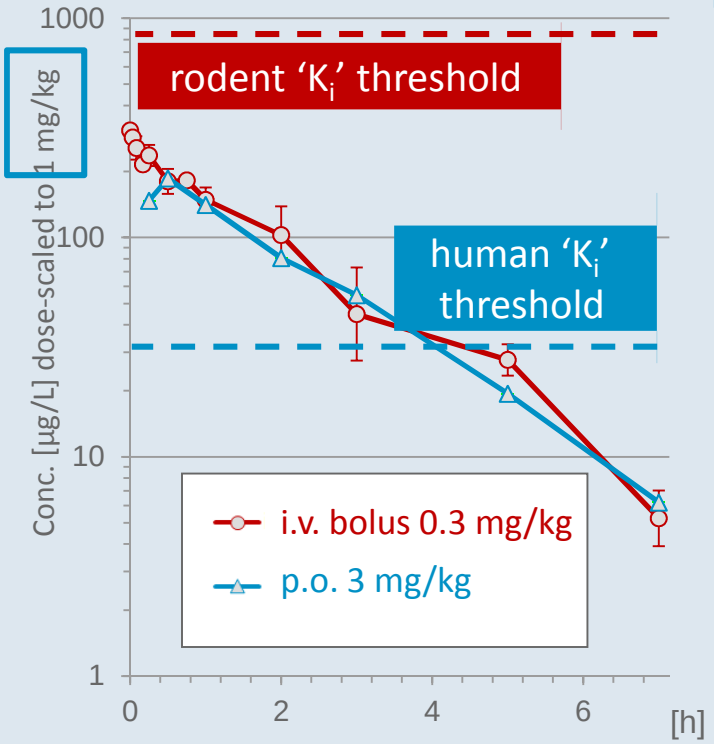


BAY-678

PK rat i.v.	$t_{1/2}$ 1.3 h, CL_{matrix} 2.0 L/h*kg, AUC_{norm} 0.50 kg*h/L, V_{ss} 3.9 L/kg
PK rat p.o.	$t_{1/2}$ 1.3 h, AUC_{norm} 0.42 kg*h/L, BA 83%
CYP inhib.	CYP 2C9 12 µM; other > 50 µM
F_U rat	~30%
Caco-2 cellperm.	$P_{app, A-B}$ 228 nm/s, $P_{app,B-A}$ 606 nm/s, $Efflux_{ratio}$ 2.7

Pharmacokinetics in Rats

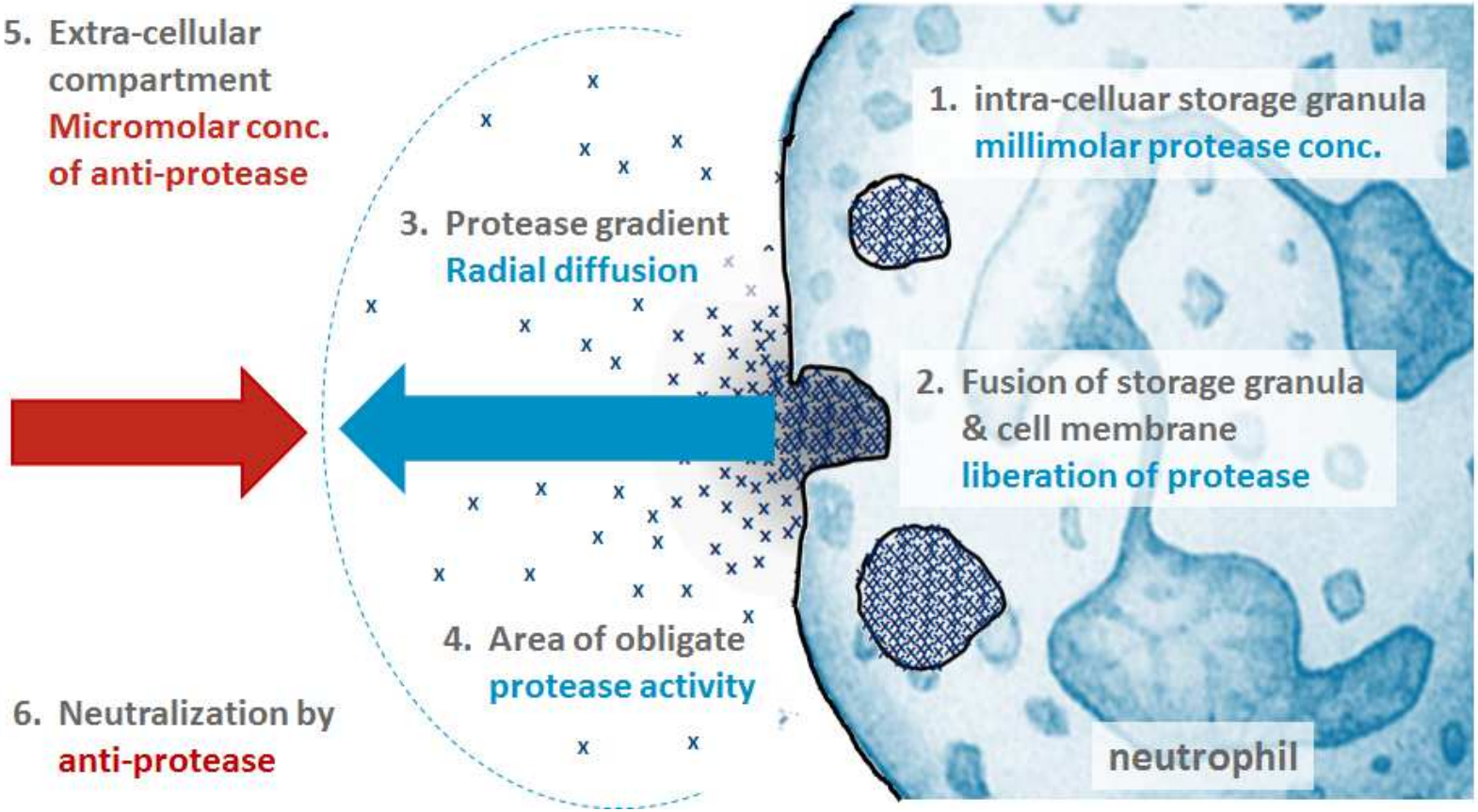
BAY-678 plasma concentration versus time



BAY-678 is cell permeable and reveals an overall good pharmacokinetics

Targeting Elastase in More Complex Settings

Model of the Kinetics of Free Extra-cellular Elastase, the Potential Driver of Inflammation and Autoimmunity



It's all about concentration & timing

Investigating BAY-678 in More Complex Settings

Beyond the Interaction with the Isolated Target

Investigating cellular action of elastase (not meaningful)

No interference with intra-cellular elastase activity (host defense) expected as this would request a very high concentration of the inhibitor (target concentration in cells is millimolar!)

Investigating extra-cellular action of elastase

Efficacy of inhibitor treatment is assessed in *in vivo* models with out-of-balance elastase activity driving presumably the onset and progression of the disease

➔ ***In vivo / ex vivo efficacy assessment of BAY-678***

In vivo / ex vivo Efficacy Assessment of BAY-678

Per oral Administration of Inhibitor (1/2)

Neutrophilic Inflammation

Efficacy demonstrated in <u>acute</u> *in vivo* models

- Protease-induced acute lung injury (ALI) in mice
  (lung hemorrhage, neutrophil count in lung lavage)
- Thread infarct model in rats
  (infarct size, cardiac function)

details on slides #20/21

Efficacy demonstrated in <u>sub-chronic</u> *in vivo* models

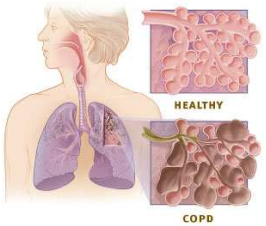
- Cigarette smoke-induced lung injury in guinea pigs
  (inflammatory cell count in lung lavage)

→ ***Anti-inflammatory mode-of-action of BAY-678***

In vivo / ex vivo Efficacy Assessment of BAY-678

Per oral Administration of Inhibitor (2/2)

COPD*



PAH

Efficacy demonstrated in <u>chronic</u> *in vivo* models

- Protease-induced lung emphysema in mice
  (lung compliance and alveolar morphometry)
- Hypoxia-induced pulmonary arterial hypertension (PAH) in mice and rats
  (heart hemodynamics & hypertrophy, biomarker, pulmonary artery muscularisation)
- Monocrotaline (MCT)-induced PAH in rats
  (heart hemodynamics & hypertrophy, biomarker)

} details on back-up slides

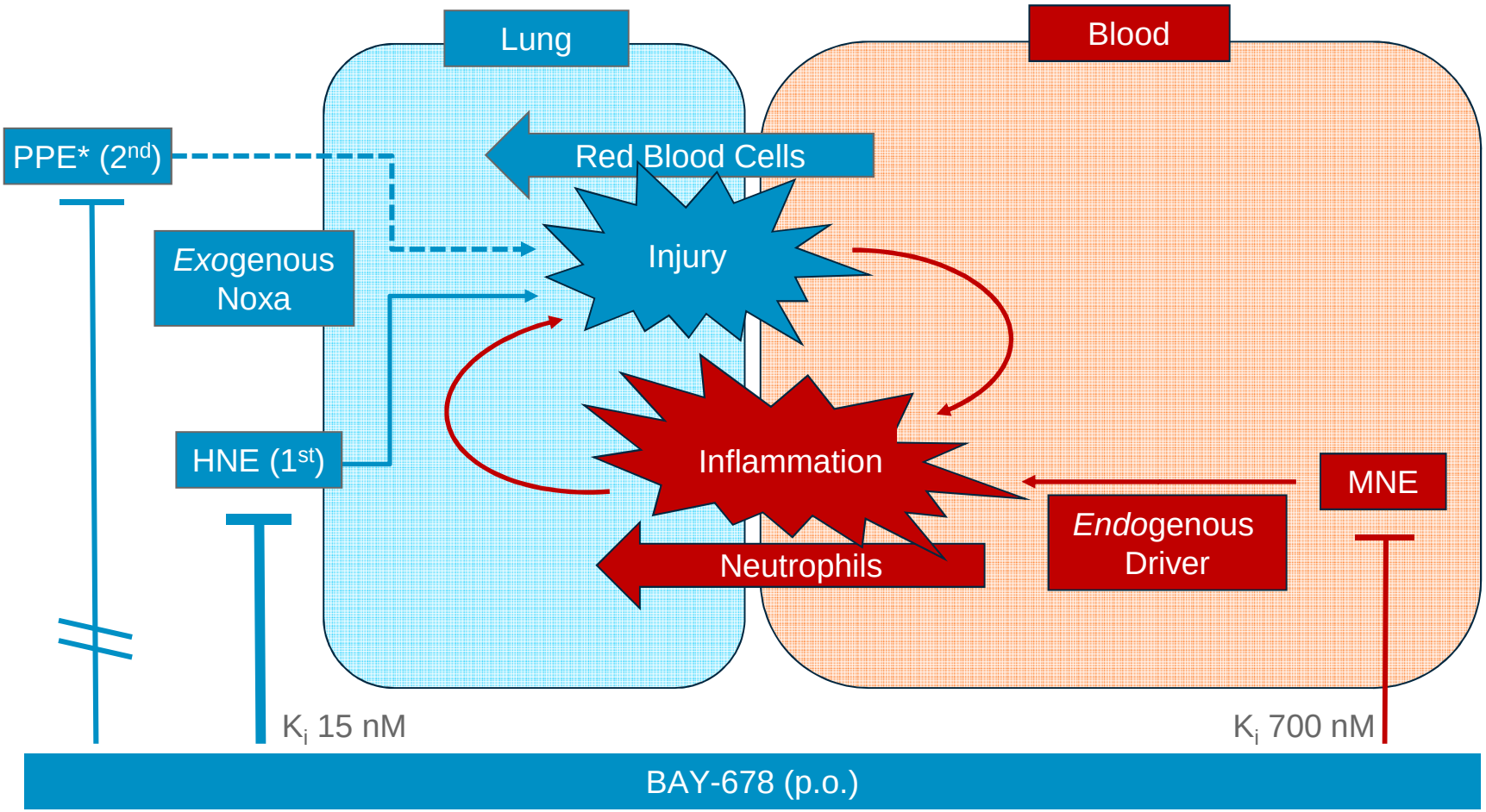
Anti-remodeling mode-of-action of BAY-678

* chronic obstructive pulmonary disease

BAY-678 in Protease-induced ALI Mice Models

Scheme of HNE-induced and PPE*-induced ALI Scenario

see also von Nussbaum F, Li V *et al. Chem. Med. Chem.* **2015**



* porcine pancreas elastase

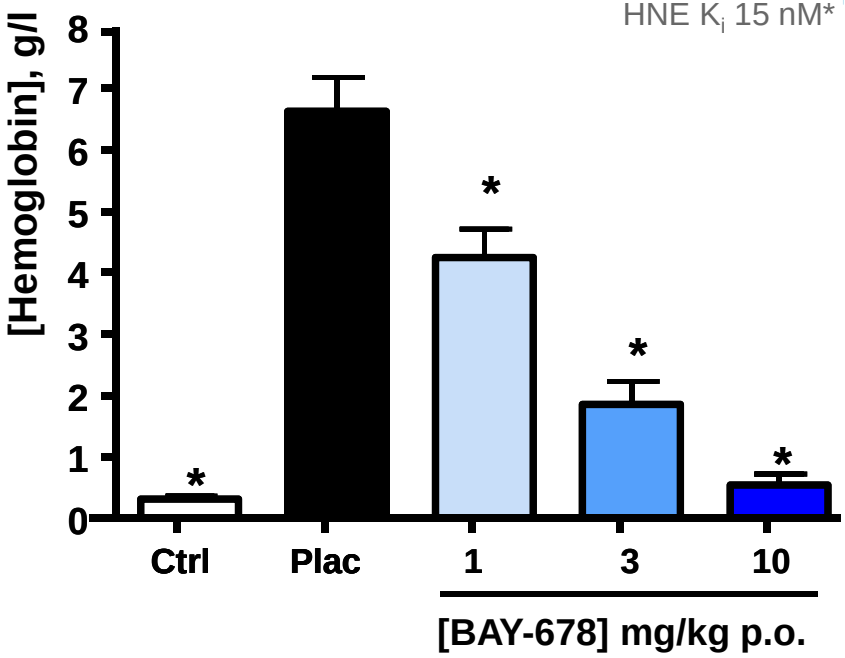
BAY-678 in Protease-induced ALI Mice Models

Lung Hemorrhage Data

see also von Nussbaum F, Li V *et al. Chem. Med. Chem.* **2015**

HNE-induced ALI
Hemoglobin count

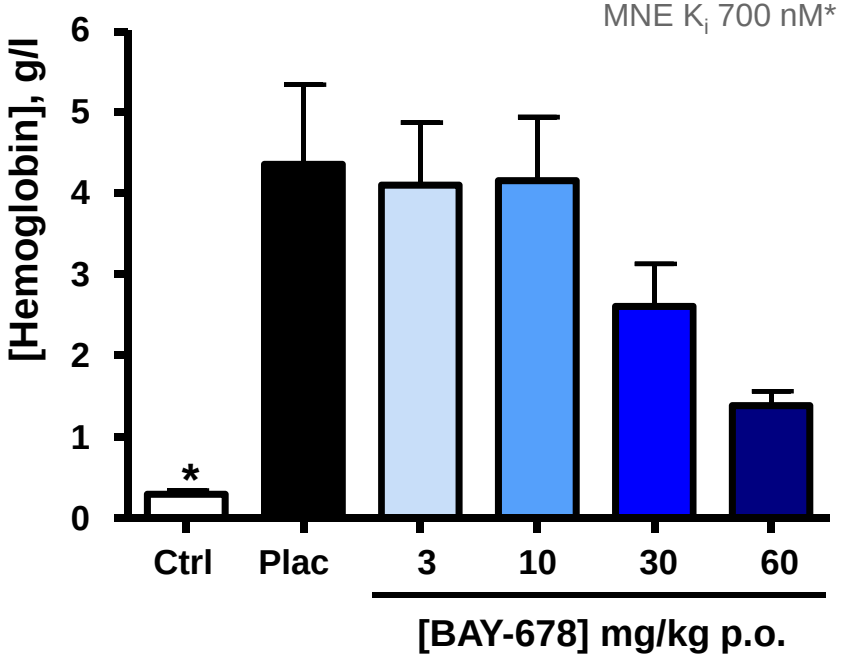
HNE K_i 15 nM*



Hitting the (exogenous) HNE target in the lung after oral administration

PPE-induced ALI
Hemoglobin count

MNE K_i 700 nM*

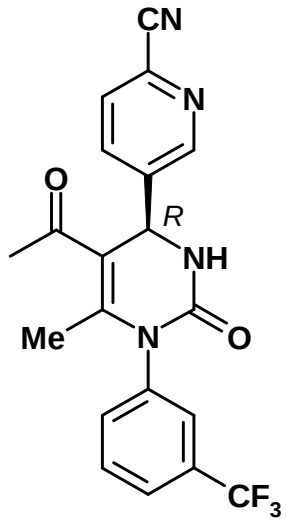


Reduction of (endogenous) MNE driven lung inflammation

* biochemical in vitro assay

Human Neutrophil Elastase Inhibitor Probe

Summary



BAY-678

BAY-678 fulfills all SGC chemical probe criteria.

We consider BAY-678 as an attractive & novel SGC probe for HNE

HNE Inhibitor Programs

Acknowledgements

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Thank you!