REVIVE
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The importance of chemical synthesis for antimicrobial research and development

Guest speakers: Anna K.H. Hirsch & Patrizio Mattei

Moderator: Ravindra Jumde

Host: Victor Kouassi

23 January 2025

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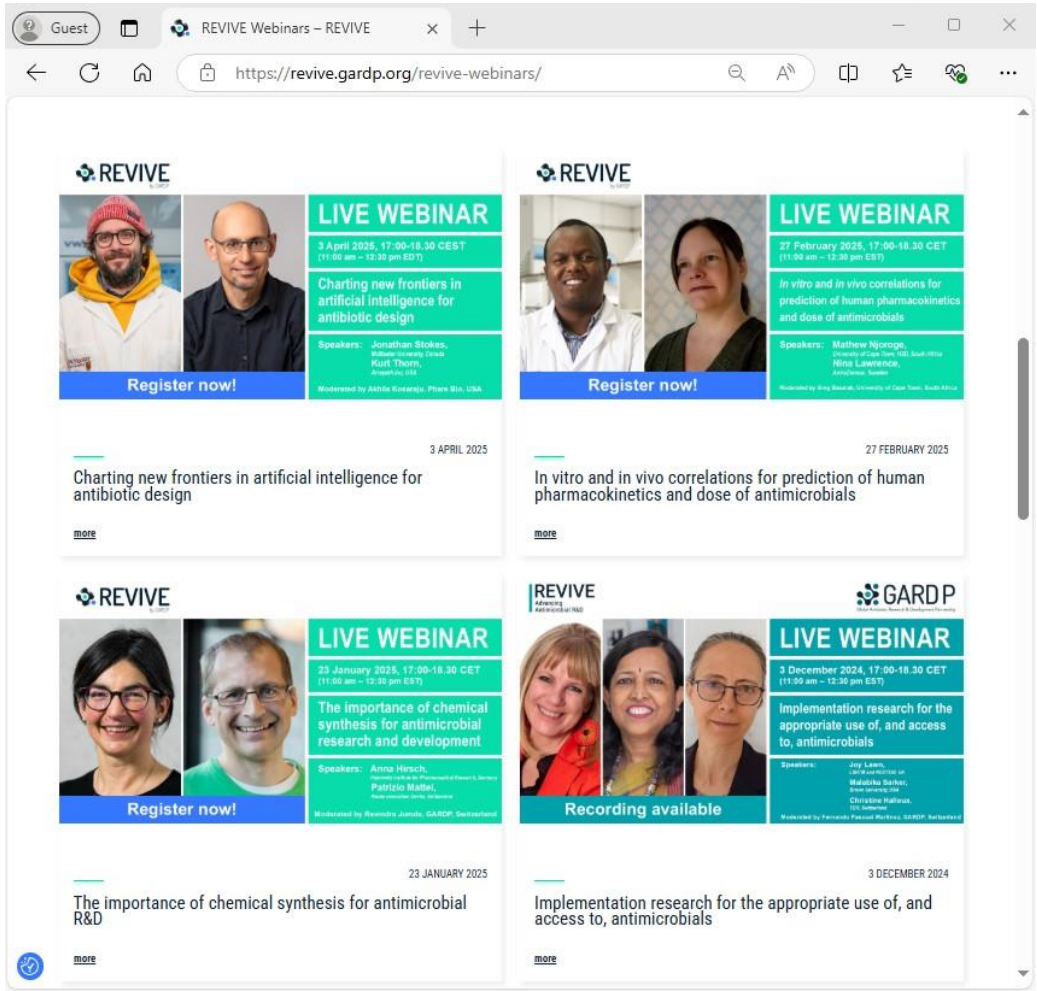

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Antimicrobial Viewpoints

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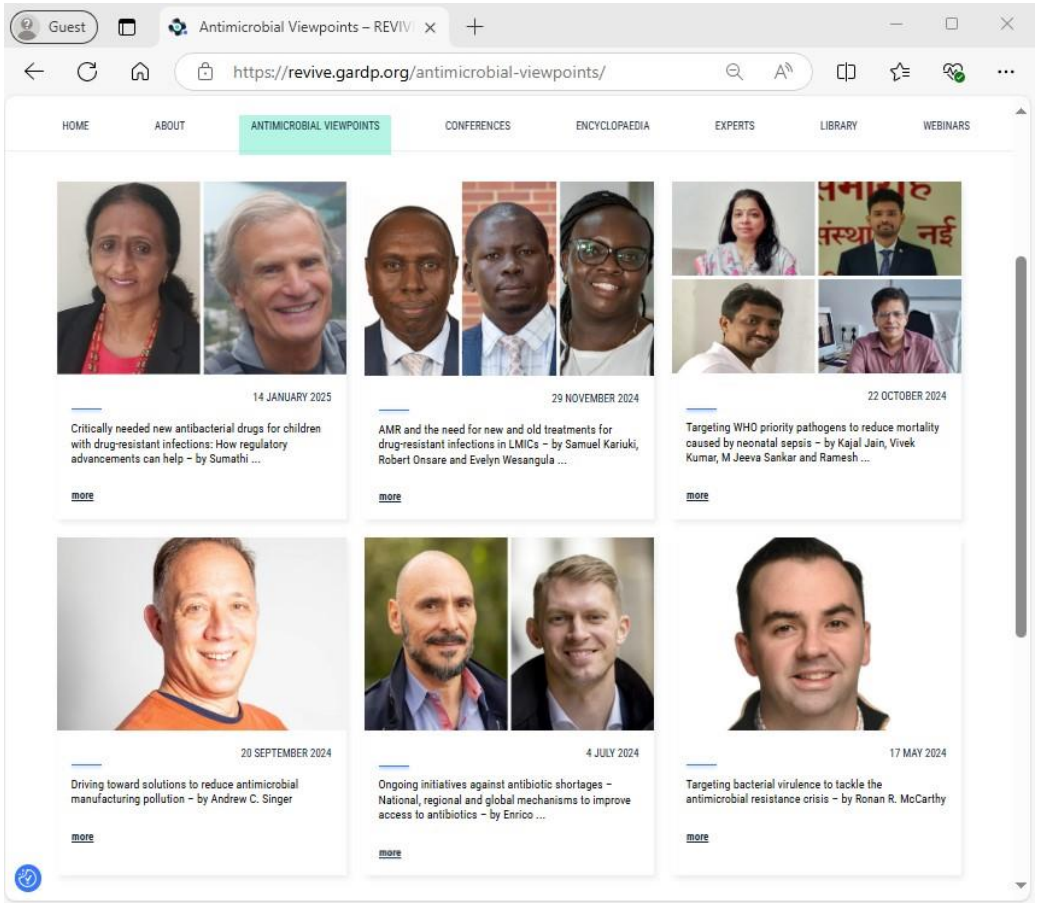

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Antimicrobial Encyclopaedia

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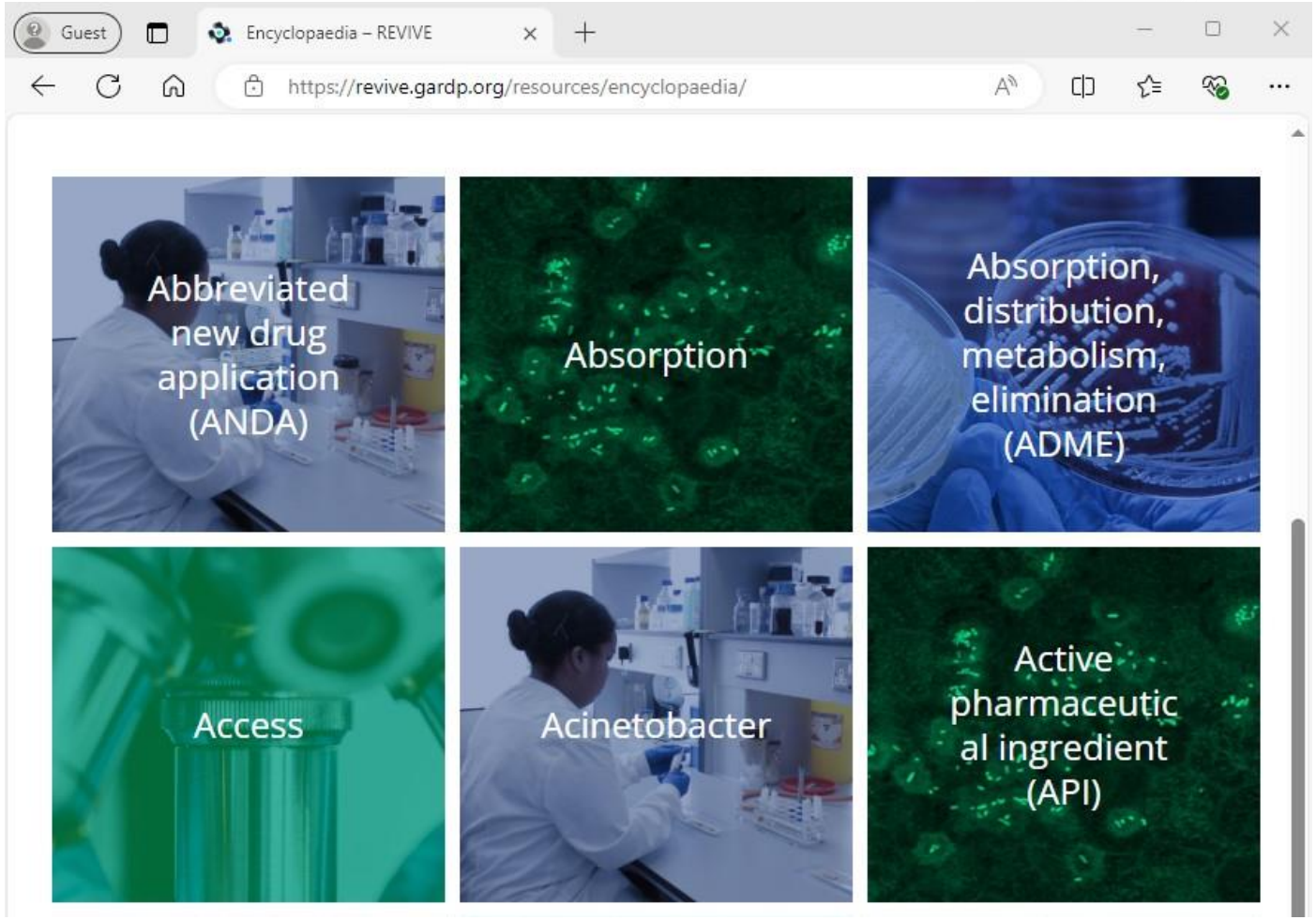

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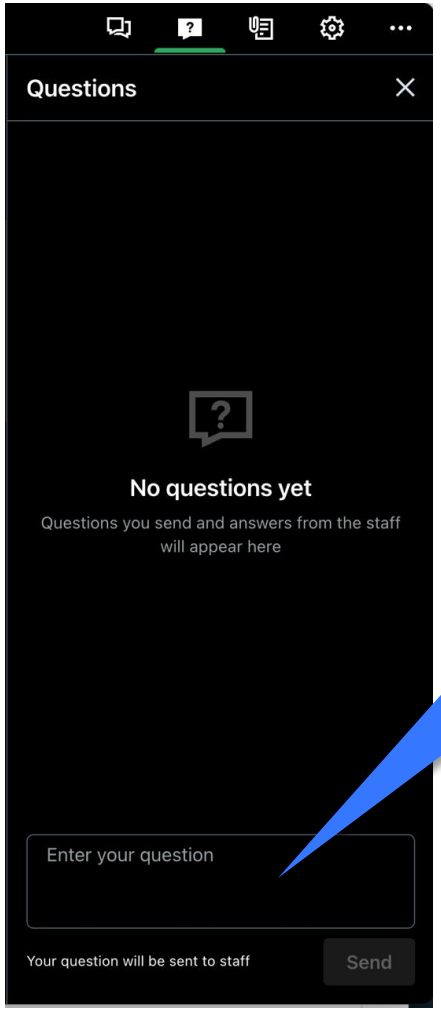
How to submit your questions

REVIVE by GARDP



If your question is addressed to a specific speaker, please include their name when submitting the question.

Please submit your questions through the box provided after clicking the ‘questions’ button. We will review all questions and respond to as many as possible after the presentation.

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Today’s speakers

REVIVE by GARDP

The importance of chemical synthesis for antimicrobial research and development

Moderator:
Ravindra Jumde
GARDP

Anna K.H. Hirsch
Helmholtz institute for Pharmaceutical Research Saarland

Patrizio Mattei
Roche Innovation Center Basel

Anna K.H. Hirsch

Anna K.H. Hirsch is a full professor of Pharmaceutical Chemistry at Saarland University and head of drug design and optimization at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS).

Her research focuses on anti-infective drug design, adopting rational approaches such as structure- and fragment-based design in combination with the protein-templated strategies of dynamic combinatorial chemistry and kinetic target-guided synthesis. She has published over 170 peer-reviewed articles and received several prestigious awards such as the 2024 RSC-BMCS Capps Green Zomaya Award. Anna completed her PhD at ETH Zurich and has held research positions at the Institut de Science et d'Ingénierie Supramoléculaires (ISIS) in Strasbourg, France and the University of Groningen, Netherlands.



Tackling underexplored anti-infective drug targets with new chemical scaffolds

Anna K. H. Hirsch
Revive Webinar, GARDP
23rd of January 2025

UNIVERSITÄT DES SAARLANDES

HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

Development of new antibiotics is lengthy and expensive

In vitro

In vivo: profiling and validation

Partnering

Screening/hit generation	Hit-to-lead	Lead optimization	N	Preclinical studies	Clinical phases I–III	Approval/ launch	Drug
>1,000–10,000 molecules				5–10	1–2	1	
1–7 years*				1–2	5–7	1–2	
5–10 m€**				3–5	>100–500	>50	

HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

UNIVERSITÄT DES SAARLANDES

close interaction with **industry, computer science** and **medicine** for **translation** into (pre)clinical development

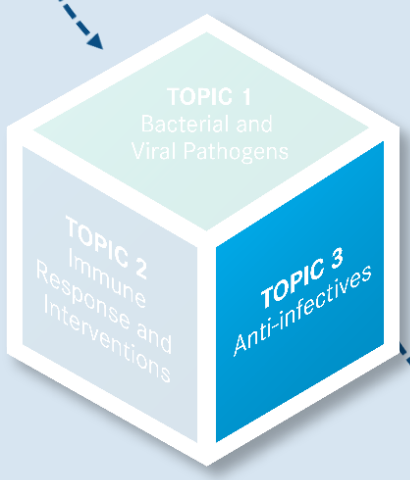
Pharma Science Hub

AMR collaborators, *Lancet* **2022**
Miethke *et al.*, *Nature Rev. Chem.* **2021**

HIPS/HZI at a glance

***Infection research** at*

HZI HELMHOLTZ Centre for Infection Research



3 Research topics

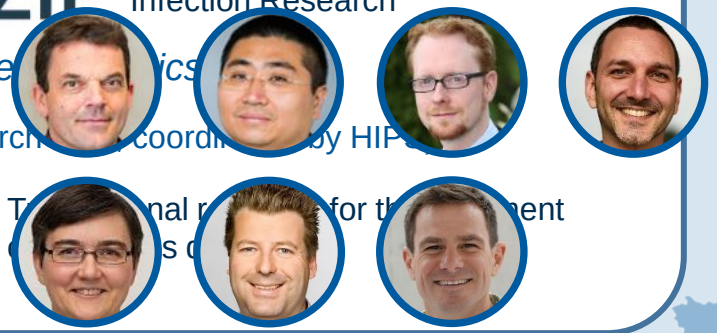
PIs in Topic 3

DZIF German Centre for Infection Research

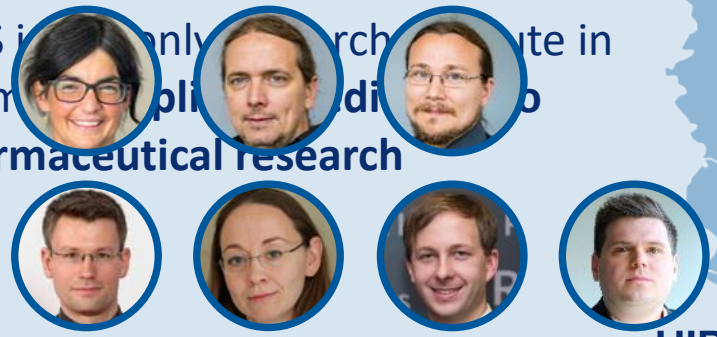
Nove… ics

(Research … coordi… by HIP…

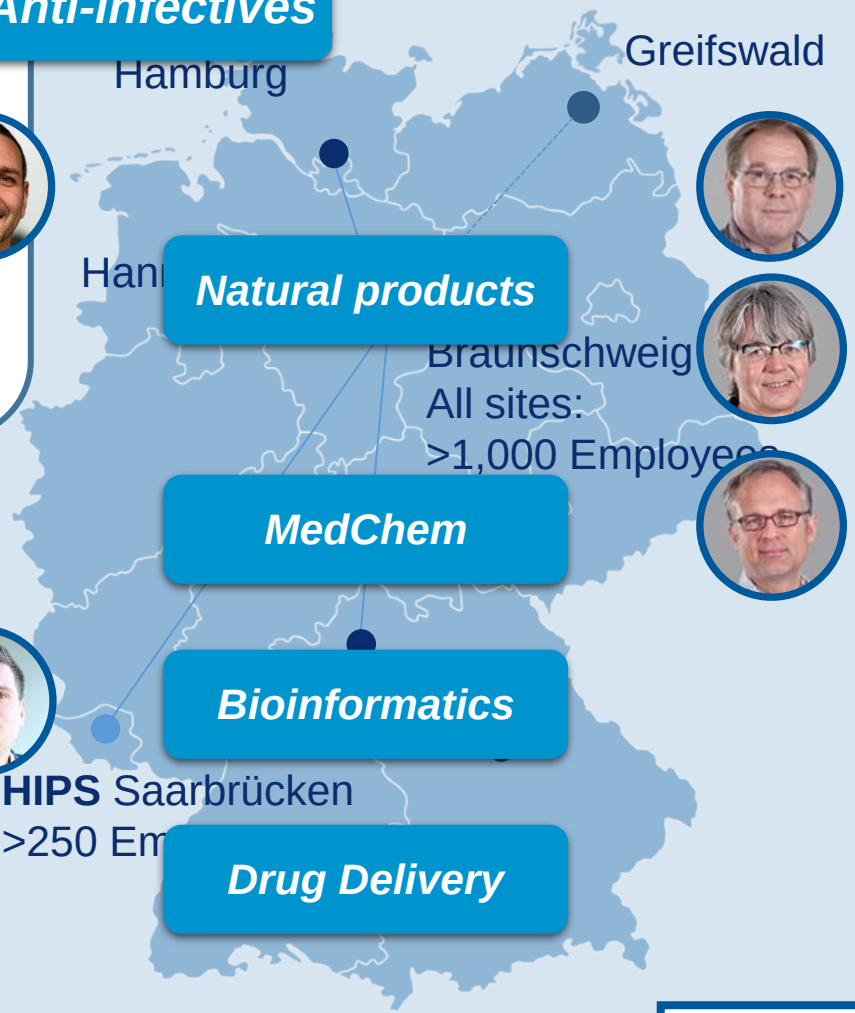
T… nal r… for th… ment … s c…



- HIPS i… only … rch … ute in Germ… **pli… di… o pharmaceutical research**



Anti-infectives



Natural products

MedChem

Bioinformatics

Drug Delivery

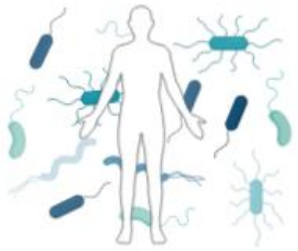
HIPS

HZI

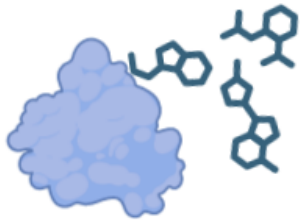
Anti-infective drug discovery at the HIPS

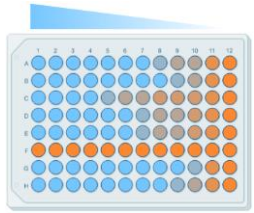
Natural products from bacteria

Natural products from microbiota



Medicinal chemistry

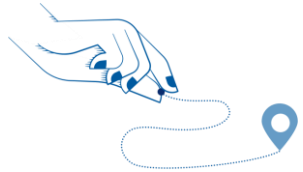




Screening

Biotechnological optimisation

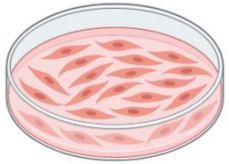
Production optimisation



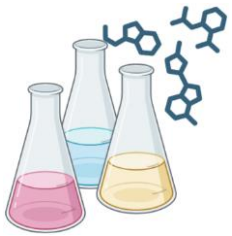
Drug delivery

From identification to clinical trials

Profiling/ mode of action



Chemical optimisation



in vivo characterisation

Translation into clnical application

Computer-assisted optimisation via AI, bio- and cheminformatics

M. Mullowney *et al.*, *Nature Rev. Drug Discov.* **2023**

General workflow

Virtual screening:
Structure- & ligand-based design
Docking
Property prediction

Fragment-based drug design

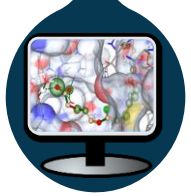
Protein-templated synthesis:
DCC & KTGS

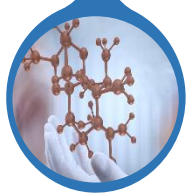
Biophysical assays:
STD-NMR, SPR
MST & ITC

Small-molecule synthesis
Structure–activity relationship studies

Enzyme- and cell-based inhibition assays
***Ex vivo* and *in vivo* models**
Co-crystallisation studies

***In vitro* ADME-T**
***In vivo* pharmacokinetic and pharmacodynamic studies**





Computer-aided drug design

Hit-identification strategies

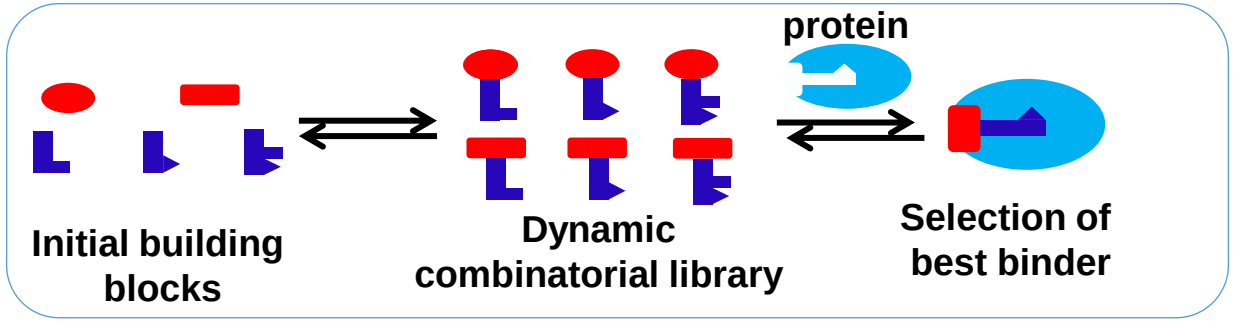
Design and synthesis

Biological assays

Multiparameter optimisation

Protein-templated strategies

Dynamic combinatorial chemistry (DCC):



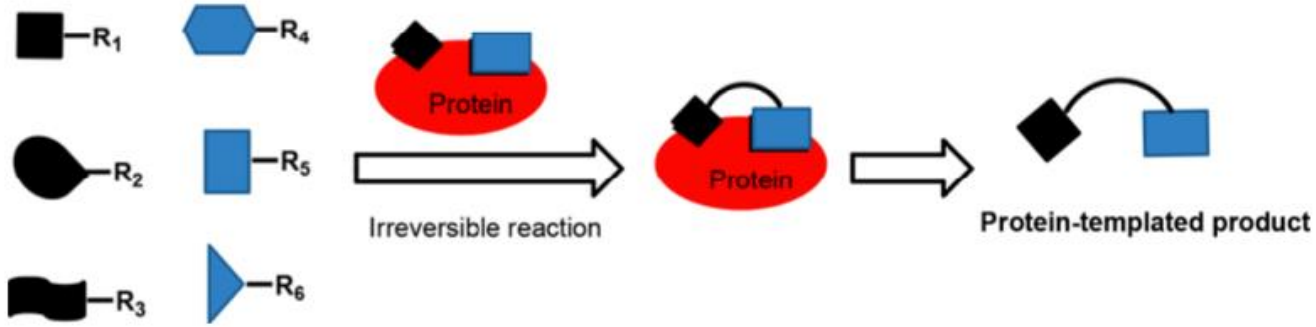
Lehn, Eliseev, Ramström, Greaney, Ernst, Vincent …

- Reaction between building blocks is reversible
- Selection and amplification of best binder(s)
- Biocompatibility of **reversible** reaction

M. Mondal *et al. Angew. Chem. Int. Ed.* **2014** & **2016**

A. M. Hartman *et al. ChemMedChem* **2020**
R. P. Jumde *et al. Chem. Sci.* **2021**
I. Exapicheidou *et al. Chem. Comm.* **2024**
G. Jézéquel *et al. Chem. Eur. J.* **2025**
W. Elgaher *et al., in preparation*.

Kinetic target-guided synthesis (KTGS):



Sharpless, Rademann, Deprez …

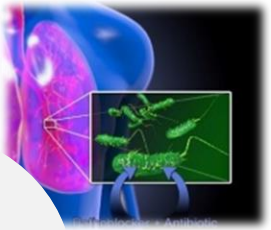
- Reaction partners bind to adjacent pockets
- Proper orientation
- Protein-templated **irreversible** reaction

M. Mondal *et al. Chem. Eur. J.* **2016**
F. Mancini *et al. Chem. Eur. J.* **2020**,

D. Bosc *et al. J. Med. Chem.* ***2020***, 3817.
M. Y. Unver, R. M. Gierse, H. Ritchie, A. K. H. Hirsch, *J. Med. Chem.* **2018**, *61*, 9395–9409.

M. Mondal, A. K. H. Hirsch, *Chem. Soc. Rev.* **2015**, *44*, 2455–2488.
O. Ramström, J.-M. Lehn, *Nat. Rev. Drug Discov.* **2002**, *1*, 26–36.

Most promising projects at the department *Drug Design and Optimisation*



LasB
IC_{50} : 1.5 nM
Target strain:
PA

LecA/B
IC_{50} : 10.8 nM
Target strain:
PA

PqsR
IC_{50} : 5 & 30 nM
(PqsR & Pyocyanin)
Target strain: PA

IspD & IspE
IC_{50} (PF): 10 nM
Target strain(s):
MT, PF, EC, AB

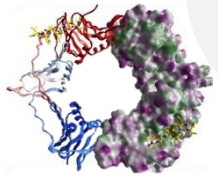
DXS
IC_{50} (PF): 0.7 µM
Target strain(s):
MT, PF, EC

Target Proteins

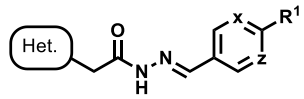
F protein
IC_{50} : 40 nM
Target virus:
RSV

Nsp10/12/14/16
IC_{50} (RdRp): 1 µM
Target virus:
SARS-CoV-2

ECF-T
MIC: 0.5 µM
Target strain(s):
SA, SP



DnaN/pORF59
MIC (MS): 0.25 µM
Target strain(s):
broad spectrum
MT, AB… & KSHV



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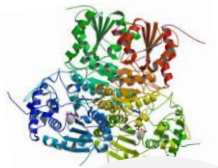

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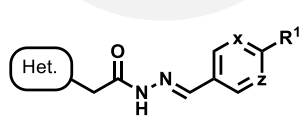

ECF-T
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Target strain(s):
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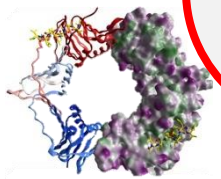

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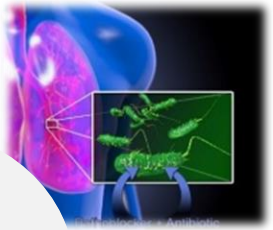

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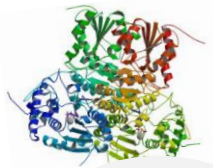

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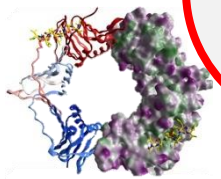

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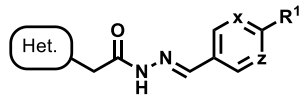

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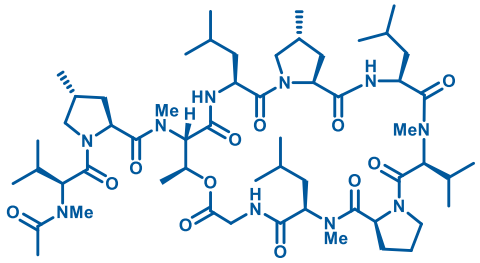
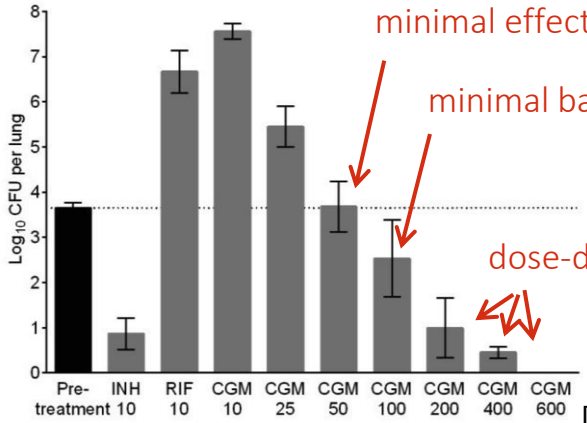
Griselimycin – a promising NP with antitubercular activity

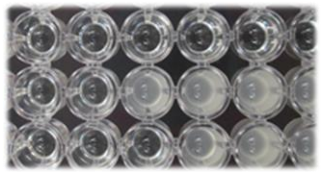
Griselimycin producer strain
Streptomyces caelicus



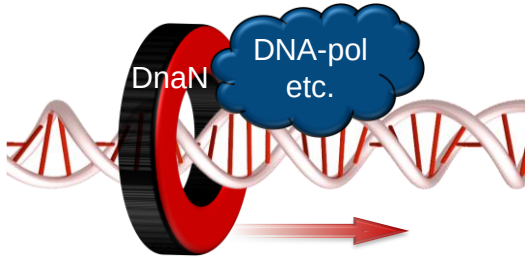
290 derivatives generated



Production & Isolation → Activity → Structure → Bio-synthesis → Semi- and total synthesis → Mode of Action → Pre-clinic → STOP



Active against drug-susceptible and -resistant *Mycobacterium tuberculosis* (Mtb)



CGM programme stopped →**unfavourable liver toxic** effects: BSEP inhibition

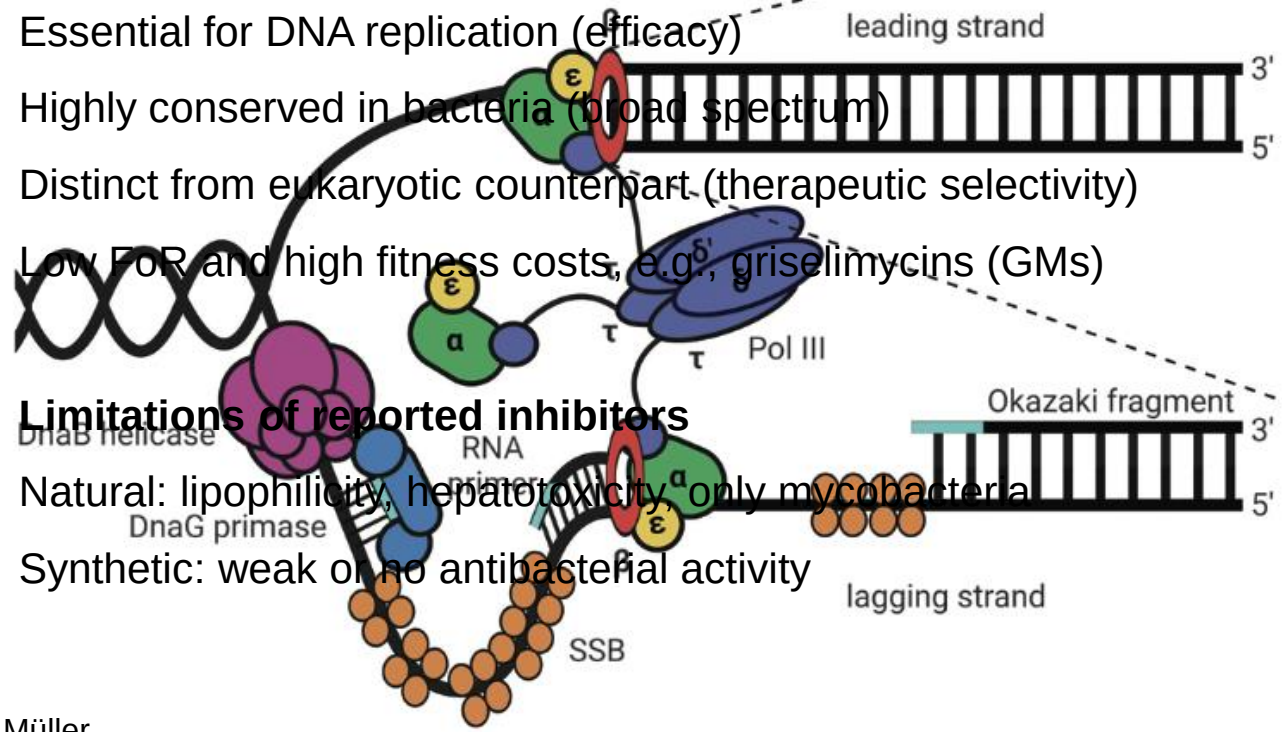
A. Kling, P. Lukat *et al.*, *Science* **2015**

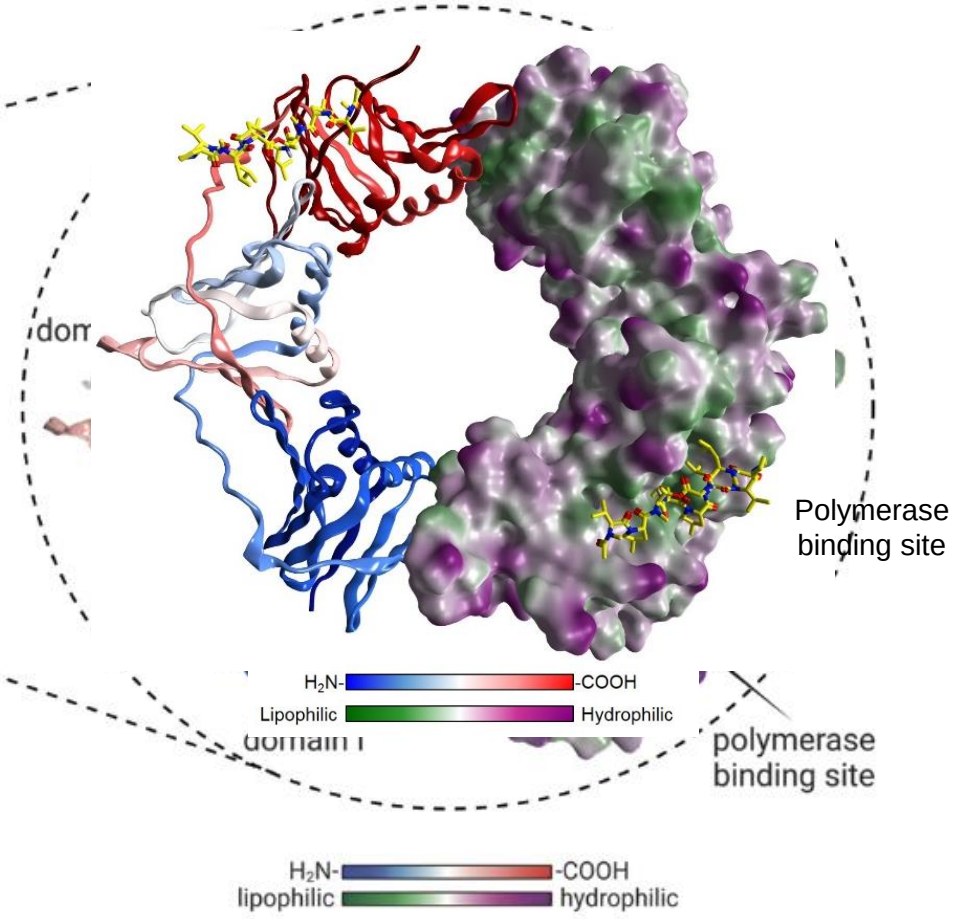
β-Sliding clamp (DnaN): an innovative target to combat AMR

- Ring-shaped homodimer of β-subunit of bacterial DNA polymerase III

- **Attractive target**:
  - Essential for DNA replication (efficacy)
  - Highly conserved in bacteria (broad spectrum)
  - Distinct from eukaryotic counterpart (therapeutic selectivity)
  - Low FoR and high fitness costs, e.g., griselimycins (GMs)

- **Limitations of reported inhibitors**
  - Natural: lipophilicity, hepatotoxicity, only mycobacteria
  - Synthetic: weak or no antibacterial activity





→ Synthetic small-molecule inhibitors → Community-acquired bacterial pneumonia

→ Genome mining with bait genes from 4-methylproline pathway: new natural products → TB

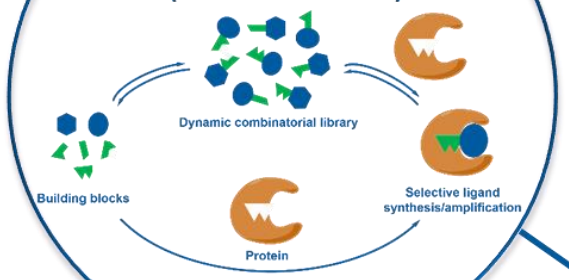
with Rolf Müller,
Wulf Blankenfeldt
Norbert Reiling
Katharina Rox
Nicholas Dixon
Mark Brönstrup
Philipp Klahn

W.A. M. Elgaher *et al., Ann. Rep. Med. Chem*. **2023**

A. Kling, P. Lukat *et al.*, *Science* **2015**

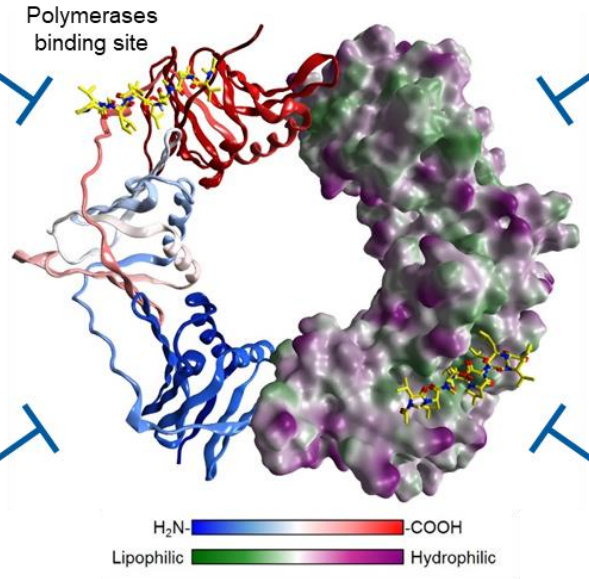
Strategies for novel DnaN inhibitors

Target-guided synthesis (DCC & KTGS)

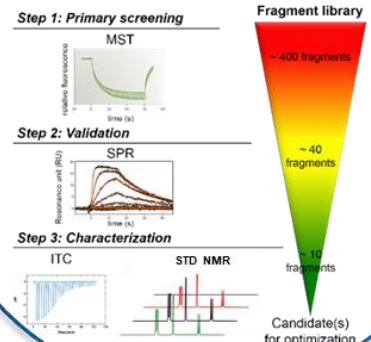


Chem. Eur. J. **2020**

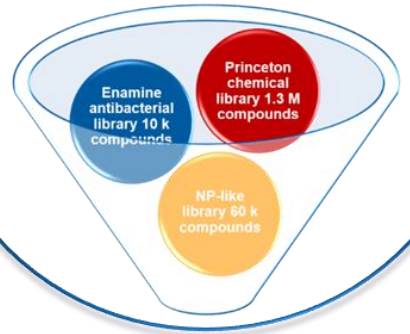
β-sliding clamp (DnaN)



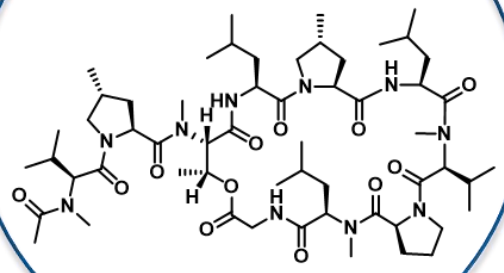
Fragment screening



Structure- & ligand-based virtual screening



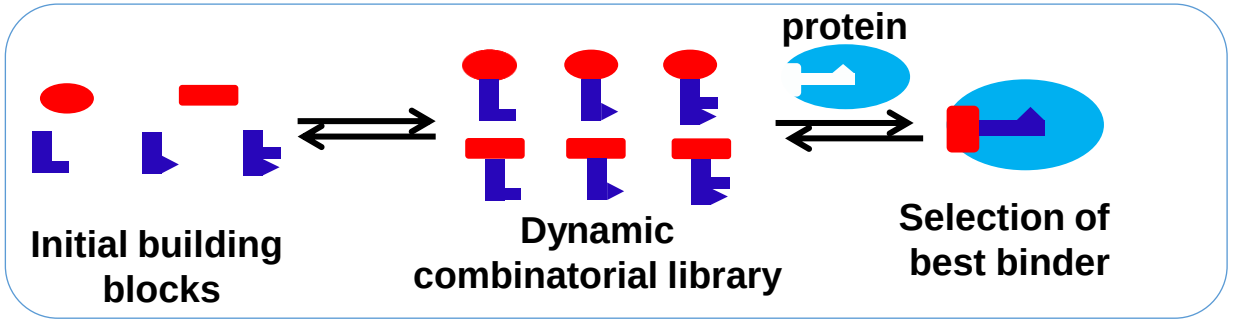
Total & semi-synthesis



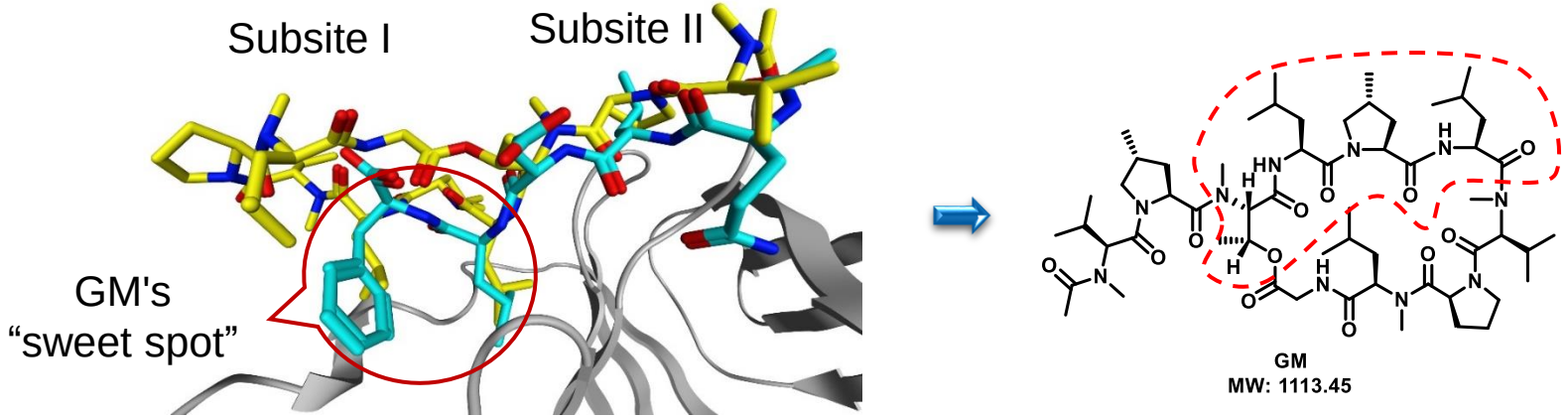
GM

Dynamic combinatorial chemistry (DCC)

- Protein-templated strategy combining synthesis and binding assay in one step
- **Reversible** reaction, *e.g.*, acylhydrazone formation



R. Jumde *et al.*, *Chem. Sci.* **2021**, *12*, 7775–7785.
M. Mondal *et al.*, *Chem. Soc. Rev.* **2015**, *44*, 2455–2488.

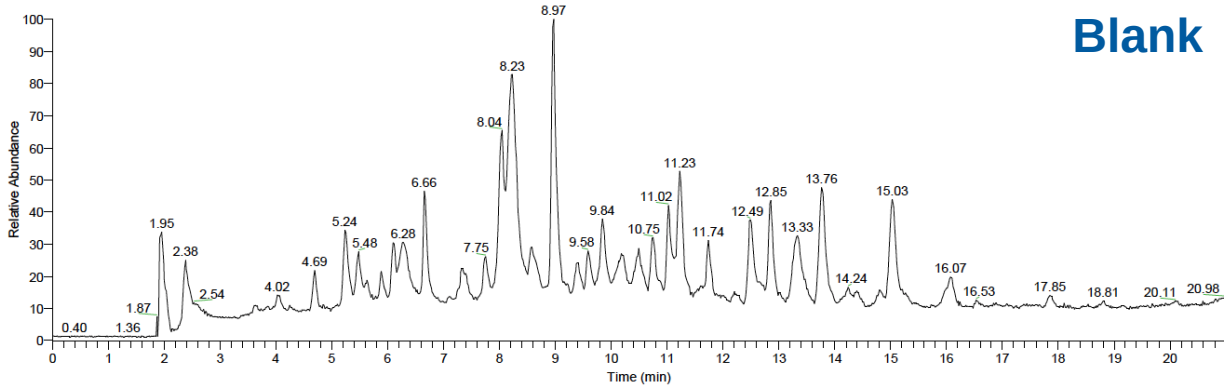


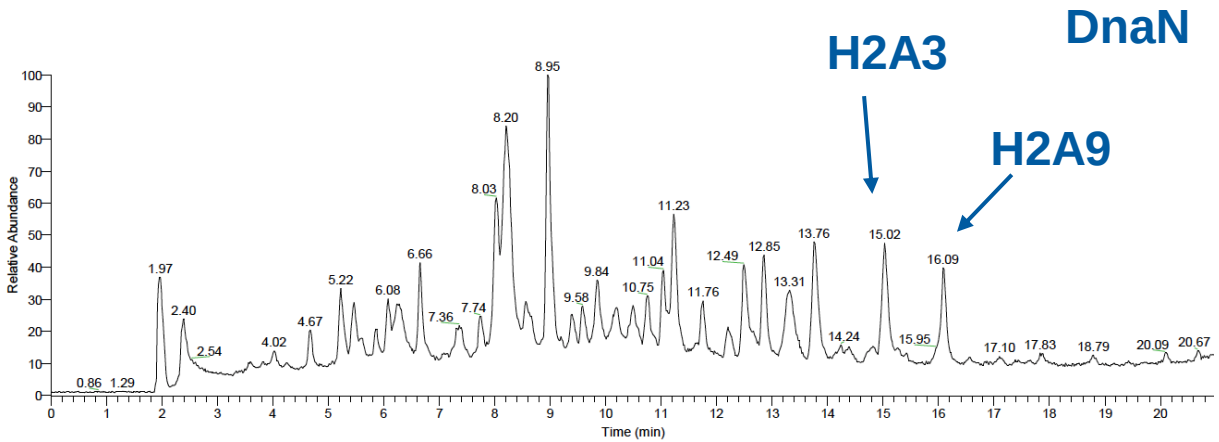
Overlay of GM (yellow) and the universal clamp-binding motif [AcQLDLF] (cyan) with DnaN (grey)

W. A. M. Elgaher, A. Ahmin *et al.* (in preparation).

DCC analysis

DCL analysis and hit identification

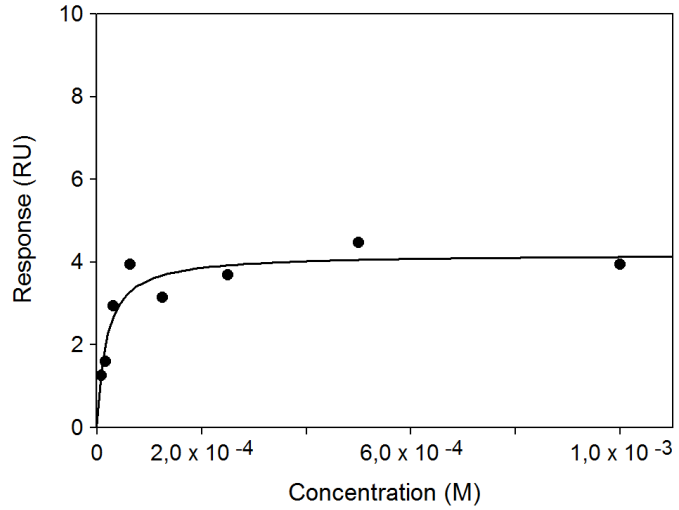


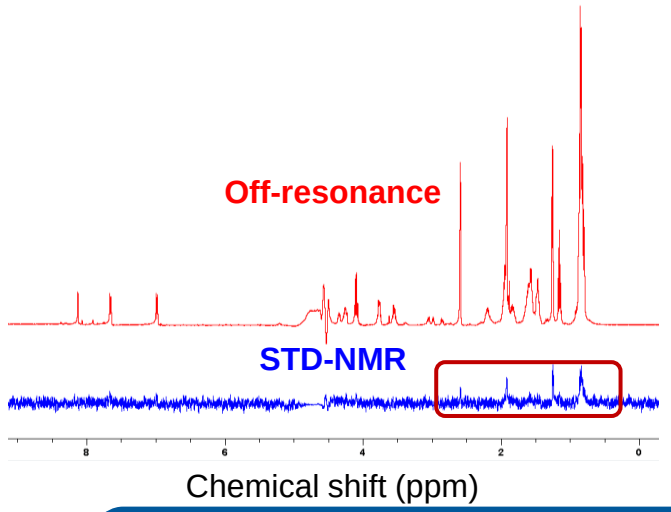


- Low micromolar affinity
- Potent antitubercular activity with no cytotoxicity
- Broadened spectrum
- Hit optimisation completed: isoniazid scaffold

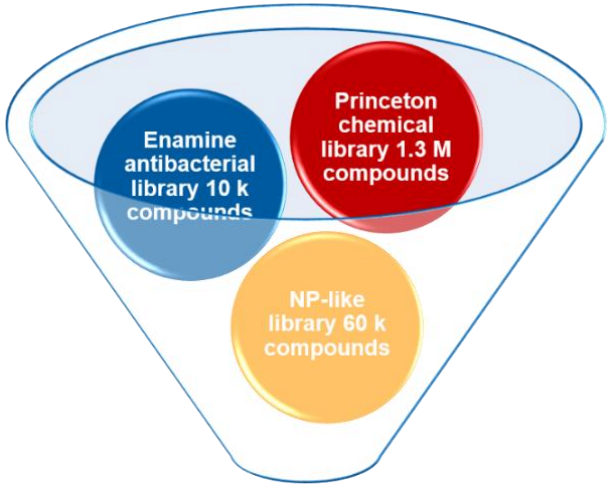
W. A. M. Elgaher, A. Ahmin *et al.* (in preparation).

Hit validation





Structure-based virtual screening

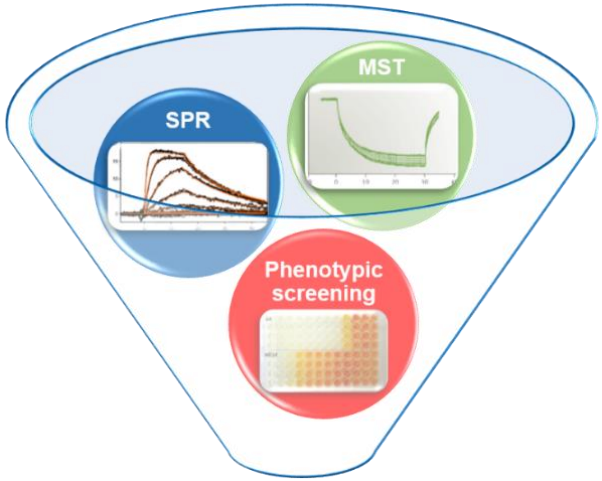


140 k hits

Hit refinement

30 hits

Hit valdation



3 validated hits

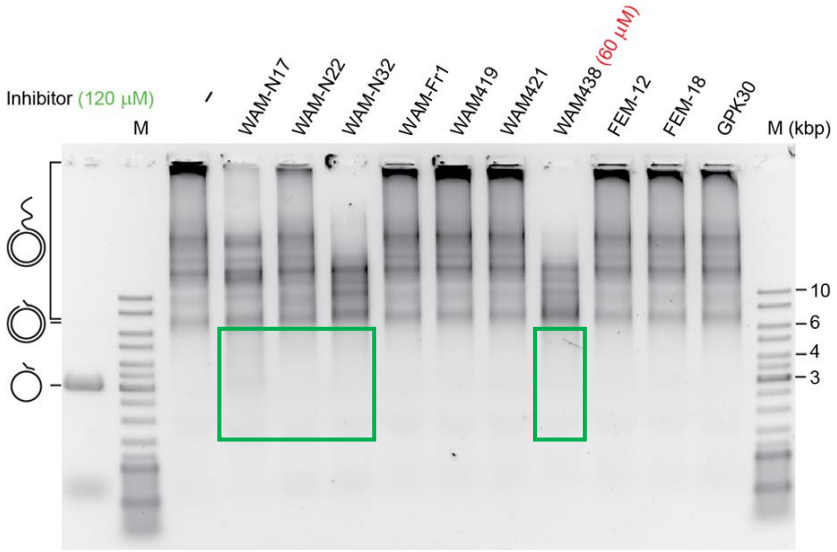
Prioritization

WAM-N17

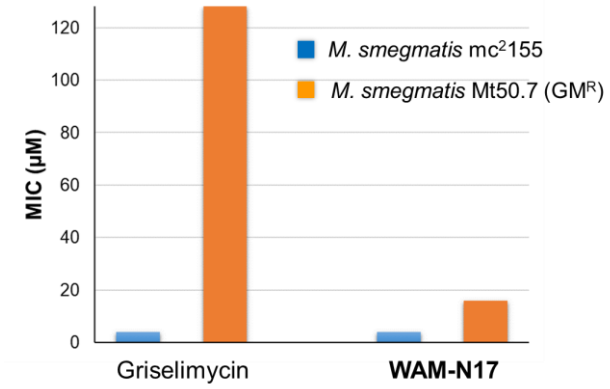
- ✓New chemical entity*
- ✓Micromolar affinity (K_D 50 ±10 µM)
- ✓Broad antibiotic activity
- ✓No cytotoxicity (IC_{50} > 100 µM)
- ✓Antibiotic activities against ESKAPE and *M. tuberculosis*

W. A. M. Elgaher, *et al*. (patent application in preparation).

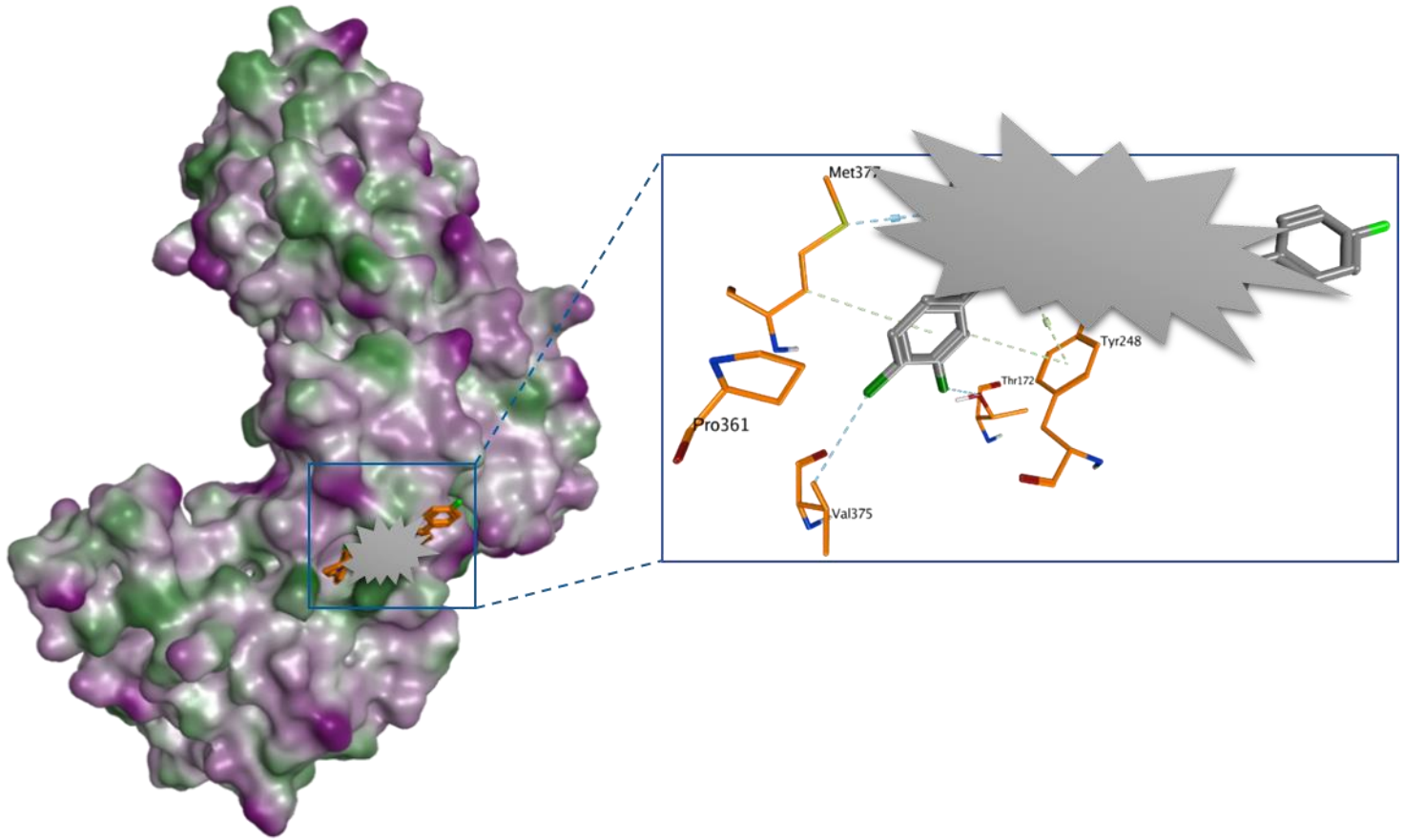
Mode of action: DnaN-dependent inhibition of DNA replication



WAM-N17 shows DnaN-dependent inhibition of *E. coli* DNA replication



WAM-N17 shows 4-fold increase of MIC against DnaN-over expressing *M. smegmatis**



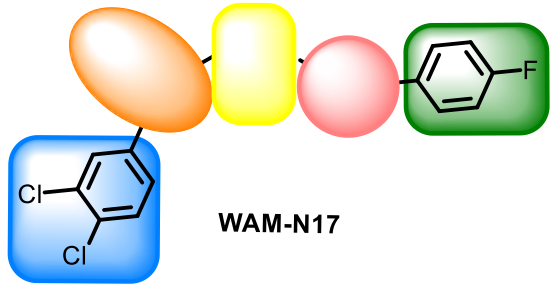
Crystal structure of **WAM-N17** bound to *Rickettsia typhi* DnaN at the polymerase binding site at 2.2 Å

W. A. M. Elgaher, *et al*. (patent application in preparation).

Hit-optimisation and SARs

- Structure–activity relationship (SAR) studies: >200 compounds

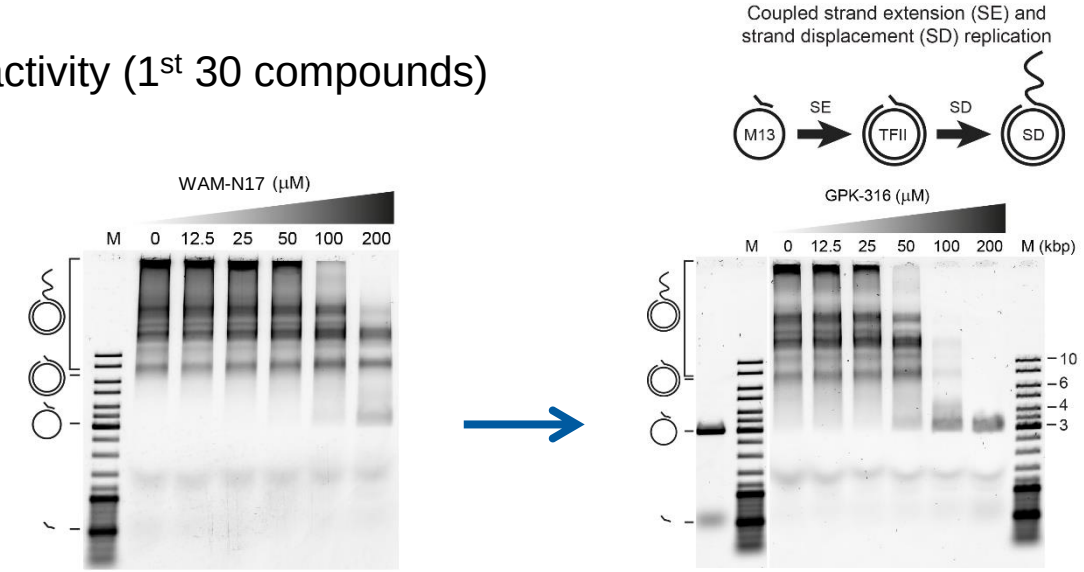
Small lipophilic substituents are favorable: 3,4-dichloro > *m*-Cl ≥ *p*-Cl > *p*-Me > *p*-F > *p*-MeO > H



WAM-N17

Various substituted arenes can be tolerated
Can be replaced by an alkyl group

✓ ~3-Fold improved inhibitory activity (1st 30 compounds)



IC_{50} 150 ± 30 μM

IC_{50} 60 ± 10 μM

✓ Binding site has been confirmed by crystal structures for two compounds in complex with *R. typhi* DnaN

W. A. M. Elgaher, *et al*. (patent application in preparation).

Antibacterial activity and *in vitro* ADMET profile

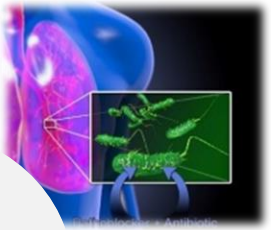
Compound	MIC (µg/mL)								CC_{50} (µM) HepG2	Mouse Liver S9 $t_{1/2}$ (min) / Cl_{int} (µL/min/mg)	Mouse Plasma $t_{1/2}$ (min) / % at 4 h
	S. aureus Newman	*S. pneumoniae* DSM20566	*S. pneumoniae* DSM11865 PRSP	*E. coli ΔacrB*	*E. coli* DSM-1116	*M. smeg.* mc^2155	*M. smeg.* Mt50.7, GM^R	*M. tb.* H37Rv			
WAM-N17	4	8	n.d.[a]	4	64	2	8	16	>37	n.d.	n.d.
WAM-N96	**2**	**1**	**1**	**1**	16	**0.5**	**1**	4–8	>37	>240 / <2.9	>240 / >100
WAM-N102	**2**	**2**	**1**	**2**	n.d.	**0.5**	**1**	4–8	>37	>120 / <5.8	>240 / 85.0
WAM-N112	**0.5**	n.d.	4	**2**	n.d.	**0.5**	**1**	**1**	29	>240 / <2.9	>240 / 87.4
WAM-N113	4	n.d.	4	**1**	n.d.	**0.125**	**2**	**2–4**	>37	>240 / <2.9	>240 / 63.2
WAM-N126	**1**	n.d.	**1–2**	**2**	**4–8**	**2**	n.d.	**2–4**	>37	>240 / <2.9	>240
WAM-N127	**2**	n.d.	**2**	4	**4–8**	**1–2**	n.d.	**2–4**	>37	>240 / <2.9	>240
WAM-N128	**0.5–1**	n.d.	**1–2**	8	32	**2**	n.d.	**2**	>37	>240 / <2.9	>240
WAM-N194	**1**	n.d.	**2**	**2**	16	n.d.	n.d.	4–8	>200	>120 / <11.6[b]	n.d.
WAM-N198	**1**	n.d.	**0.5**	**2**	16	n.d.	n.d.	4	20	>120 / <11.6[b]	n.d.

[a] not determined; [b] data for mouse liver microsomes.

- ✓ 8–16-Fold improved antibiotic activity
- ✓ Broad spectrum, including MDR pathogens
- ✓ No significant cytotoxicity
- ✓ High plasma- and metabolic stability

W. A. M. Elgaher, *et al*. (patent application in preparation).

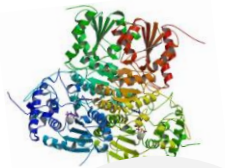
Most promising projects at the department *Drug Design and Optimisation*



LasB
IC_{50} : 1.5 nM
Target strain:
PA

LecA/B
IC_{50} : 10.8 nM
Target strain:
PA

PqsR
IC_{50} : 5 & 30 nM
(PqsR & Pyocyanin)
Target strain: PA



IspD & IspE
IC_{50} (PF): 10 nM
Target strain(s):
MT, PF, EC, AB

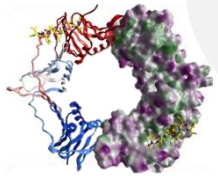
DXS
IC_{50} (PF): 0.7 µM
Target strain(s):
MT, PF, EC

Target Proteins

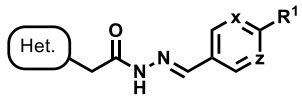
F protein
IC_{50} : 40 nM
Target virus:
RSV

Nsp10/12/14/16
IC_{50} (RdRp): 1 µM
Target virus:
SARS-CoV-2

ECF-T
MIC: 0.5 µM
Target strain(s):
SA, SP

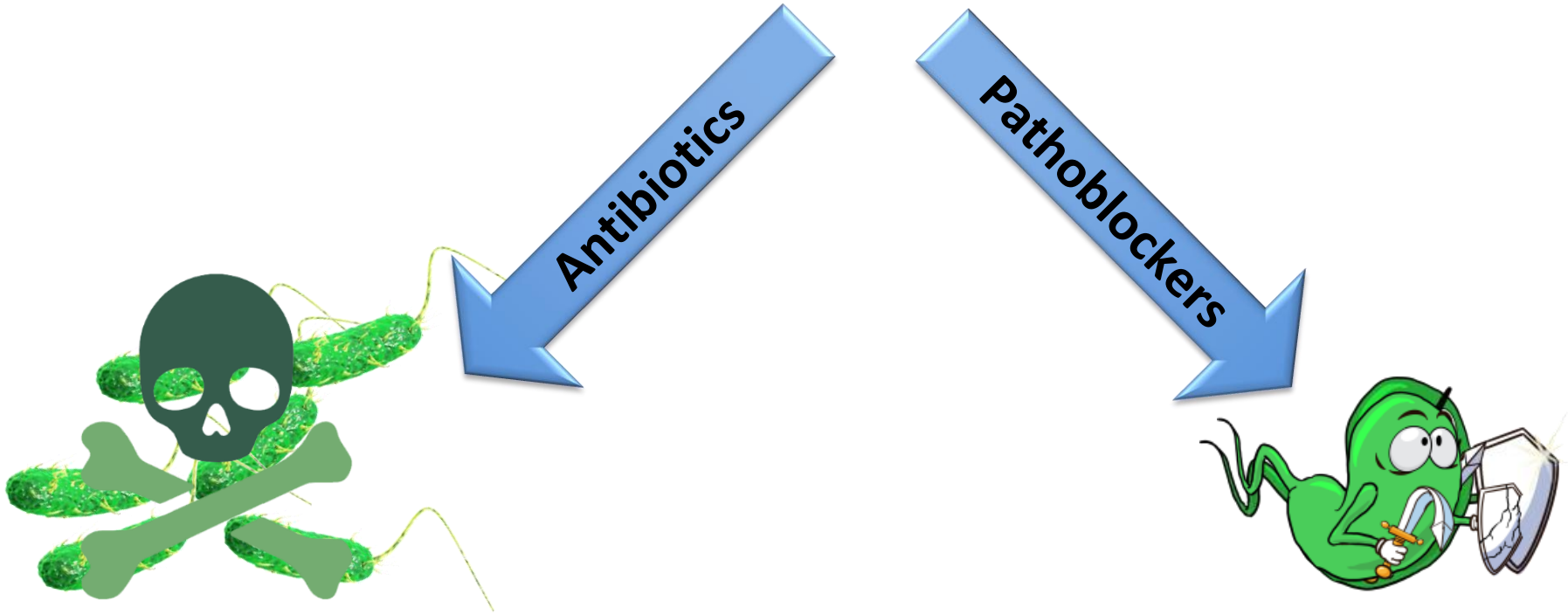


DnaN/pORF59
MIC (MS): 0.25 µM
Target strain(s):
broad spectrum
MT, AB… & KSHV



PA = *Pseudomonas aeruginosa*; CH = *Clostridium histolyticum*; MT = *Mycobacterium tuberculosis*; MS = *Mycobacterium smegmatis*; TB = *Trypanosoma brucei;* SA = *Staphylococcus aureus*; PF = *Plasmodium falciparum*; YT = *Yersinia pseudotuberculosis;* PF = *Plasmodium falciparum*; SP = *Streptococcus pneumoniae;* BC = *Bacillus cereus;* EnC = *Enterobacter cloacae;* KP = *Klebsiella pneumoniae;* EC = *Escherichia coli;* AB = *Acinetobacter bamannii;* SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2; RSV = respiratory syncitial virus; KSHV: Kaposi's sarcoma-associated herpesvirus

Classical antibiotics *vs* pathoblocker approach



bacteriostatic/
bactericidal effects

- prone to resistance development

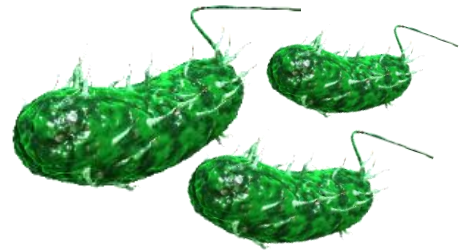
do not kill bacteria but disable virulence/pathogenicity traits

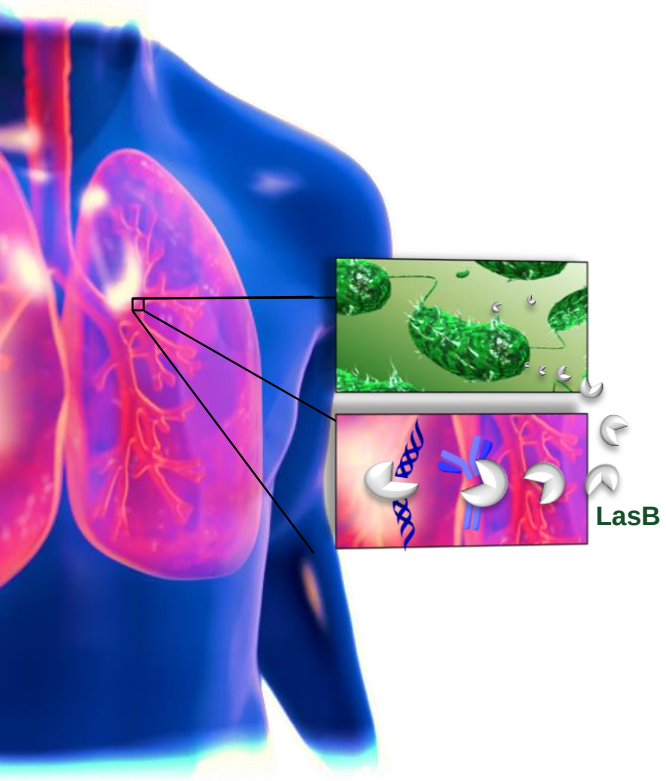
- low selection pressure towards resistance
- preserve commensal bacteria

PoC: *Clostridium difficile* Toxin B (Bezlotoxumab, Merck, Sharp & Dohme); approved in 2017

Pseudomonas aeruginosa LasB as target

- ***Pseudomonas aeruginosa*** classified as high priority by the WHO
- Causes infections of the lungs (*e.g.,* in HAP/VAP patients), skin/wounds, eyes, urinary tract, …

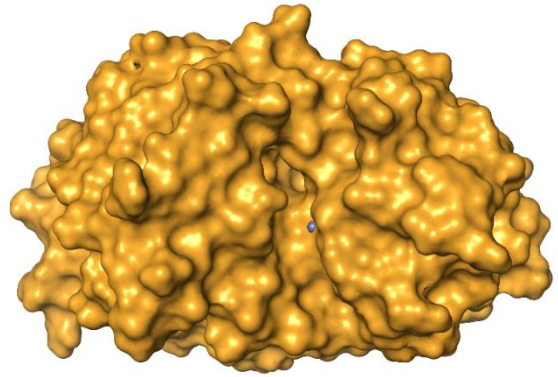




Target enzyme: Elastase (LasB)

- Secreted zinc-metalloprotease
- Degrades elastin and collagen (→ tissue penetration)
- Degrades components of the host immune system (→ immune evasion)
- Validated target enzyme, highly conserved across clinical isolates

→ Virulence factor LasB as excellent target protein



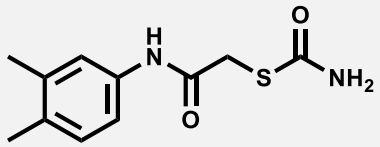
V. Camberlein *et al.*, *Antibiotics* **2022**
J. Zupetic *et al.*, *Chest* **2021**
F. Bastaert *et al.*, *Front Immunol*. **2018**

LasB inhibitor hit discovery and hit-to-lead optimisation

Hit Discovery

Functional FRET-based screening of a focused protease inhibitor library

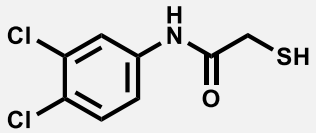
(*TimTec ActiTarg-P library* with 1192 compounds)



Hit compound

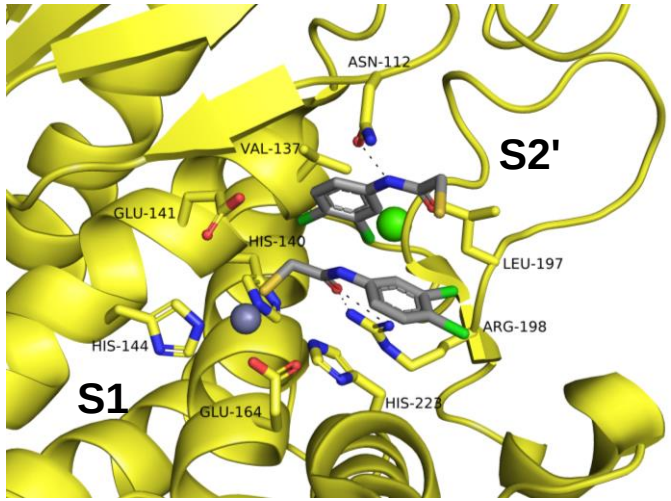
IC_{50} = 16.0 ± 1.9 µM

MedChem optimisation
Further experiments (esp. X-ray)



Thiol as active compound

IC_{50} = 6.6 ± 0.3 µM



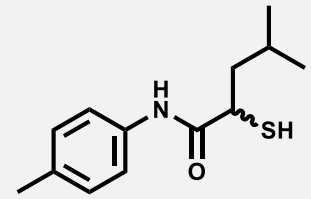
Structure-based H2L optimisation

Kany *et al*., *ACS Infect. Dis.* **2018**

Hit-to-Lead

Rational design of 55 derivatives

- Successful fragment merging/linking
- Advantageous α-substitution
- 16-fold improvement of IC_{50}
- High selectivity over human Zn enzymes
- No cytotoxicity



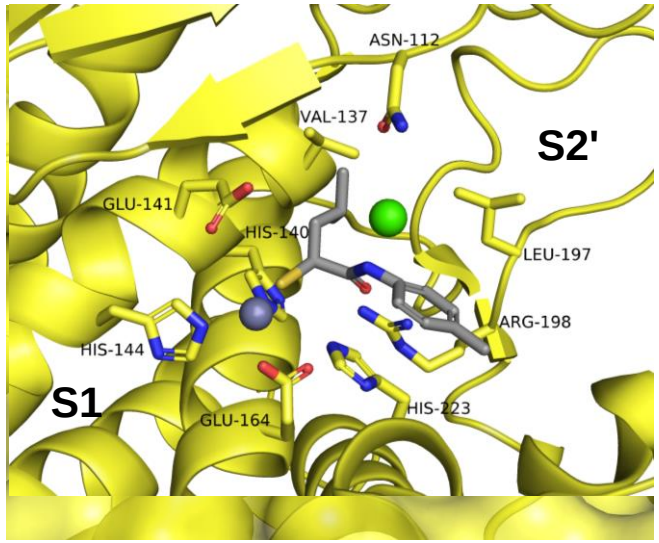
Optimised hit

IC_{50} = 0.4 ± 0.1 µM

Off-Target	Inh. @ 100 µM
MMP-1	n.i.
MMP-2	n.i.
MMP-3	n.i.
MMP-7	n.i.
MMP-8	22%
MMP-14	n.i.
HDAC-3	n.i.
HDAC-8	n.i.

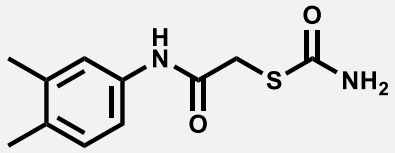
Cell Line	LD_{50} (µM)
HepG2	>100
A549	>100

n.i. = <10% inhibition

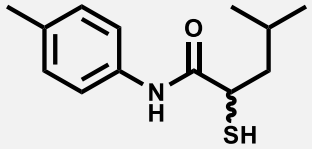


Kaya *et al*. *ACS Infect. Dis.* **2022**
Kaya *et al*., *Angew. Chem.* **2022**

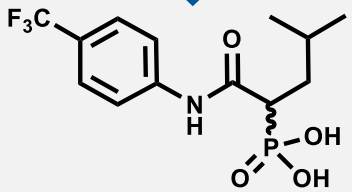
Hit-to-lead optimisation



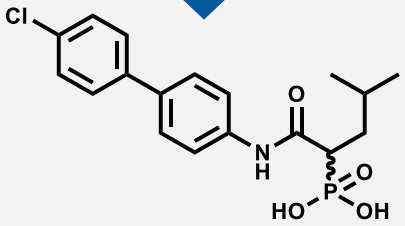
IC_{50} = 16.0 ± 1.9 µM



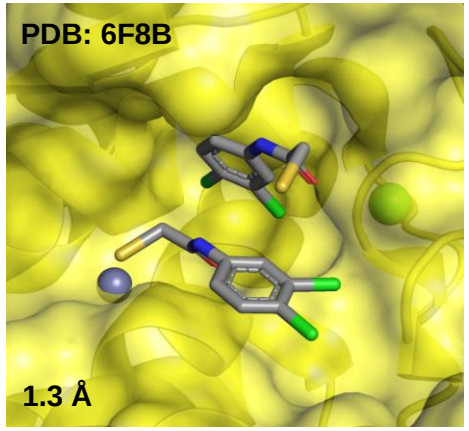
IC_{50} = 0.4 ± 0.1 µM

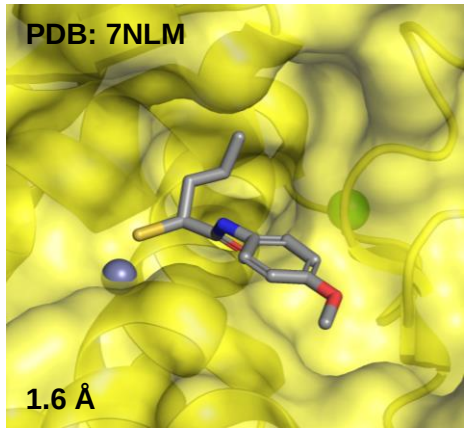


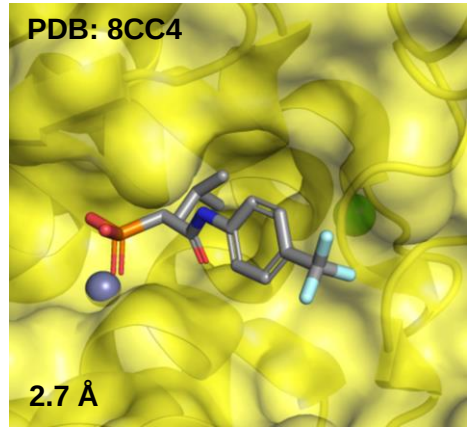
IC_{50} = 26.3 ± 7.6 nM

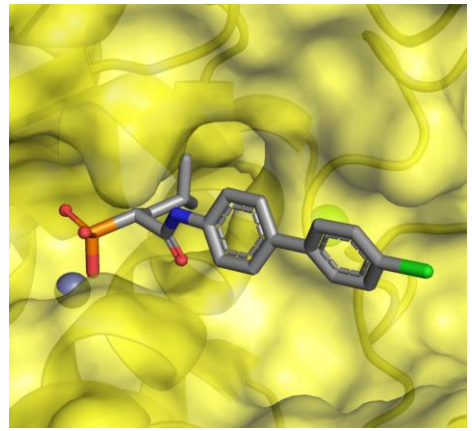


IC_{50} = 7.8 ± 0.3 nM







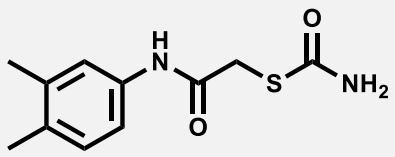


Initial fragment hit → **Substrate-inspired optimisation** → **Zinc-binding group replacement**

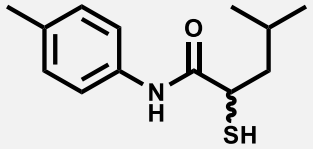
J. Konstantinovic *et al.*, *ACS Centr. Sci.* **2023**
D. Kolling, J. Haupenthal, A. K. H. Hirsch, J. Köhnke, *ChemMedChem* **2023**
C. Kaya *et al.*, *Angew. Chem. Int. Ed.* **2022**
C. Kaya *et al.*, WO 2022/043322A1, **2020**

with
Katharina Rox
Jesko Köhnke
Markus Bischoff
Jean-Michel Sallenave

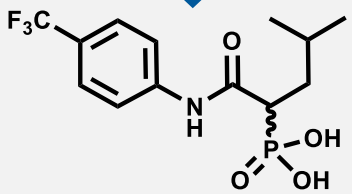
Further hit-to-lead optimisation



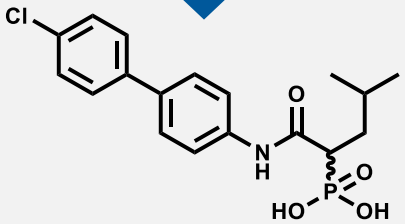
IC_{50} = 16.0 ± 1.9 µM



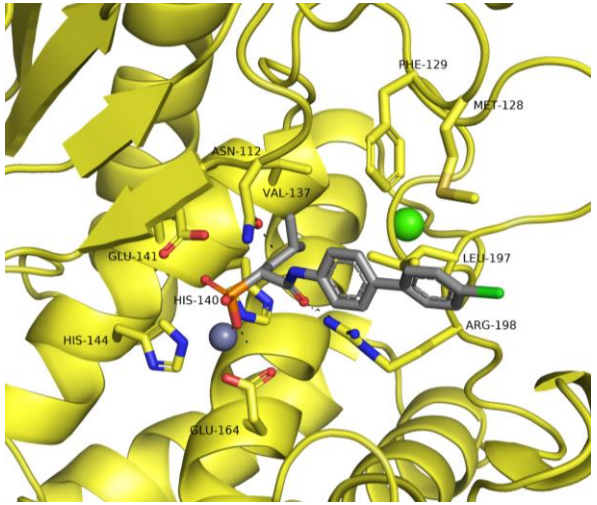
IC_{50} = 0.4 ± 0.1 µM



IC_{50} = 26.3 ± 7.6 nM

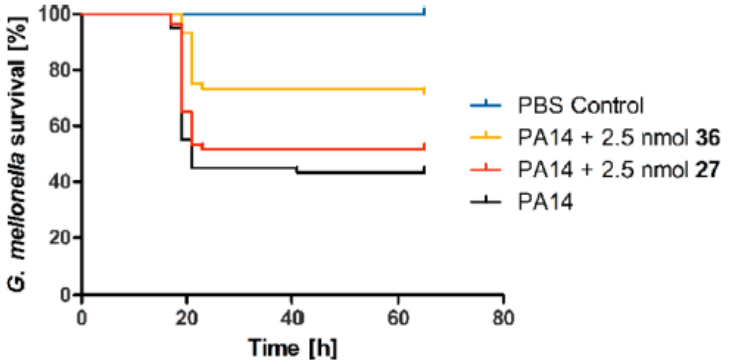


IC_{50} = 7.8 ± 0.3 nM



- >550 derivatives, >1000-fold improvement
- Potent inhibitors across five chemical classes
- High selectivity toward human off-targets
- No interference with standard-of-care antibiotics
- Favourable *in vitro* ADMET parameters
- *In vivo*: well tolerated in mice, promising *in vivo* PK profiles

→ Good prerequisites for carrying out *in vivo* PD experiments

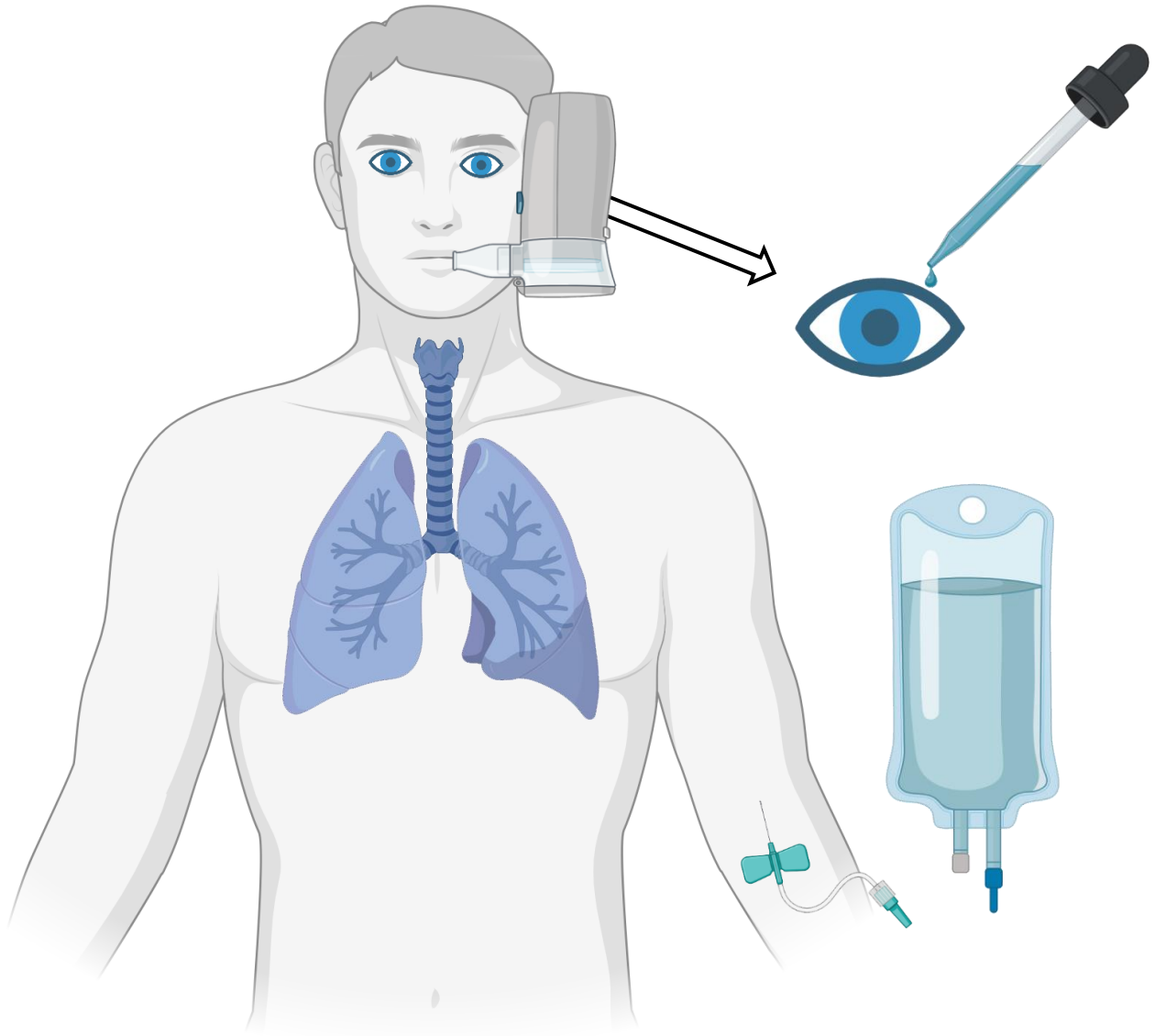


First trial in a simple *in vivo* infection model:

LasB inhibitors active in *Galleria mellonella* larvae

J. Konstantinovic *et al.*, *ACS Centr. Sci.* **2023**
C. Kaya *et al.*, *Angew. Chem. Int. Ed.* **2022**
C. Kaya *et al.*, WO 2022/043322A1, **2020**

Target indications and routes of administration



Lungs as target organ

IV route of administration
→ Indication **Hospital-acq. / ventilator-assoc. pneumonia**

Inhalation
→ Indication **Cystic Fibrosis**

Eyes as target organ

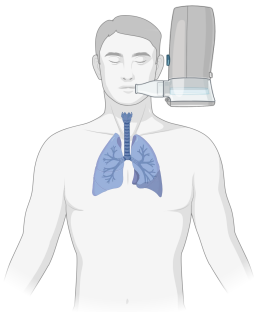
Topical administration, eye drops
→ Indication ***Pseudomonas* keratitis**

Status (based on experiments in mice)

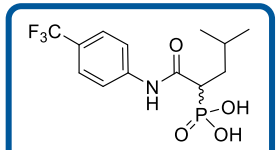
HAP/VAP: *in vivo* PoC **provided**
CF: *in vivo* PoC **provided**
Keratitis: *in vivo* PoC **provided**

→ Further *in vivo* studies planned, to (further) optimize efficacy

PoC = proof of concept

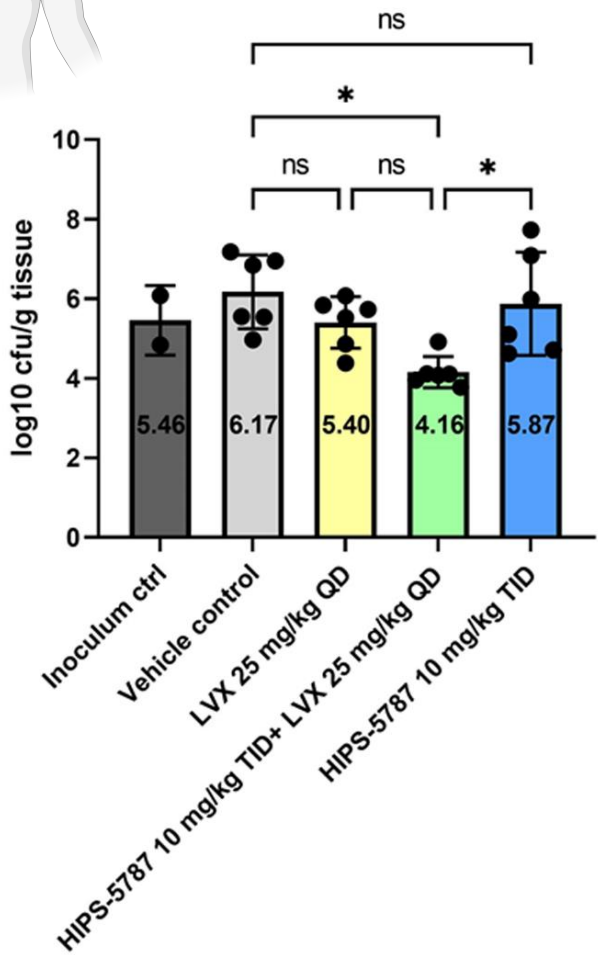


In vivo efficacy studies – HIPS-5787 effective after inhalation



HIPS-5787

CFUs in lungs



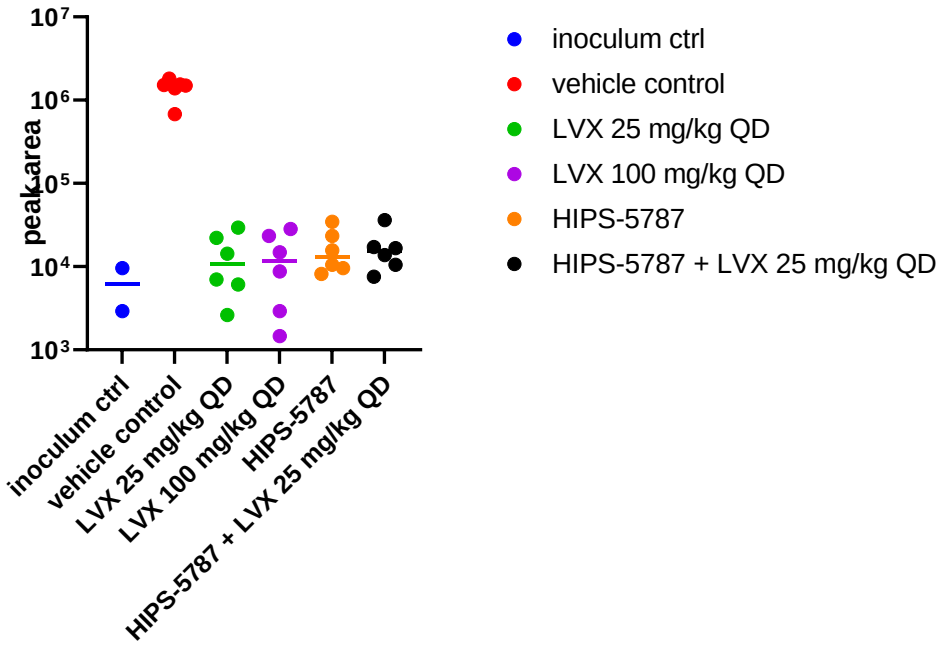
Efficacy of **HIPS-5787** after inhalation

Performed with *P. aeruginosa* **DSM-1117** and **neutropenic** mice

→ Significant CFU reduction for combination with levofloxacin (LVX)

→ Repetition of this experiment could confirm this result

LasB levels in blood



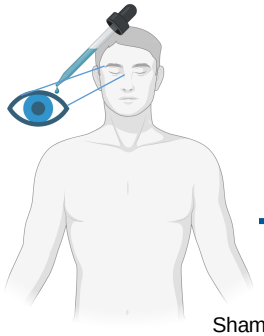
LasB levels in blood strongly reduced

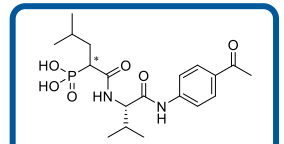
→ Decreased dissemination, even in absence of LVX → **target engagement**

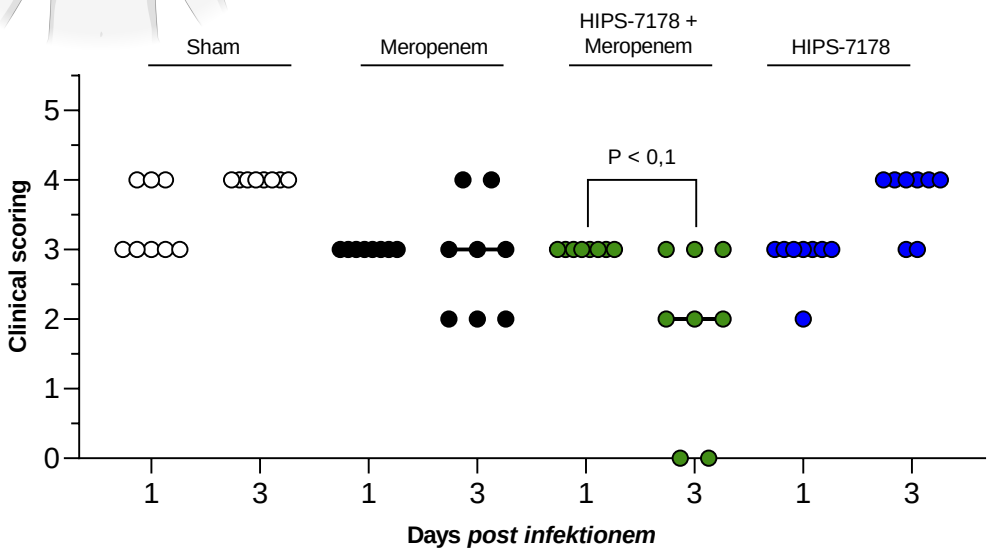
→ ***In vivo* proof-of-concept provided**

J. Konstantinovic *et al.*, *ACS Centr. Sci.* **2023**

In vivo studies – topical treatment of *Pseudomonas* keratitis



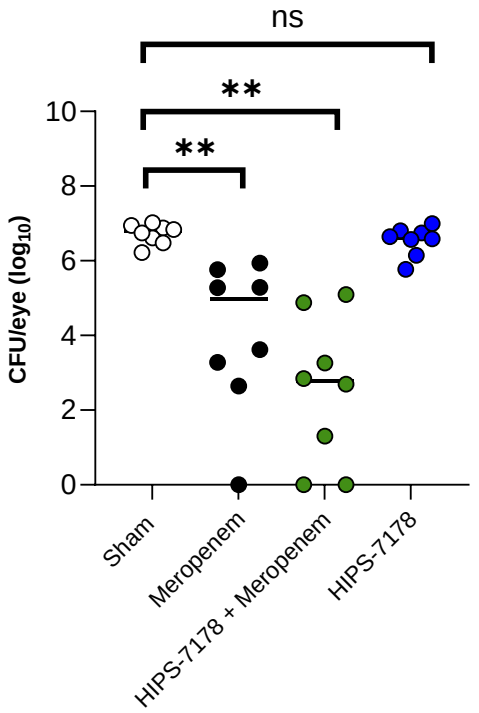




Clinical scores improved after single treatment with meropenem and **HIPS-7178**, and combination of both

In correlation with this finding:

Body weight of mice least affected after combination treatment



→ No effect of LasB inhibitor alone on **number of CFUs in the eye**

→ Synergistic and significant effect for combination with meropenem

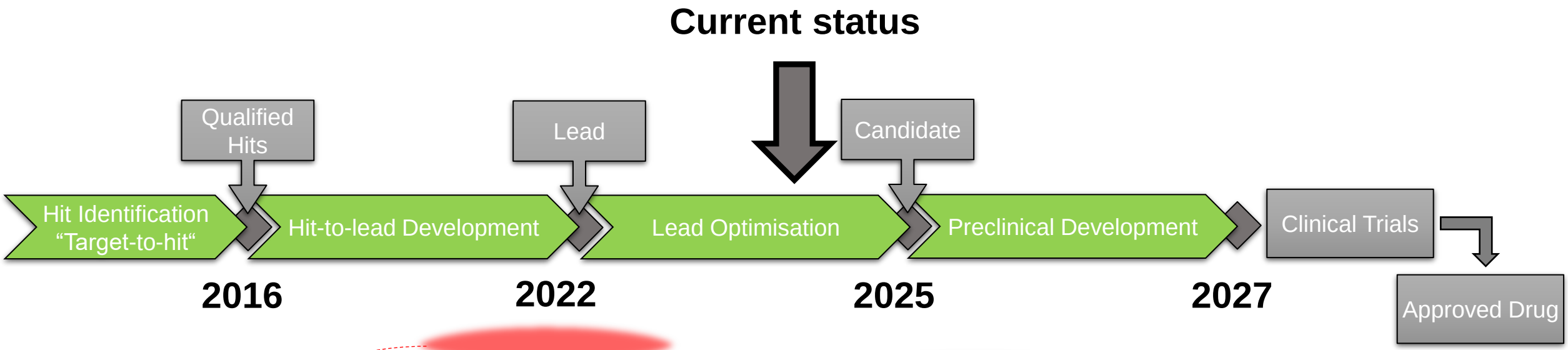
Effects on cellular and humoral immune response:

- Reduced **immune cells in eye**, especially after combination treatment
- No major effects on immune cells in blood
- **Inflammation markers** in eye: significant reduction of IL-1beta levels *via* **HIPS-7178** treatment, and mero/**HIPS-7178** combination

Female C57BL/6 N mice infected with 5x10^7 CFUs of *P. aeruginosa* PA54,
9 treatments (every 8 h for 72 h); **HIPS-7178** at 1 mg/mL, meropenem at 0.5 mg/mL

A. F. Kiefer, C. Schütz *et al.*, *Adv. Sci.* **2025**.

LasB project status



CARB-X
Combating Antibiotic-Resistant Bacteria

10/2020 – 05/2023

Bundesministerium für Bildung und Forschung

03/2021 – 02/2024

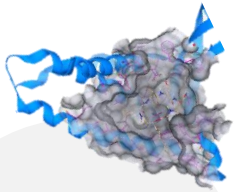
INCATE

01/2024 – 12/2024

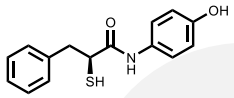
DZIF
Deutsches Zentrum für Infektionsforschung

03/2024 – 08/2026

Conclusions



LspA
IC_{50} : 47 nM
Target strain(s):
PA, EC

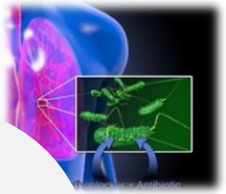


ColH/Q1
IC_{50} : 10 nM
Target strain(s):
CH, BC

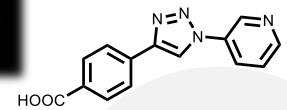
CARB-X
Combating Antibiotic-Resistant Bacteria

LasB
IC_{50} : 1.5 nM
Target strain:
PA

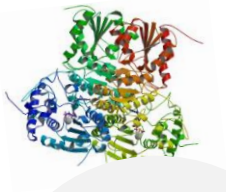
LecA/B
IC_{50} : 10.8 nM
Target strain:
PA



PqsR
IC_{50} : 5 & 30 nm
(PqsR & Pyocyanin)
Target strain: PA



LANA
K_D: 5 µM
Target virus:
KSHV



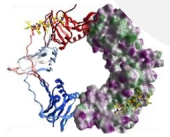
IspD & IspE
IC_{50} (PF): 10 nM
Target strain(s):
MT, PF, EC, AB

DXS
IC_{50} (PF): 0.7 µM
Target strain(s):
MT, PF, EC

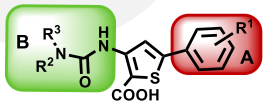
- **Target-based anti-infective drug discovery**
- **Multiple hit-identification strategies**
- **Early multiparameter optimisation**

RNAP
IC_{50} : 6 µM
MIC (SA): 1 µM
Target strain(s):
broad spectrum
SA, MT…

ECF-T
MIC: 0.5 mM
Target strain(s):
SA, SP

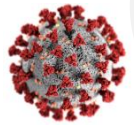


DnaN
MIC (MS): 0.5 µM
Target strain(s):
MT, AB

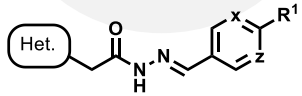


F protein
IC_{50} : 40 nM
Target virus:
RSV

VolkswagenStiftung



Nsp10/12/14
IC_{50} (RdRp): 1 µM
Target strain(s):
SARS-CoV-2



PA = *Pseudomonas aeruginosa*; CH = *Clostridium histolyticum*; MT = *Mycobacterium tuberculosis*; MS = *Mycobacterium smegmatis*; TB = *Trypanosoma brucei;* SA = *Staphylococcus aureus*; PF = *Plasmodium falciparum*; YT = *Yersinia pseudotuberculosis;* PF = *Plasmodium falciparum*; SP = *Streptococcus pneumoniae;* BC = *Bacillus cereus;* EnC = *Enterobacter cloacae;* KP = *Klebsiella pneumoniae;* EC = *Escherichia coli;* AB = *Acinetobacter bamannii;* SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2; RSV = respiratory syncitial virus

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Dr Virgyl Camberlein
Dr Ravindra Jumde
Dr Sebastian Adam
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M. Bischoff (UKS)
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Prof. R. Voulhoux (CNRS, Marseille)

Zebrafish toxicity
Y.-M. Park
Prof. R. Müller (HIPS)

Cell permeability
Prof. C.-M. Lehr (HIPS)

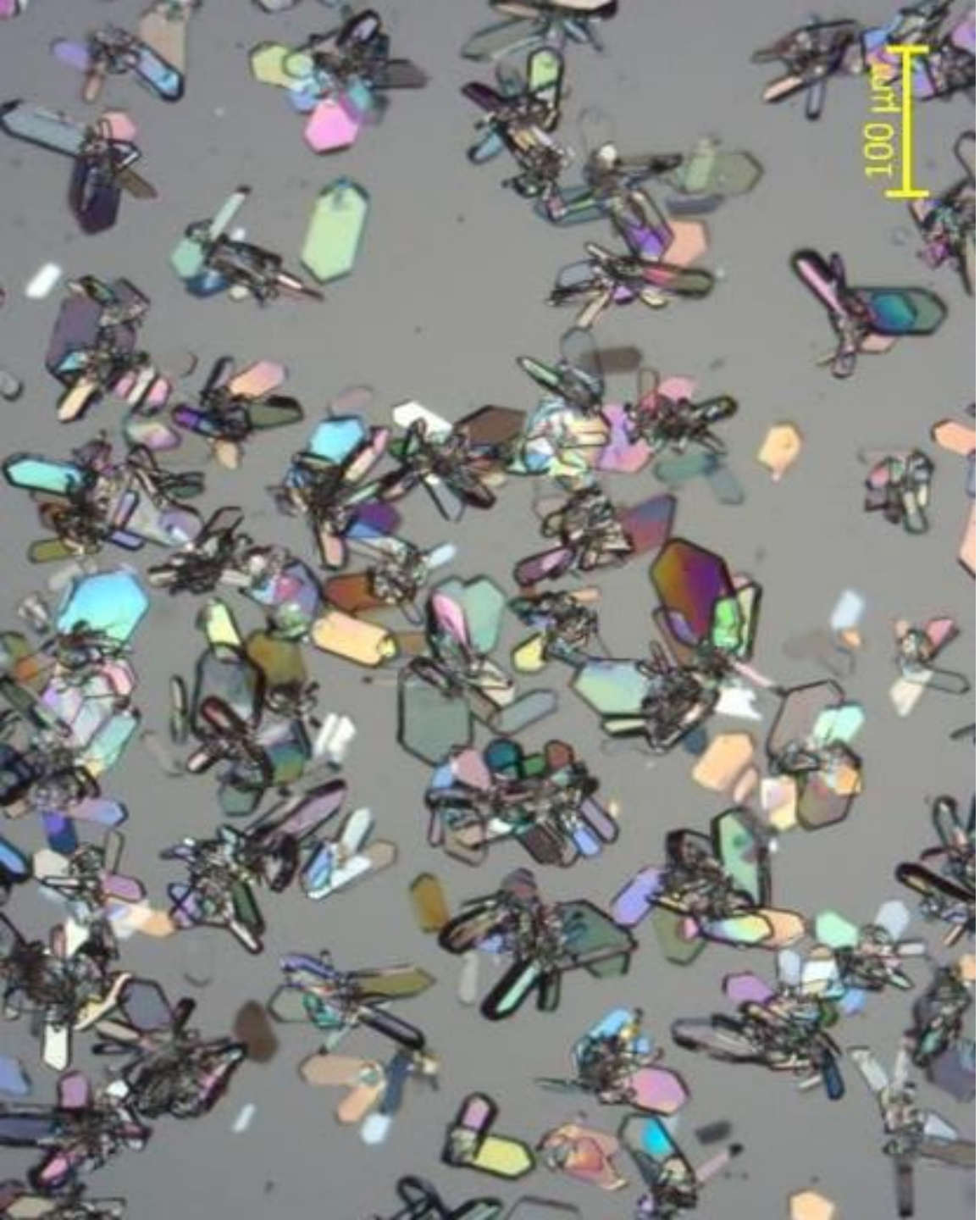
PK/PD
Dr K. Rox (HZI)

HELMHOLTZ RESEARCH FOR GRAND CHALLENGES
erc European Research Council
VolkswagenStiftung
Bundesministerium für Bildung und Forschung
CARB-X Combating Antibiotic-Resistant Bacteria
MARIE CURIE ACTIONS
MeP Anti

Patrizio Mattei

Patrizio Mattei is a Distinguished Scientist, Medicinal Chemistry at the Roche Innovation Center Basel, Pharma Research & Early Development (pRED). His research interests have been mainly devoted to the areas of metabolic diseases, ophthalmology, and more recently antibiotics. Patrizio led the medicinal chemistry team that discovered the novel antibiotic, zosurabalpin.

Before joining Roche in 1999, Patrizio received his doctoral degree from ETH Zürich and was a postdoctoral research fellow at The Scripps Research Institute in La Jolla, California.



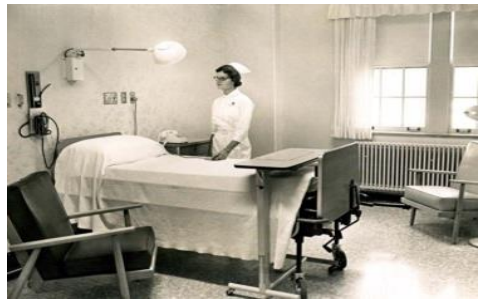
Zosurabalpin, a tethered macrocyclic peptide antibiotic selective for Acinetobacter: The importance of chemical synthesis

REVIVE webinar by GARDP, January 23th 2025

Dr. Patrizio Mattei
Small Molecule Research, Therapeutic Modalities
Pharma Research & Early Development
Roche Innovation Center Basel

Acinetobacter baumannii, carbapenem-resistant (CRAB)

Evolution from a rare infection to a challenging hospital pathogen



World Health Organization

1960s-70s: Emergence, parallel rise in ICU care

2003: Outbreaks in soldiers during Iraq war

2006→: Global spread of carbapenem resistance

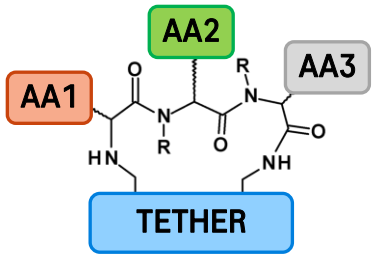
2017 and again, 2024: WHO #1 Priority Pathogen

- No new chemical class of antibiotics since the carbapenems (1980s)
- Our ambition: Significantly improve treatment, reset the clock on resistance
  - Inhibit previously undrugged targets, overcome pre-existing resistance mechanisms

The discovery of zosurabalpin

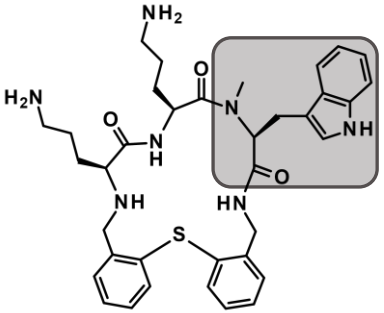
Optimization of a phenotypic high-throughput screening (HTS) hit without mechanistic insight

tranzyme pharma



HITCREATE™ Library
(R = H, Me)

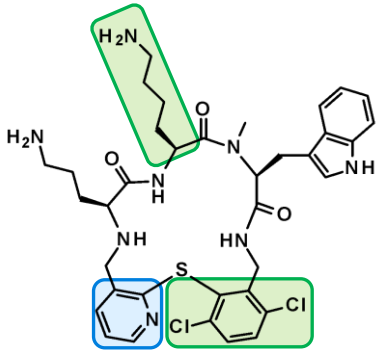
Phenotypic screen for bacterial growth inhibition



RO7036668
HTS hit

- Selective for Acinetobacter
- Not potent enough

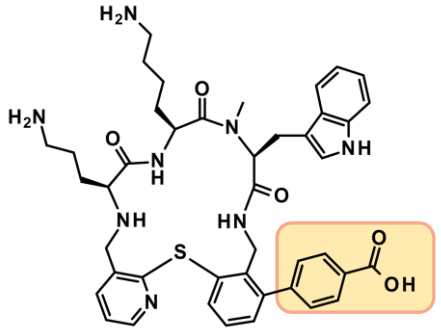
Optimize potency



RO7075573
1st generation lead

- Active in vivo (mouse)
- Poor plasma compatibility
- Causes organ toxicity

Minimize plasma precipitation, focus on zwitterionic compounds



zosurabalpin
clinical development

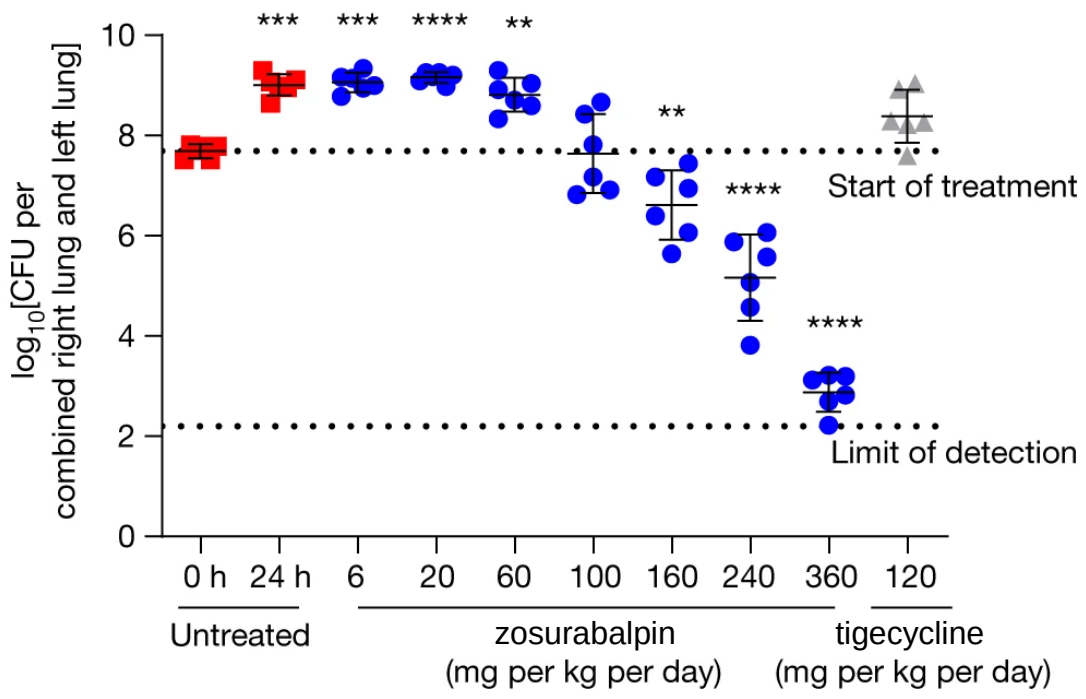
- Active in vivo (mouse)
- Favourable PK and safety profile (humans)

C. Zampaloni, P. Mattei, K. Bleicher, et al. A novel antibiotic class targeting the lipopolysaccharide transporter. *Nature* **2024**, *625*, 566–571.

Zosurabalpin is efficacious in mice[a] and was well tolerated in humans

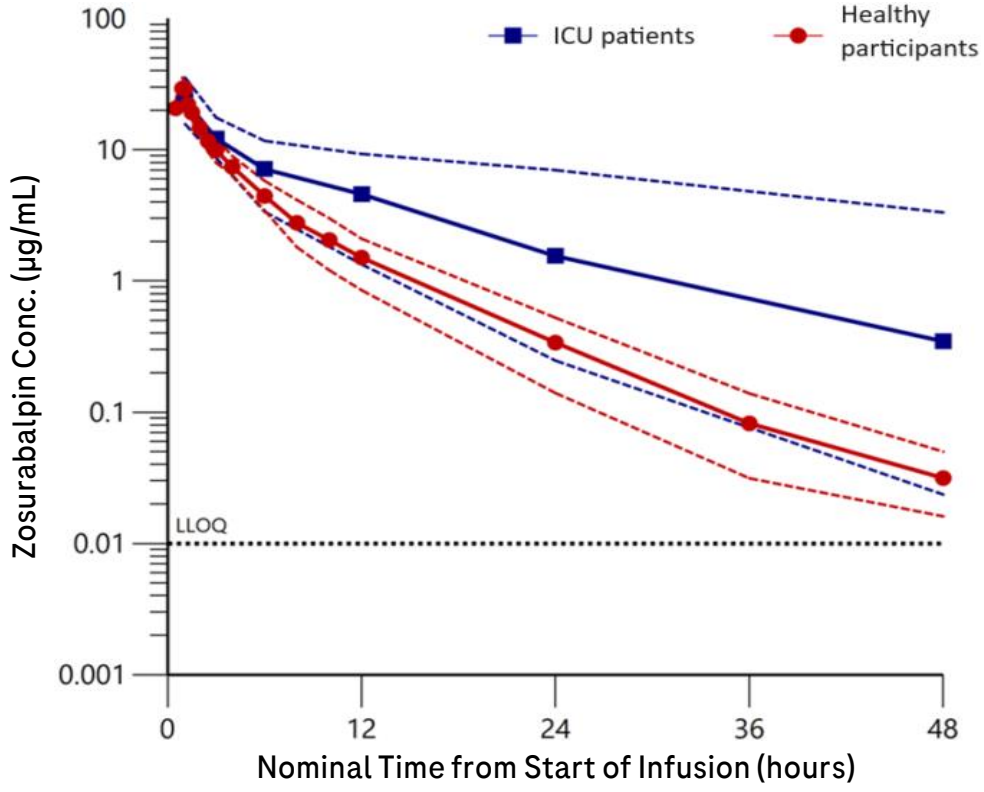
Effectively treats pan-drug resistant and virulent *A. baumannii* pneumonia in neutropenic mice

24-hour Acute Lung Infection Model[b] induced by pan-drug resistant *A. baumannii* ACC001073



Statistical analysis: one way ANOVA followed by Dunnett's post hoc test vs 0h

PK and safety of single dose zosurabalpin (600 mg) in healthy people and ICU patients[c] (phase 1)



[a] C. Zampaloni et al., *Nature* **2024**, *625*, 566. This work is licensed under CC BY 4.0. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

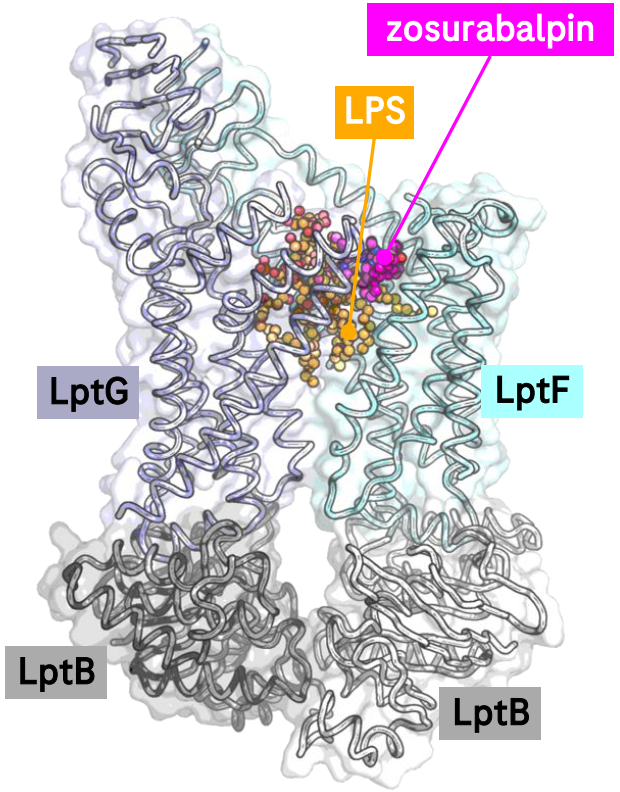
[b] Study conducted at CRO, Aptuit Verona/Evotec

[c] E. Cottreel et al. ESCMID Global, Barcelona, 28 April 2024, Poster number: P2419.

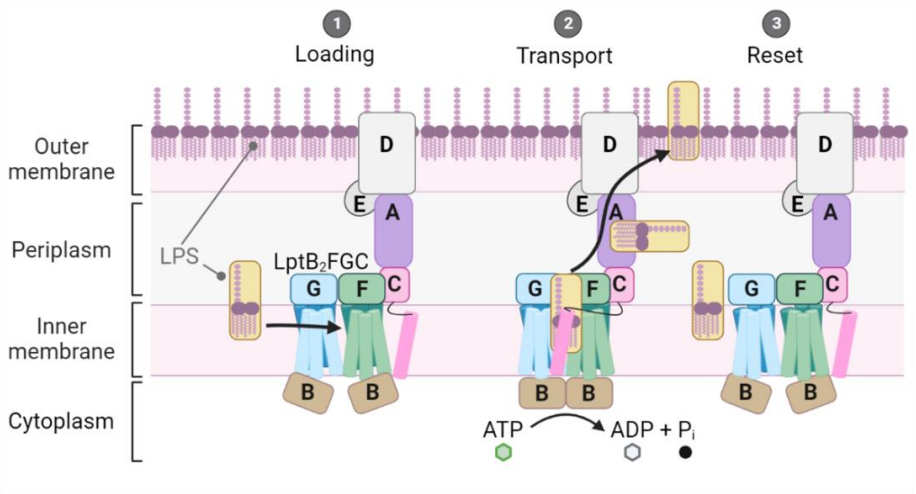
Mechanism of action: Inhibition of lipopolysaccharide (LPS) transport

Zosurabalpin (**ZAB**) binds to LPS-loaded $LptB_2FG$ intermediate, trapping LPS on its way to the outer membrane

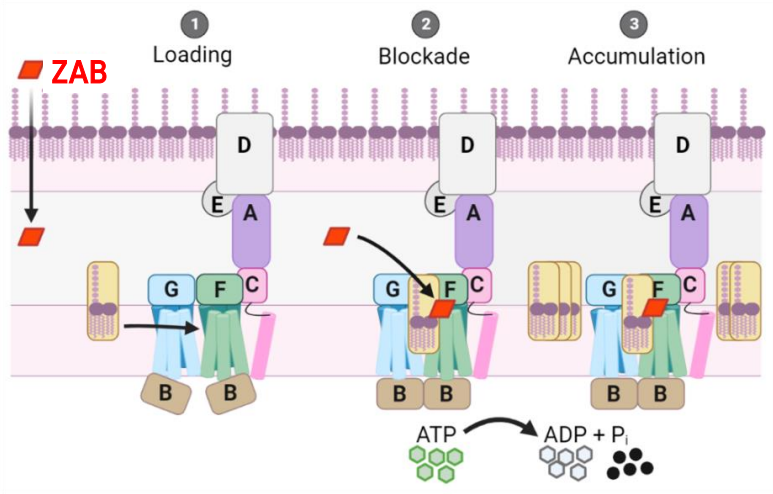
Acinetobacter baylyi[a] $LptB_2FG$ bound to LPS and zosurabalpin[b]



LPS transport in *Acinetobacter*



Impaired LPS transport in the presence of zosurabalpin

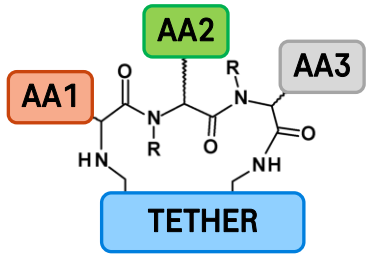


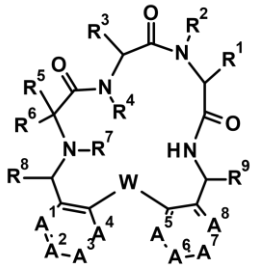
- Blocking LPS transport leads to *Acinetobacter* cell death.
- Binding site formed by *Acinetobacter* LPS-$LptB_2FGC$ is distinct from that of other gram negative bacteria.

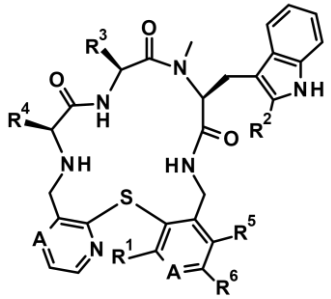
[a] *A. baylyi* $LptB_2FG$ is about 85% identical to *A. baumannii* and is similarly susceptible to zosurabalpin.
[b] Cryo-electron microscopy structure, PDB ID: 8frn. K. Pahil et al., *Nature* **2024**, *625*, 572.

Chemical synthesis: The small molecule drug candidate

Before a drug candidate can be tested, chemists must produce it



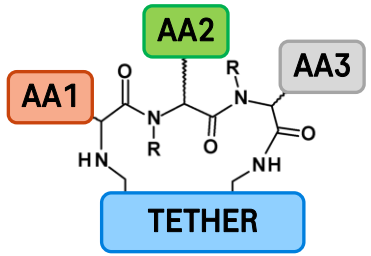


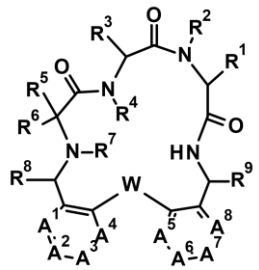


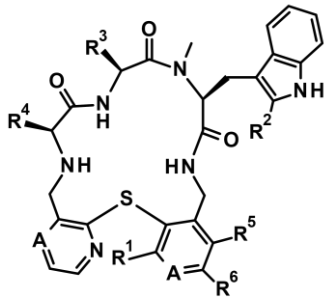
Stage of the project	HTS	Lead Identification	Lead Optimization
Number of test compounds	ca. 45000	a few hundred	a few hundred, including zosurabalpin
Required quantity	few mg	few mg	milligrams to grams
What for?	phenotypic screening (library was used for other projects as well)	in vitro testing, first animal studies	in vitro testing, animal studies, preformulation
Who?	chemists at Tranzyme	medicinal chemists	medicinal chemists

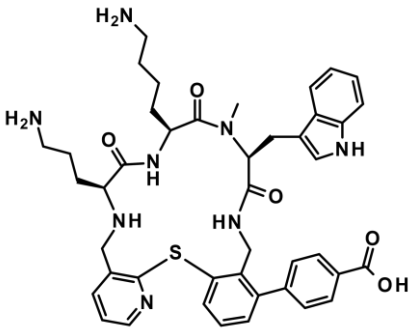
Chemical synthesis: The small molecule drug candidate

Before a drug candidate can be tested, chemists must produce it









Stage of the project	HTS	Lead Identification	Lead Optimization	Phase 0-3	Market
Number of test compounds	ca. 45000	a few hundred	a few hundred, including zosurabalpin	1	1
Required quantity	few mg	few mg	milligrams to grams	kilograms	kilograms to tons
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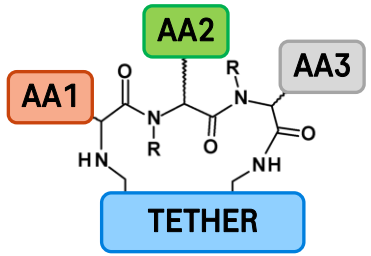
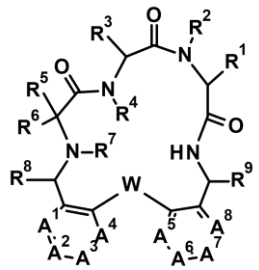
Who?	chemists at Tranzyme	medicinal chemists	medicinal chemists	process chemists	manufacturing chemists

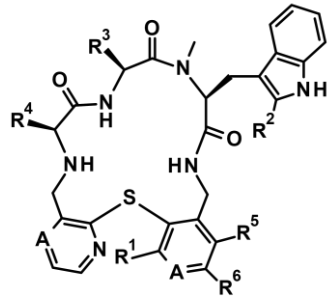
Clinical candidate selection

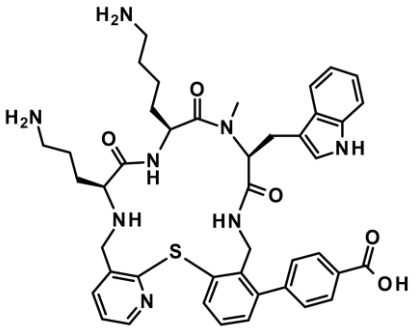
Chemical synthesis considerations

Different chemical synthesis requirements at any project stage, different chemistry skills required









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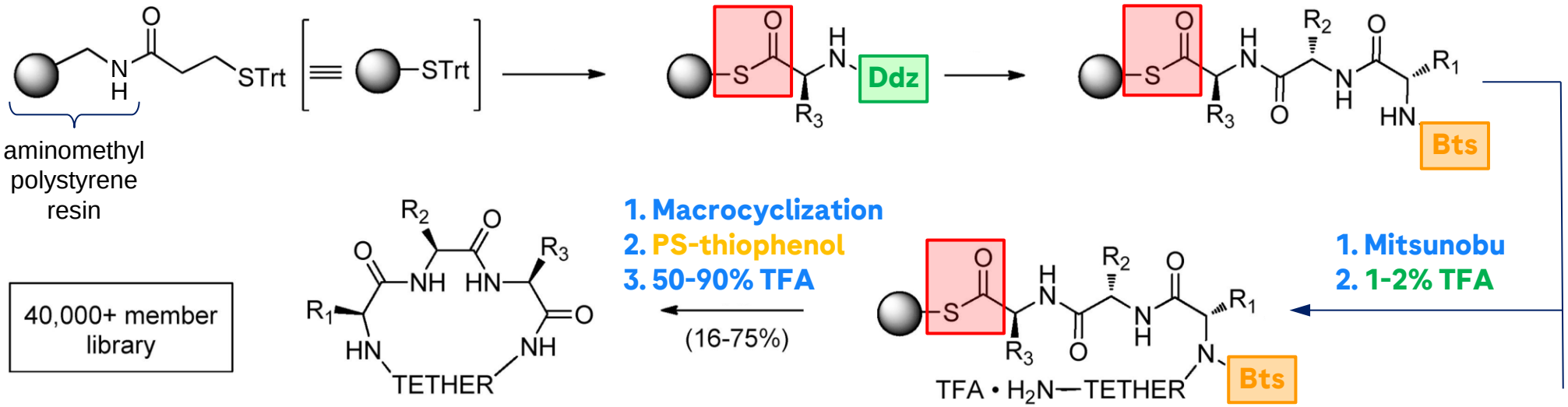
Who?	chemists at Tranzyme	medicinal chemists	medicinal chemists	process chemists	manufacturing chemists
Typical considerations in chemical synthesis	• versatility • building blocks • speed	• diversity • customization • purification	• focus • key intermediates • initial scale-up	• scalability • timelines • regulatory control	• supply • cost • robustness

Synthesis of the HTS library

Solid phase peptide synthesis, solid supported reagents, cyclative release mechanism

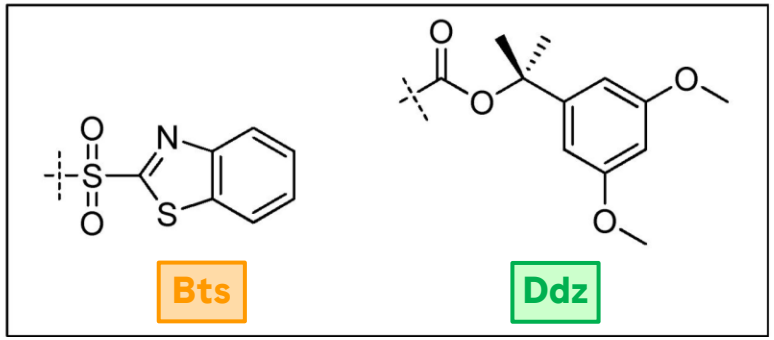
- versatility
- building blocks
- speed

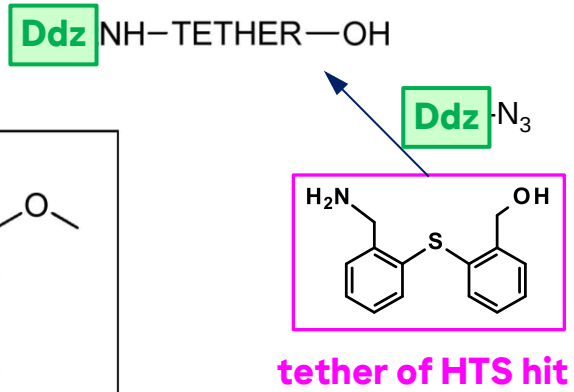
tranzyme pharma



- high tripeptide diversity
- off-the-shelf tethers

- **thioester is not stable enough**





tether of HTS hit

E. Marsault et al., *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4731-4735.

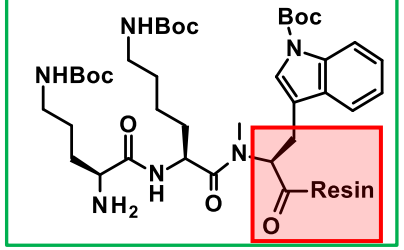
Medicinal Chemistry synthesis of zosurabalpin

Methodology suitable up to gram amounts

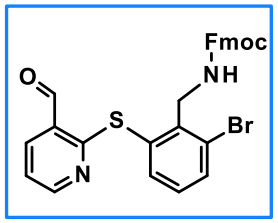
- focus
- key intermediates
- initial scale-up

Roche

solid phase peptide synthesis on 2-chloro-tritylchloride resin using Fmoc amino acids

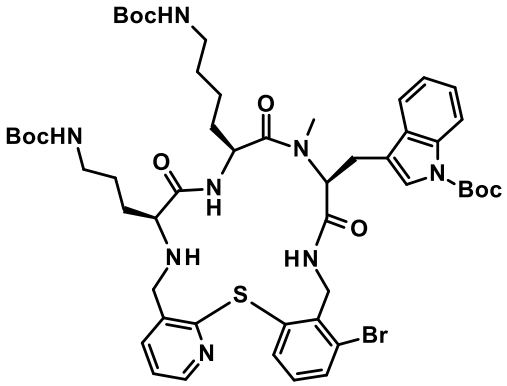


tether



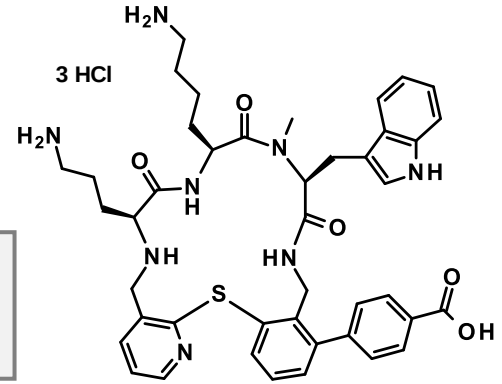
1. Reductive amination
2. Fmoc deprotection
3. Cleavage off resin (HFIP)
4. Macrocyclization

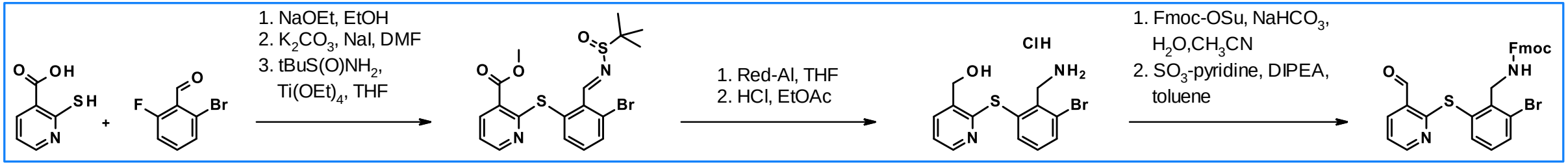
- **epimerization during HFIP cleavage after >10 min**
- chromatography



1. Suzuki coupling
2. Deprotection (TFA)
3. Salt change (TFA to HCl)

- trifluoroacetic acid
- chromatography
- product is hygroscopic



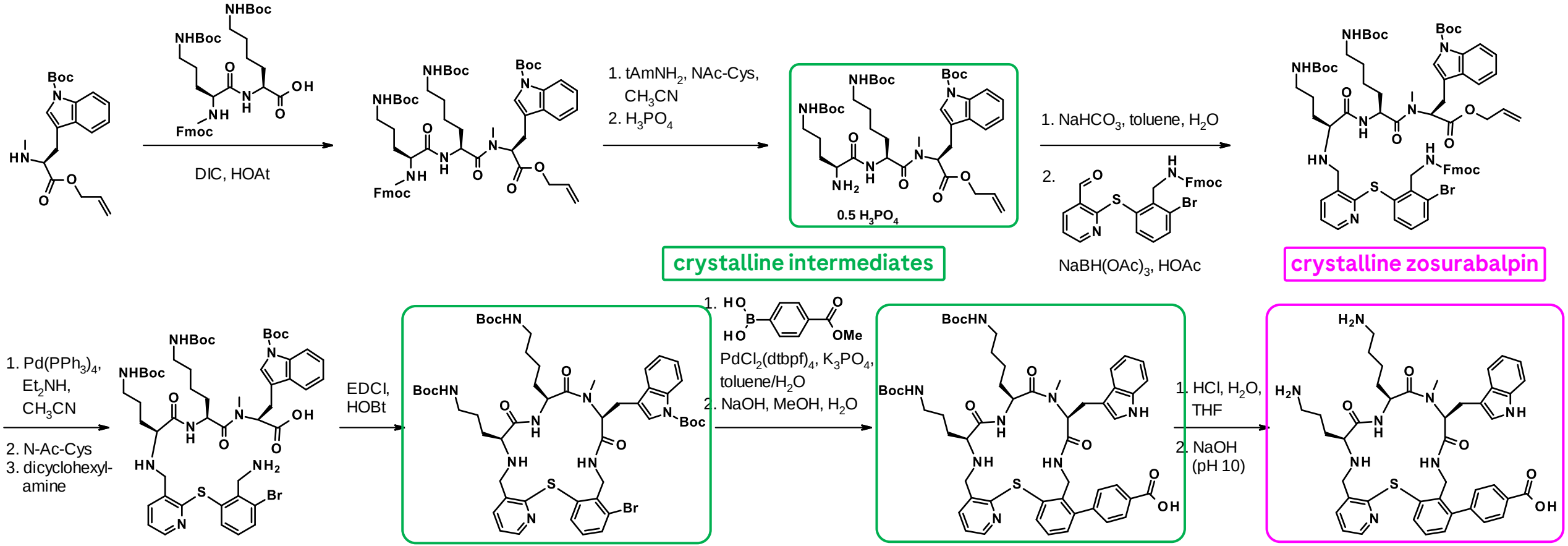


C. Zampaloni et al., *Nature* **2024**, *625*, 566-571.

Scalable synthesis[1,2] of zosurabalpin

Multi-kg batches delivered for clinical studies

- scalability
- timelines
- regulatory control



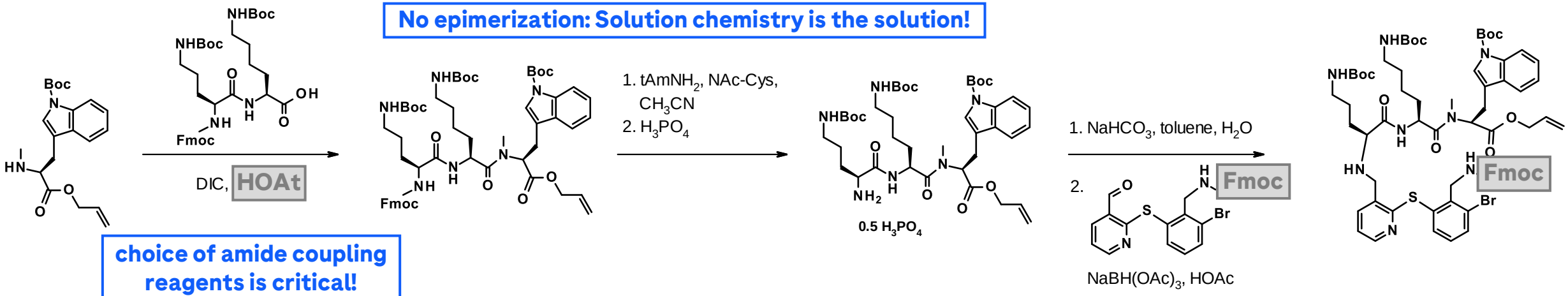
[1] Adam, Jean-Michel; Bliss, Fritz; Dott, Pascal Jean Claude; Hoffmann-Emery, Fabienne Roxane; Larsson, Ulf Goeran; Puentener, Kurt, WO2023/152347.
[2] Bigler, Raphael; Bliss, Fritz Theodor; Fantasia, Serena Maria; Hoffmann-Emery, Fabienne Roxane; Santandrea, Ernesto, WO2024/033278.

- scalability
- timelines
- regulatory control

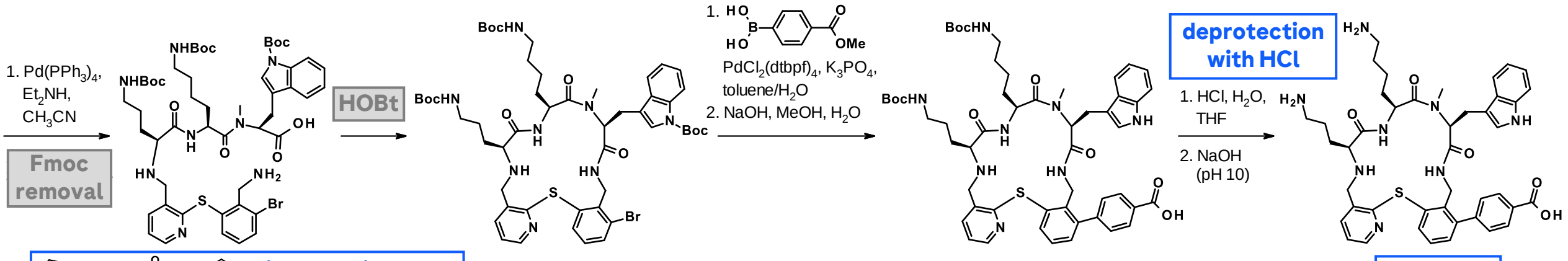
Scalable synthesis[1,2] of zosurabalpin

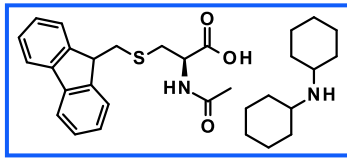
Multi-kg batches delivered for clinical studies

No epimerization: Solution chemistry is the solution!



choice of amide coupling reagents is critical!





dihexylamine salt of dibenzofulvene-NAc-Cys adduct (s)

parent molecule

[1] Adam, Jean-Michel; Bliss, Fritz; Dott, Pascal Jean Claude; Hoffmann-Emery, Fabienne Roxane; Larsson, Ulf Goeran; Puentener, Kurt, WO2023/152347.
[2] Bigler, Raphael; Bliss, Fritz Theodor; Fantasia, Serena Maria; Hoffmann-Emery, Fabienne Roxane; Santandrea, Ernesto, WO2024/033278.

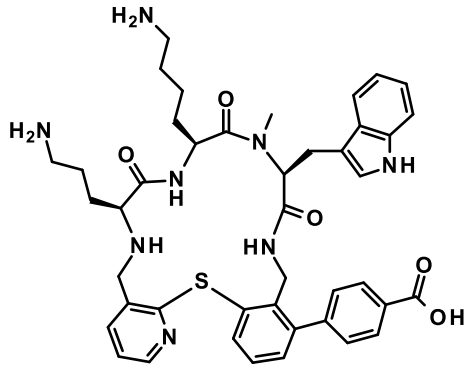
Commercial synthesis of zosurabalpin

- supply
- cost
- robustness

Roche

starting materials

work in progress!



zosurabalpin

Summary

- Zosurabalpin is a novel antibiotic to treat difficult-to-treat *Acinetobacter baumannii* infections.
  - The discovery of zosurabalpin started with the identification of a high-throughput screening (HTS) hit from a library of ca. 45000 compounds, which were all **made by chemists**.
  - The optimization from the HTS hit to zosurabalpin went through several hundred new drug candidates. **Medicinal chemists** produced them.
- In a drug discovery project, the role of chemical synthesis undergoes a **fundamental change at clinical candidate selection**:
  - Early project stages (HTS to lead optimization) are **chemical diversity** oriented processes. Medicinal chemists invent and produce several new drug candidates in sufficient amounts for in vitro and animal testing.
  - After clinical candidate selection, the task of chemical synthesis is handed over to **process chemists**, who create a synthesis of the selected molecule to supply the material for clinical studies, with the potential to be later technically developed into a **large-scale production process**.

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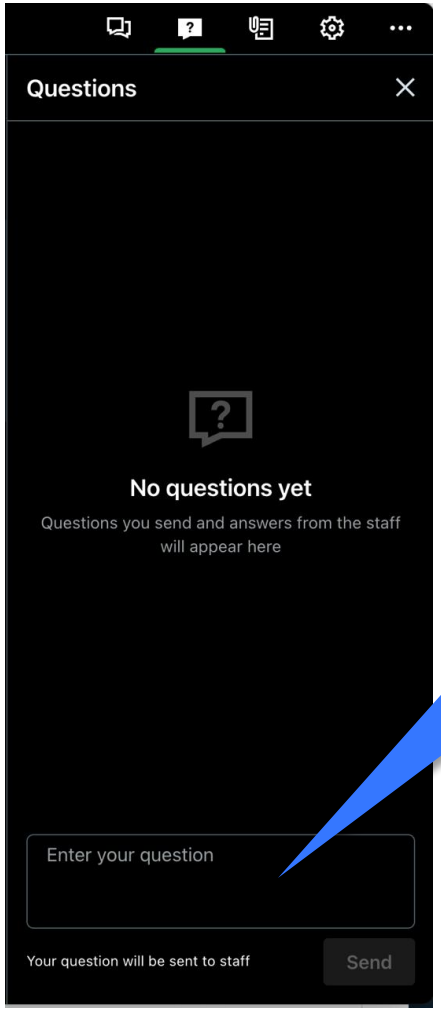
Doing now what patients need next

How to submit your questions

REVIVE by GARDP



If your question is addressed to a specific speaker, please include their name when submitting the question.

Please submit your questions through the box provided after clicking the ‘questions’ button. We will review all questions and respond to as many as possible after the presentation.

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REVIVE by GARDP

The importance of chemical synthesis for antimicrobial research and development

Moderator:
Ravindra Jumde
GARDP

Anna K.H. Hirsch
Helmholtz institute for Pharmaceutical Research Saarland

Patrizio Mattei
Roche Innovation Center Basel

Upcoming webinars

REVIVE by GARDP

In vitro and in vivo correlations for prediction of human pharmacokinetics and dose of antimicrobials

- With Mathew Njoroge & Nina Lawrence
- 27 February 2025, 17:00-18:30 CET

Charting new frontiers in artificial intelligence for antibiotic design

- With Jonathan Stokes & Kurt Thorn
- 3 April 2025, 17:00-18:30 CEST

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Thank you for joining us