

Adressing underexplored anti-infective targets

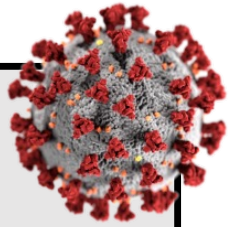
Anna K. H. Hirsch
IASOC 2022
24th of September 2022

@Helmholtz_HIPS
@HirschLab

UNIVERSITÄT DES SAARLANDES

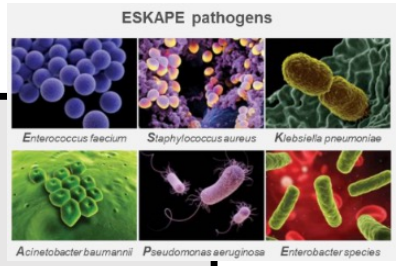
HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

Antimicrobial Resistence (AMR) – the silent pandemic



Covid-19

- viral pathogen
- rapid global spread
- Containment of the pandemic through vaccines
- No therapeutic agents available
- **high economic interest**

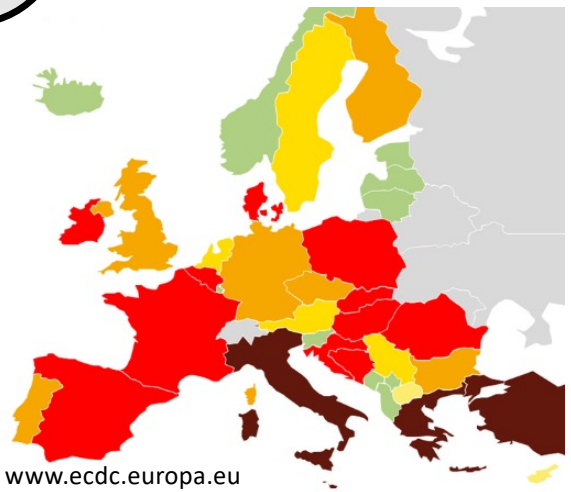


AMR

- several bacterial pathogens
- slow but steady spread
- Vaccination not possible
- Therapeutic agents available (antibiotics), but loss of efficacy due to resistance
- **hardly any economic interest**

World Health Organization

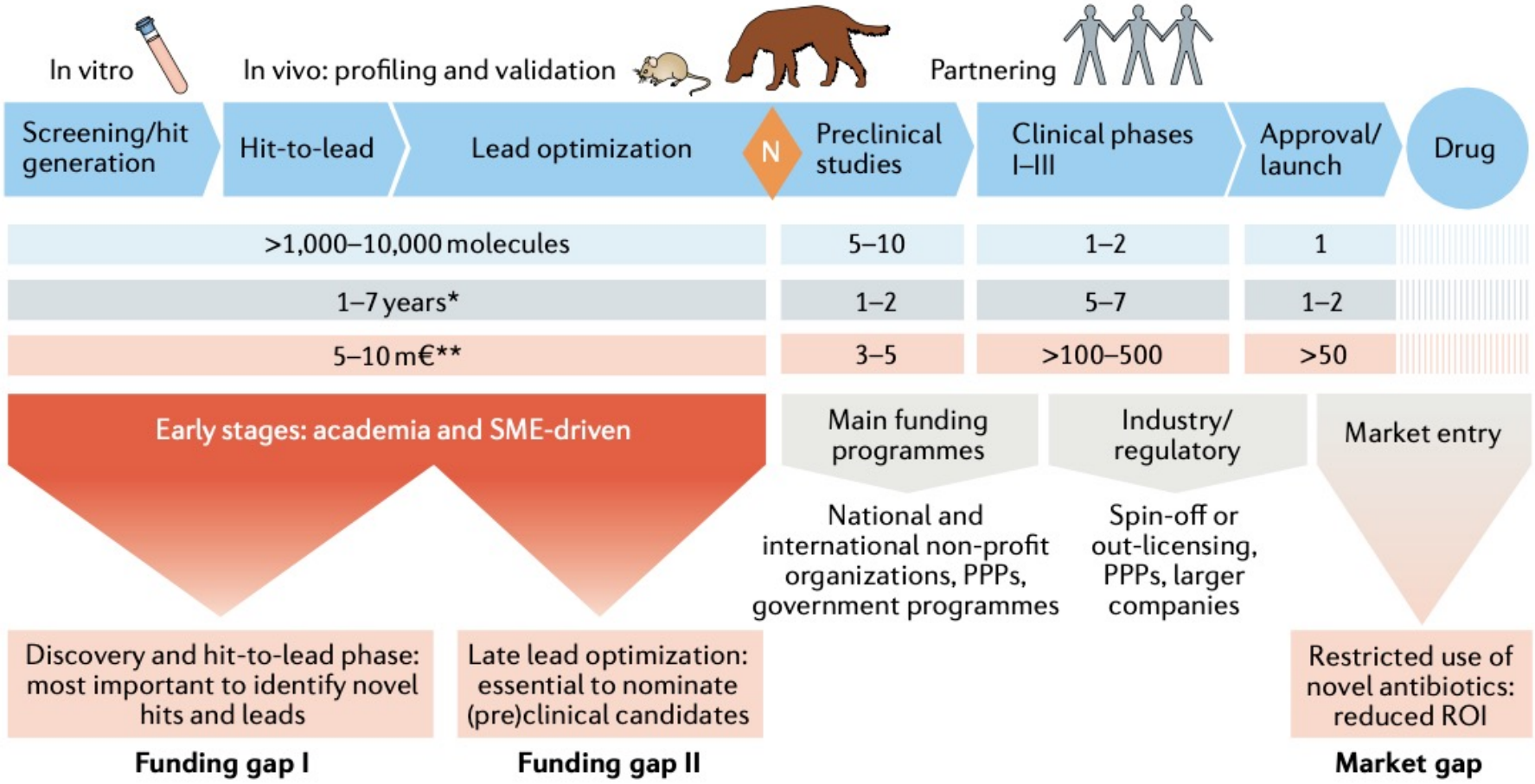
Spread of AMR According to WHO **Very threatening**



www.ecdc.europa.eu

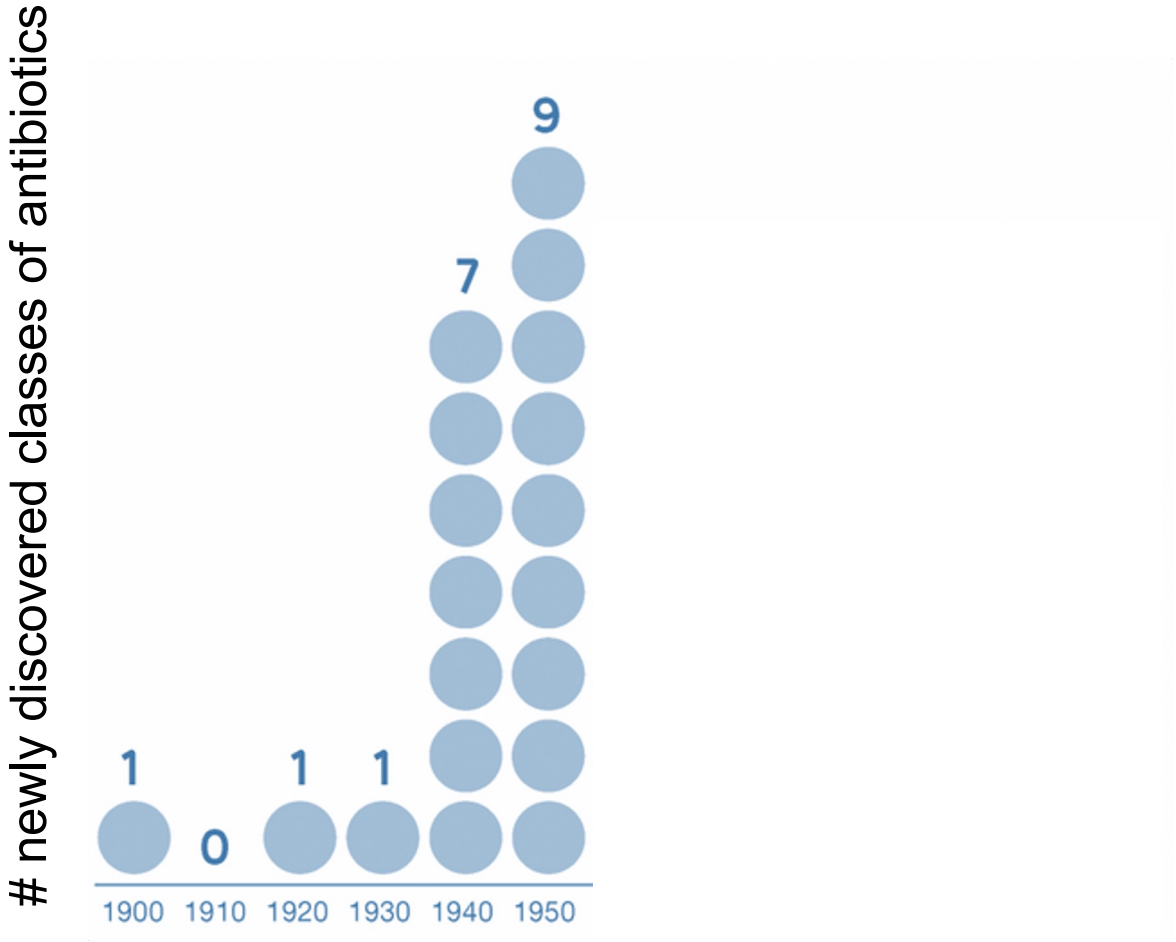
Antibiotics market is economically not viable, therefore **drug discovery** in **academia** and **public private partnerships** is essential for AMR!

Antimicrobial drug development



M. Miethke, …, A. K. H. Hirsch, …, R. Müller, *Nature Rev. Chem.* **2021**, *5*, 726–749.

No new Antibiotics



Development gap!

Quelle: wellcome.org

Challenge: Appreciation of Antibiotics

Sales of the anti-cancer drug Glivec in 2013

4.000 Mio €

772 Mio €

Total value of **ALL** antibiotics prescribed in Germany in 2012

Annual funding of AMR research EU+USA

400 Mio €

Annual marketing expenses

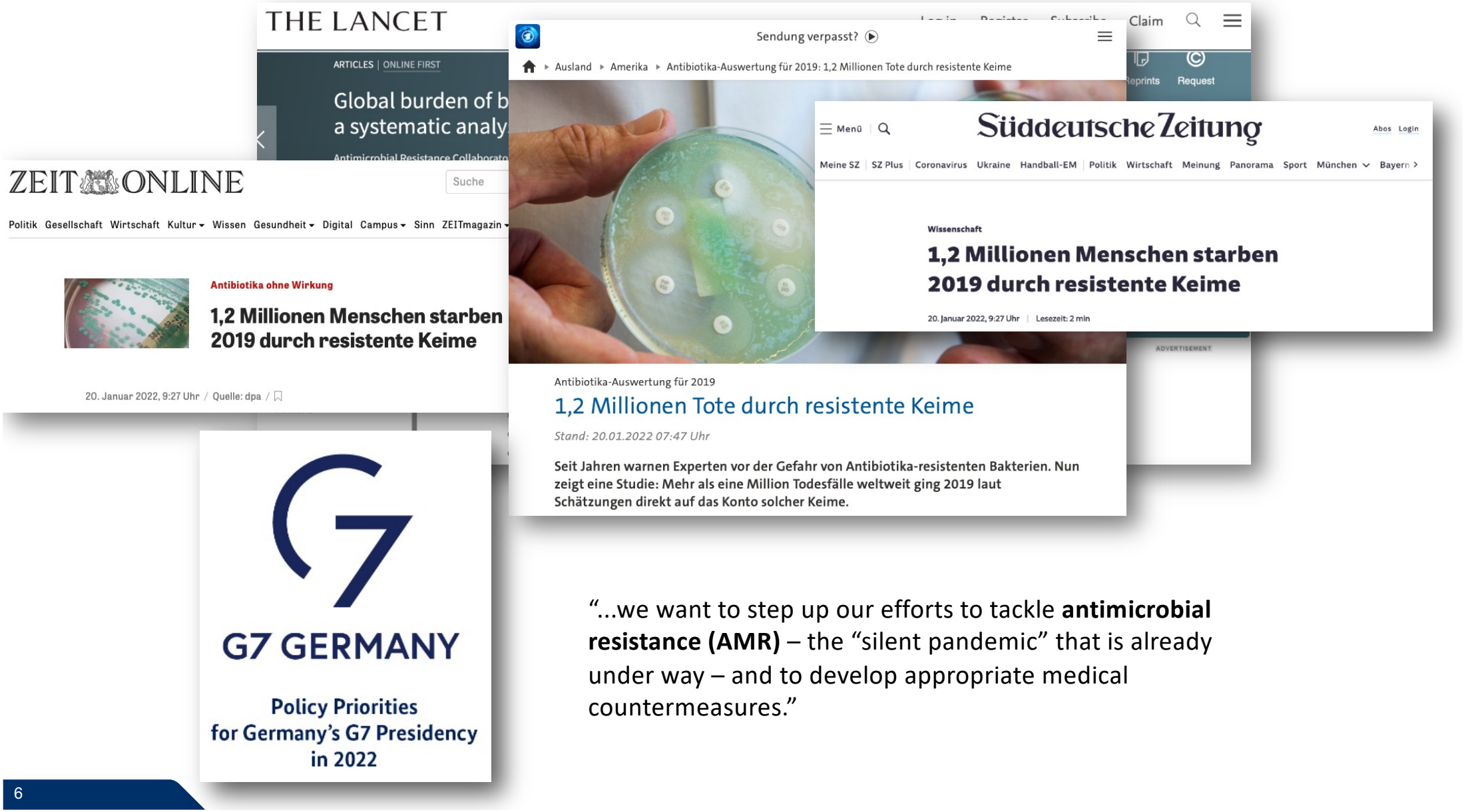
98.000 Mio $

Appreciation of antibiotics is in no sensible relationship to their significance for society!

Annual R&D expenses of the 10 largest pharma companies

65.000 Mio $

Sources: Die Zeit, brandeins



"...we want to step up our efforts to tackle **antimicrobial resistance (AMR)** – the "silent pandemic" that is already under way – and to develop appropriate medical countermeasures."

Helmholtz Institute for Pharmaceutical Research Saarland (HIPS)

HELMHOLTZ

Six Research Fields:


Energy


Earth & Environment


Matter


Key Technologies


Health


Aeronautics, Space & Transport

HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

- Founded in 2009
- Currently 200 employees
- 3 Departments with
  - 6 Research groups
  - 2 Junior research groups
- Located in Saarbrücken
- Belongs to HZI (Braunschweig)

Infection Research Programme at

HZI HELMHOLTZ Centre for Infection Research

3 Major Research Topics

Anti-Infectives

Bacterial and Viral Pathogens

Immune Response and Interventions

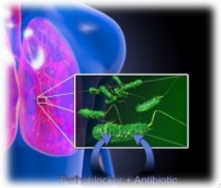
DZIF German Centre for Infection Research

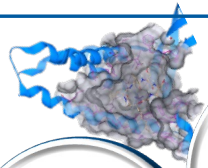
Novel Antibiotics

(Thematic Translational Unit, coordinated at HIPS)

Translational Infection Research for Treating Infectious Diseases

Projects at the department *Drug Design and Optimisation*



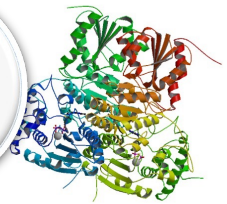


LspA
IC_{50}: 47 nM
Target strain(s):
PA, EC

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

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

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



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IC_{50}: 1.5 nM
Target strain(s):
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IC_{50} (PF): 170 nM
Target strain(s):
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F protein
IC_{50}: 40 nM
Target strain(s):
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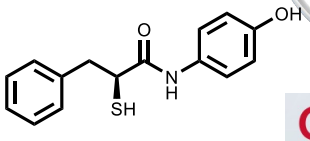
Nsp10/14
K_D: 17 µM
Target strain(s):
SARS-CoV-2



CARB-X
Combating Antibiotic-Resistant Bacteria

Computer-aided drug design ⟷ Multiparameter optimisation

In vitro assays

Bacterial assays

Biophysical screening and hit characterisation

Nsp12
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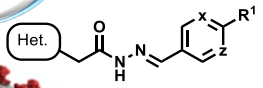

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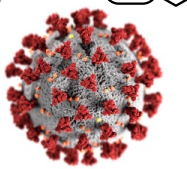

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Target strain(s):
SA, SP

DnaN
MIC (MS): 0.5 µM
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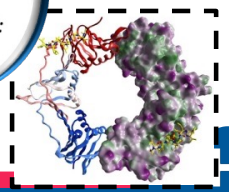

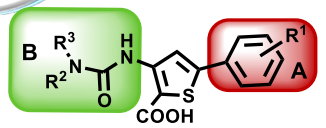

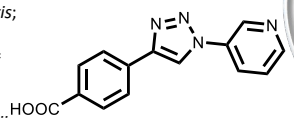

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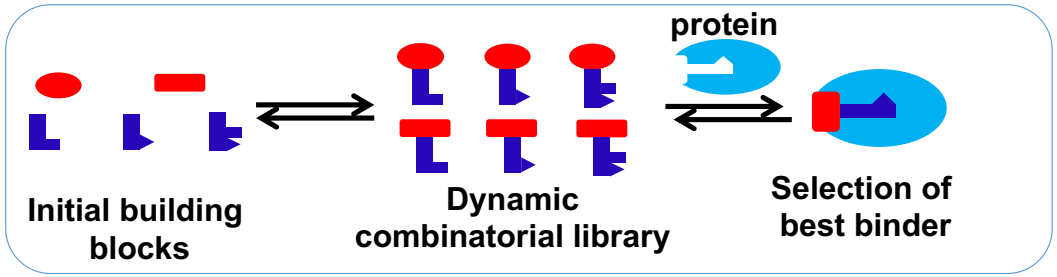
Protein-templated strategies

Dynamic combinatorial chemistry (DCC):



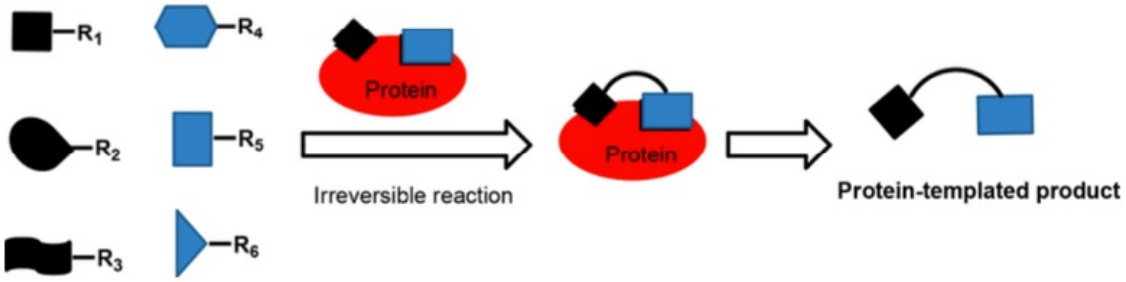
Lehn, Eliseev, Ramström, Greaney, Ernst, Vincent …

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

M. Mondal, …, A. K. H. Hirsch, *Angew. Chem. Int. Ed.* **2014** & **2016**

A. M. Hartman, …, A. K. H. Hirsch, *ChemMedChem* **2020**
R. P. Jumde, …, A. K. H. Hirsch, *Chem. Sci.* **2021**
W. Elgaher, …, A. K. H. Hirsch, *in preparation*.

Kinetic target-guided synthesis (KTGS):



Sharpless, Rademann, Deprez …

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

M. Mondal, …, A. K. H. Hirsch, *Chem. Eur. J.* **2016**
F. Mancini, …, A. K. H. Hirsch, *Chem. Eur. J.* **2020**,

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

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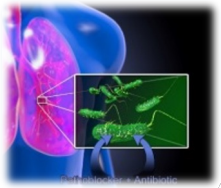

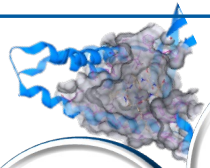

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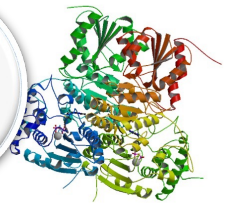

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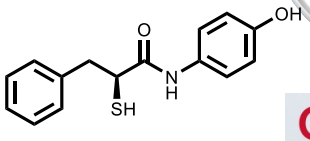

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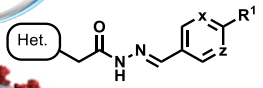

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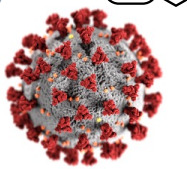

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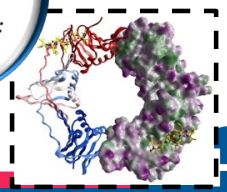

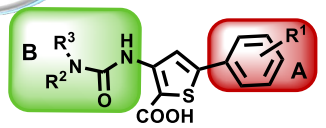

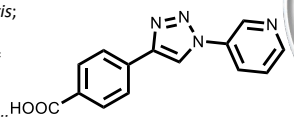

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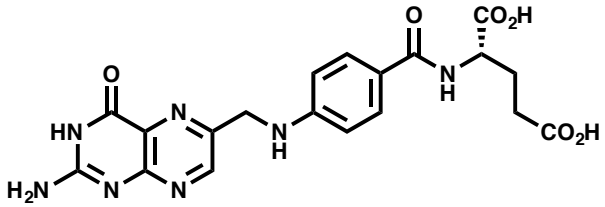
Energy-coupling factor (ECF) transporters

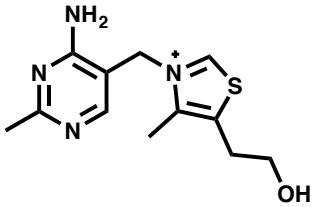
❑ *The Basics*

- Abundant class of importers for micronutrients in bacteria
- Subclass of ATP-binding cassette (ABC) transporters
- Transmembrane proteins
- Structure: 4 subunits (A, A', T, S)

Energising module



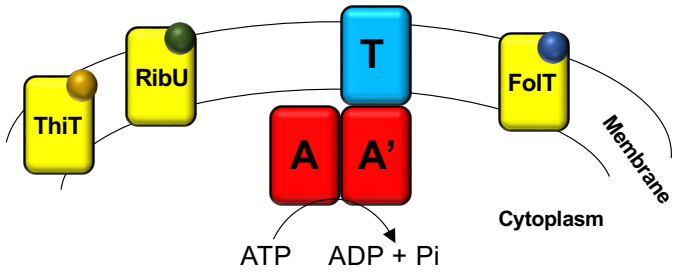
Folic acid (vitamin B9)



Thiamine (vitamin B1)



Leticia Monjas, Eleonora Diamanti, Mostafa Hamed

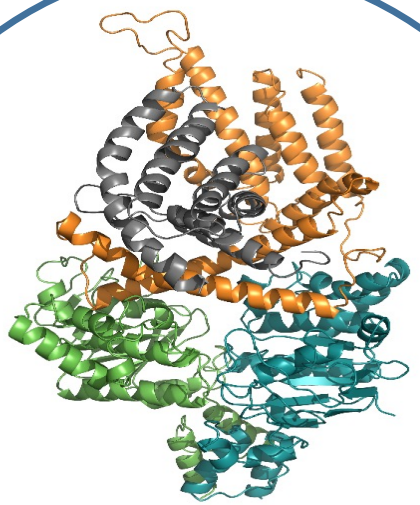


D. A. Rodionov *et al. J. Bacteriol*. **2009**, *191*, 42.
D. J. Slotboom. *Nature Rev. Microbiol.* **2014**, *12*, 79-87.

Multiple hit-identification strategies

Dynamic combinatorial chemistry

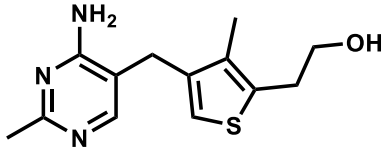
ThiT



ECF transporter
vitamin transport
in bacteria

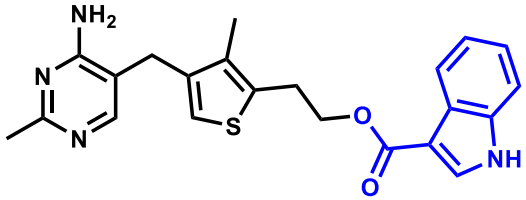
Structure-based design

ThiT



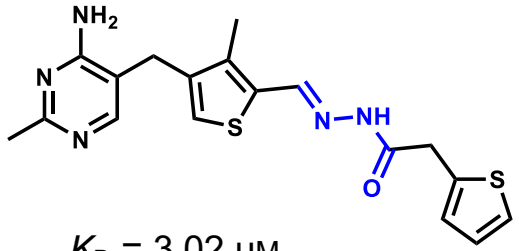
K_D = 4.23 ± 1.69 nM

ChemBioChem **2015**, *16*, 819
MedChemComm **2016**, *7*, 966



K_D = 20.1 ± 3.3 nM

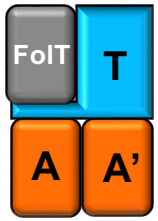
MedChemComm **2017**, *8*, 1121



K_D = 3.02 µM

ChemMedChem **2017**, *12*, 1693

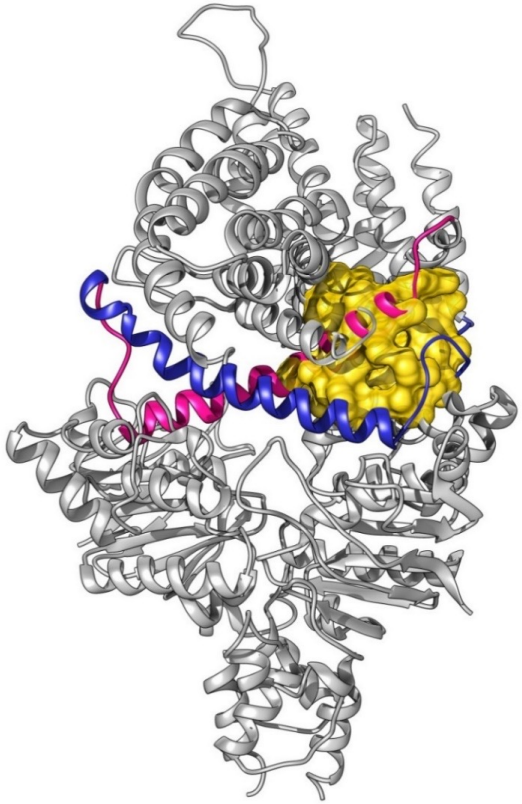
Virtual screening



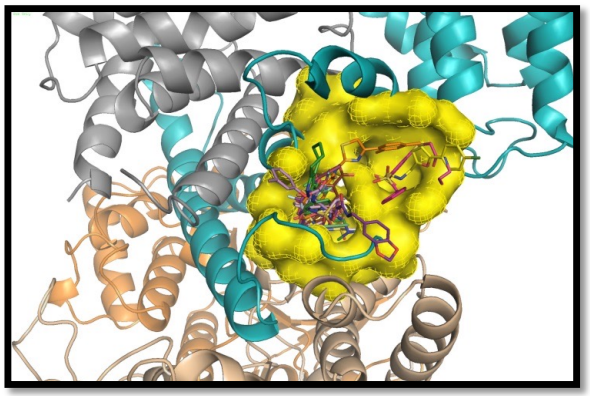
ChemRxiv **2022**

ECF-FolT

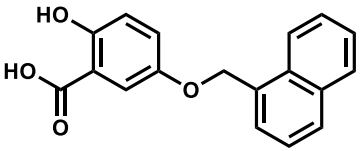
Identification of a druggable pocket and novel inhibitors



Crystal structure from *Lactobacillus delbrueckii* in the *apo*-state

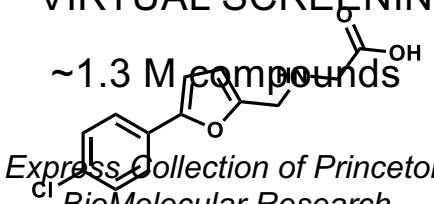


P2 pocket,
allosteric pocket



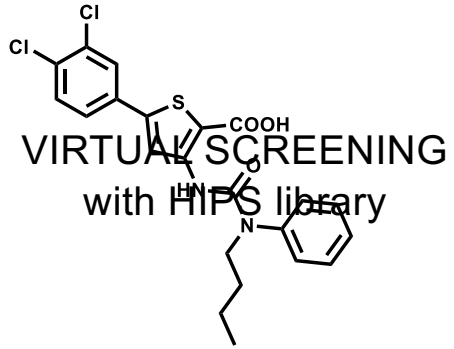
HIPS 6790
L. delbrueckii, IC_{50} = 282 ± 108 µM
L. casei, IC_{50} = 315 ± 15 µM

VIRTUAL SCREENING
~1.3 M compounds
Express Collection of Princeton BioMolecular Research



HIT 2
L. delbrueckii, IC_{50} = 1.2 mM
L. casei, not active
Int. J. Mol. Sci. **2022**

VIRTUAL SCREENING
with HIPS library

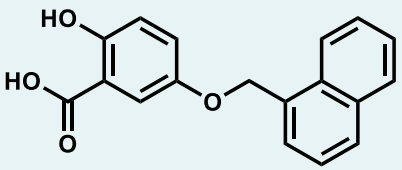


HIPS 440
L. casei, IC_{50} = 2.3 µM
J. Med. Chem. **2022**

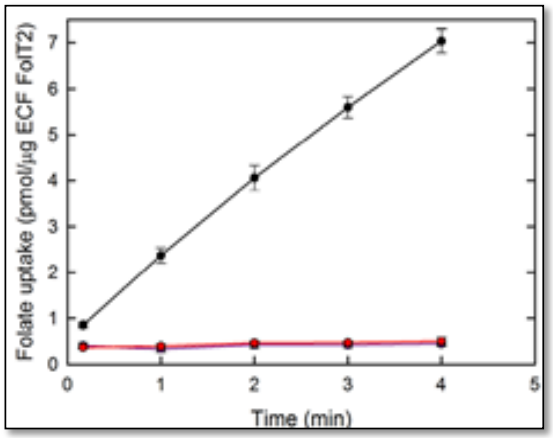
S. Bousis, I. Setyawati, E. Diamanti, D. J. Slotboom, and A. K. H. Hirsch, Adv. Therap. **2018**, 1800066
D. J. Slotboom *et al. Nat. Commun*. **2016**, *7*, 1.

Fragment HIT 1 validation

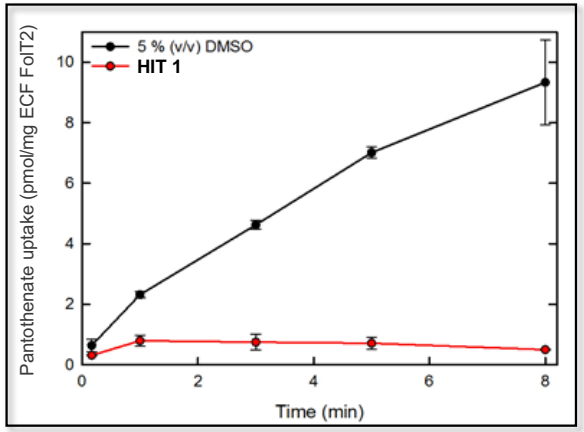
HIT 1 [2-hydroxy-5-(naphthalen-1-ylmethoxy)benzoic acid] profile

					Antibacterial profile			Cytotoxicity	
Code	**Chemical Structure**	**MW**	**clog*P***	**IC_{50}**	***S. aureus* Newman**	***S. pneumoniae* DSM-11865 – (PRSP)**	***E. faecium* -vanA-**	**HEK293**	**HepG2**
HIT 1		294.3	3.5	282 ± 108 µM	%Inh. @100 µM= 85	MIC = 32 µM	MIC = 256 µM	%Inh. @100 µM =21±6	%Inh. @100 µM =5±7

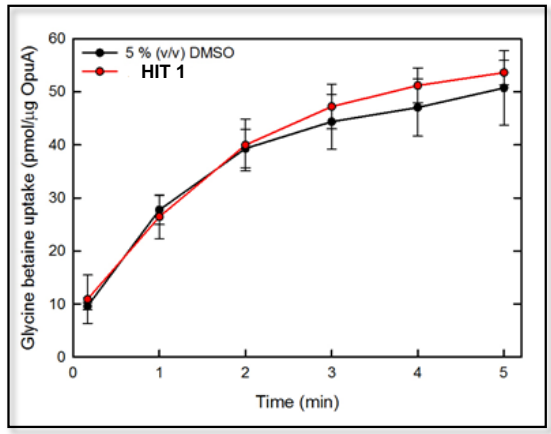
Fragment HIT 1 validation



Full inhibition of folate uptake



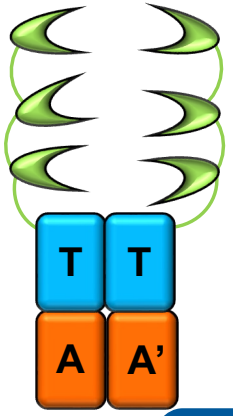
Full inhibition of pantothenate uptake



Selective for ECF TRANSPORTERS

ALLOSTERIC inhibitor

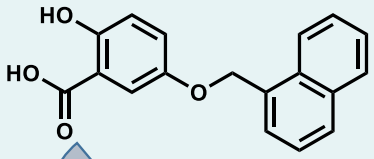
- Same energising module
- Different S-component



- Different type ABC transporter

HIT 1 optimisation

- **OH** and **COOH** are crucial
- **O** atom in the bridge could be replaced
- **Naphthyl** moiety not essential for activity

		In vitro	Antibacterial profile			
Code	**Chemical Structure**	**IC_{50}**	***S. pneumoniae* DSM-11865 (PRSP)**	***E. faecium* DSM-17050 (VREF; vanA)**	***E. faecalis* DSM-20478**	**HepG2** % growth inh. @100 µM
HIT 1		300 µM	MIC = 32 µM	MIC > 75 µM	MIC > 75 µM	20 ± 2

Optimised HIT 1

	IC_{50}	***S. pneumoniae* DSM-20566**	***S. pneumoniae* DSM-11865 (PRSP)**	***E. faecium* DSM-17050 (VREF; vanA)**	***E. faecium* DSM-20477**	***E. faecalis* DSM-20478**	**HepG2** % growth inh. @100 µM	**$t_{1/2}$ [min] mouse liver S9 /Cl_{int}** [µl/min/mg]
HIPS 6920	55 µM	8 – 16 µM	8 µM	32 µM	32−64 µM	32 µM	< 20	>120 / < 5.8

E. Diamanti, …, A. K. H. Hirsch, *ChemRxiv* **2022**, doi.org/10.26434/chemrxiv.11728710.v2

New screening assay

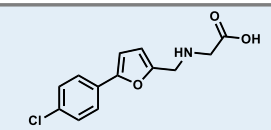
NEW ASSAY

- 96-well plate format
- Multiparameter assay
- No protein reconstitution required


Spyridon Bousis


Steffen Winkler

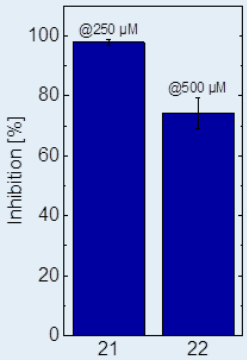

Jörg Haupenthal



HIT 2: IC_{50} = 1.2 mM

~ 50 derivatives (synthesised/ purchased)

22
Optimised hit 2



21
Optimised hit 1

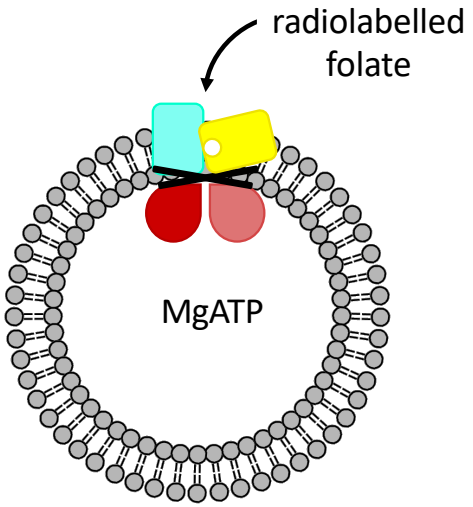
21: %Inh.@250 µM = 98±1%

22: %Inh.@500 µM = 74±5%

IN VITRO ASSAY

- Purified ECF_FolT from *L. delbrueckii* is expressed in *E. coli* and embedded in liposomes

ASSAY CONDITIONS:
PBS 50 mM
pH = 7.5



A. F. Kiefer, S. Bousis, M. M. Hamed, E. Diamanti, J. Haupenthal, A. K. H. Hirsch, *J. Med. Chem.* **2022**, *65*, 8869.

In silico screening of the HIPS library

HIPS library: 2,000 compounds

MONA

- ■ MW = 250–500 g mol^{-1}; ✘ Ester derivatives
- ✘ Fused aromatic tricyclic or bigger
- ➢ 700,000 compounds

KNIME Analytic platform

DOCKING by using LeadIT
ECF FolT2 in the *apo* state (PDB ID 5D7T)

HYDE

PAINS filter and Eli Lilly rules
✘ Promiscuous and reactive compounds

SeeSAR

- ✘ Red-flagged torsion
- ✘ Only 1 pose

TOP 1,000 poses

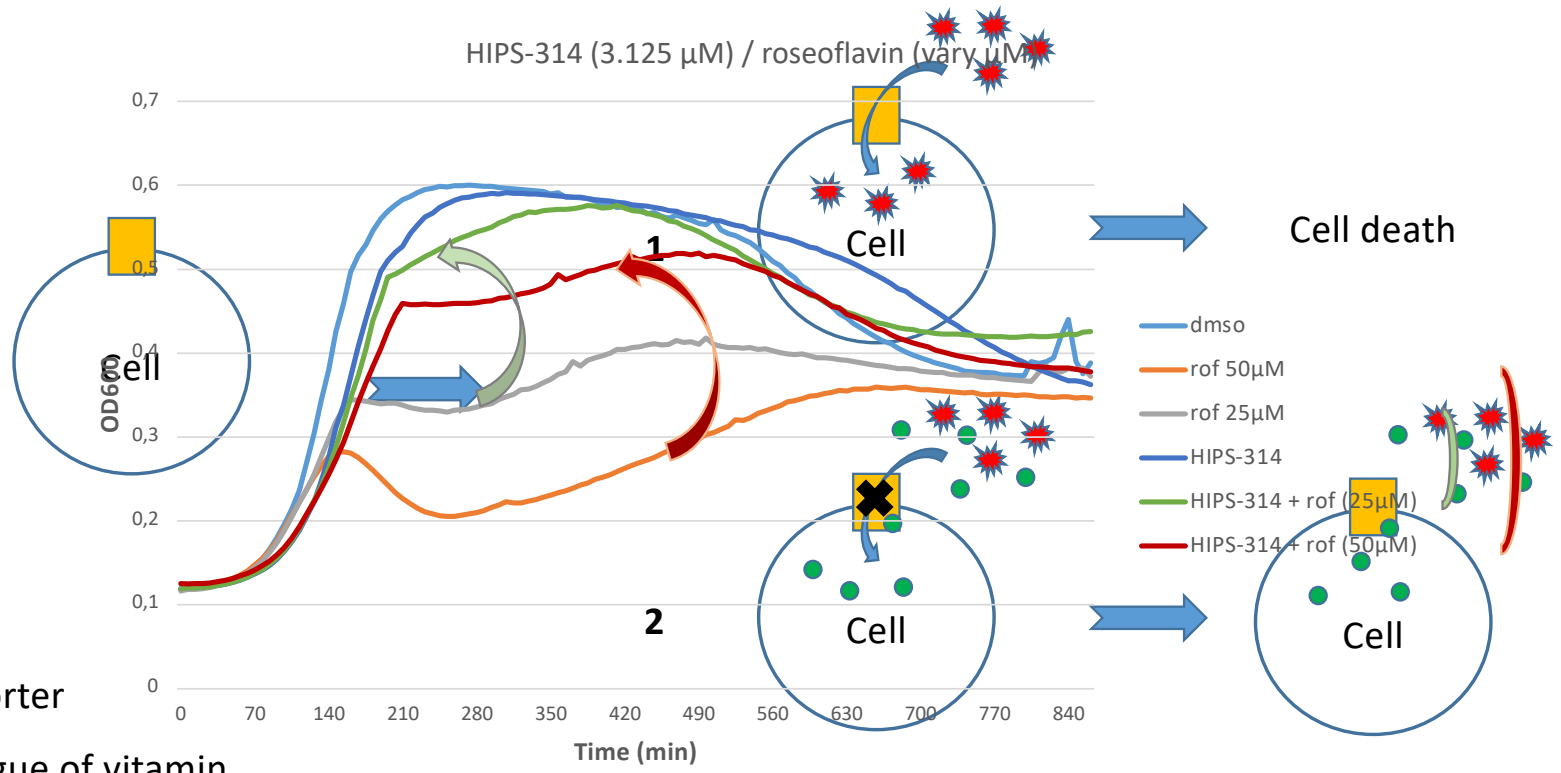
5 hits identified

2 hits validated
IC_{50} = 30–40 µM
IC_{50} = 9 µM

Properties (HIPS-314)		
	IC_{50} (cell-based assay)	30–40 µM
MIC µg/mL	*S. aureus* Newman	80% @ 3 µg/mL
	S. pneumoniae DSM-20566	0.5
	S. pneumoniae PRSP	1
	E. faecalis DSM-20478	7.8
	E. faecium DSM-20477	3.9
%growth inh. @48 h	A549	9.4 @ 100 µM
	HepG2	10.8 @ 100 µM
	HEK293	43 @ 100 µM

S. Bousis, S. Winkler, J. Haupenthal, F. Fulco, E. Diamanti, A. K. H. Hirsch, *Int. J. Mol. Sci.* **2022**, *23,* 2637.

In cellulo target validation



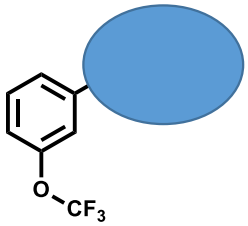
ECF transporter

Toxic analogue of vitamin

Inhibitor

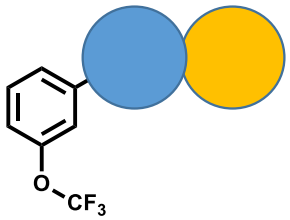
- Excellent metabolic stability > 120 min)
- No cytotoxicity
- *In vivo* PK study: oral bioavailability

Antimicrobial profile & cytotoxicity



HIPS-314

Properties	HIPS 314
(transport-activity assay) *L. delbrueckii*	> 250 µM
(cell-based assay) *L. casei*	% inh. @ 50 µM = 62
S. aureus Newman (MIC µg/mL)	80% @ 3 µg/mL
S. pneumoniae DSM-11865 (PRSP) (MIC µg/mL)	0.5
S. pneumoniae DSM-*20566* (MIC µg/mL)	1
E. faecalis DSM-20478 (MIC µg/mL)	1
E. faecium DSM-17050 (VREF; vanA) (MIC µg/mL)	8
C. perfringens (MIC µg/mL)	0.7
C. difficile DSM 1296 ATCC 9689 (MIC µg/mL)	4.9
C. difficile DSM28645 (CD630Δerm) (MIC µg/mL)	4.9
C. difficile serial No. 01104431 (MIC µg/mL)	4.9
C. sporogenes (MIC µg/mL)	9.8
Pseudomonas Aeruginosa (MIC µg/mL)	> 24
HepG2 (% growth @ 48h)	11% @ 100 µM
A549 (% growth @ 48h)	9% @ 100 µM
Hek293 (% growth @ 48h)	42% @ 100 µM



HIPS-314

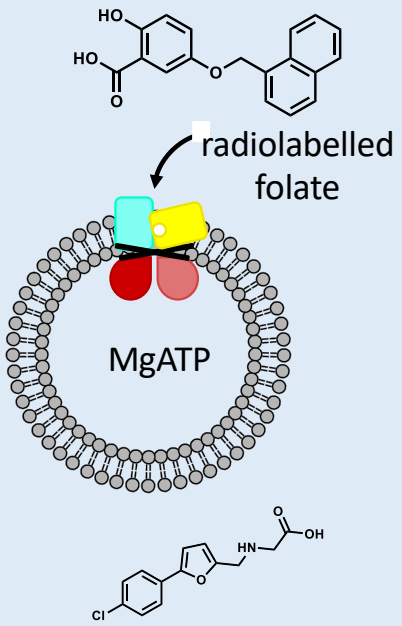
	MIC [µM]				
Compound	E. faecalis DSM-20478	E. faecium DSM-17050	E. faecium DSM-20477	S. pneumoniae DSM-11865	S. pneumoniae DSM-20566
HIPS314	32	32	64-32	16	8
HIPS314NB	>128	>128	>128	>128	>128

→ Check purity
→ Check chemical identity

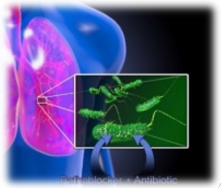
Summary – ECF transporters

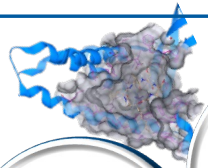
- Two novel chemical classes identified as hits by SBVS
- Structure–activity relationships established
- MD simulations corroborated allosteric mode of inhibition
- Good oral bioavailability
- New whole-cell screening assay

→ Further hit-to-lead optimisation focusing on multiple parameters



Projects at the department *Drug Design and Optimisation*



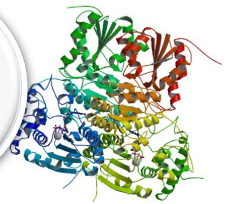


LspA
IC_{50}: 47 nM
Target strain(s):
PA, EC

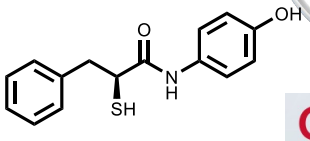
PqsR
IC_{50}: 5 & 30 nM
(PqsR & Pyocyanin)
Target strain(s): PA

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

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



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



LasB
IC_{50}: 1.5 nM
Target strain(s):
PA

IspE
IC_{50} (PF): 170 nM
Target strain(s):
MT, PF, EC, AB

F protein
IC_{50}: 40 nM
Target strain(s):
RSV

Nsp10/14
K_D: 17 µM
Target strain(s):
SARS-CoV-2

Nsp12
IC_{50} (RdRp): 1 µM
Target strain(s):
SARS-CoV-2

Computer-aided drug design ⟷ Multiparameter optimisation

In vitro assays

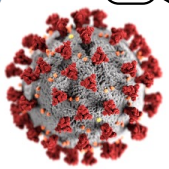
Bacterial assays

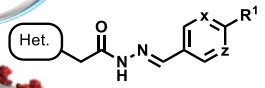
Biophysical screening and hit characterisation

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

Mß-lact
IC_{50} (VIM-1):
80 nM
Target strain(s):
PA, KP, EnC

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

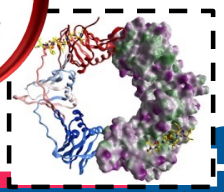


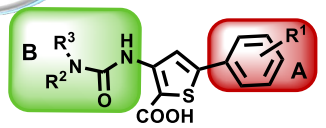


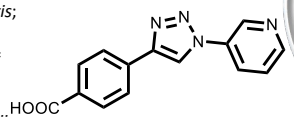
LANA & CsrA
IC_{50}: 17 µM
Target strain(s):
broad spectrum
PA, YT, SA...

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

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



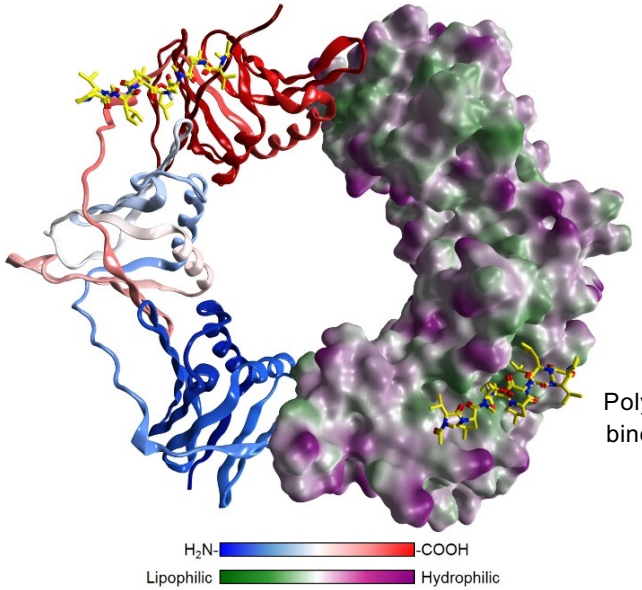




PA = *Pseudomonas aeruginosa*; CH = *Clostridium histolyticum*; MT = *Mycobacterium tuberculosis*; MS = *Mycobacterium smegmatis*; TB = *Trypanosoma brucei;* SA = *Staphylococcus aureus*; PF = *Plasmodium falciparum*; YT = *Yersinia pseudotuberculosis;* PF = *Plasmodium falciparum*; SP = *Streptococcus 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

β-Sliding clamp (DnaN)

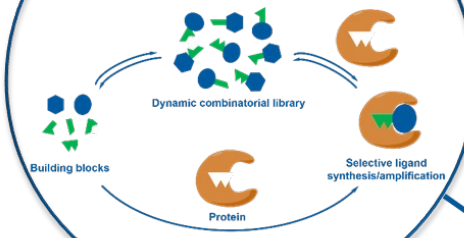
- Ring-shaped homodimer of β-subunit of bacterial DNA polymerase III
- **Attractive target**:
  - Essential for DNA replication (efficacy)
  - Highly conserved in bacteria (broad spectrum)
  - Distinct from eukaryotic counterpart (therapeutic selectivity)
  - Low frequency of resistance and high fitness costs, e.g., griselimycins (GMs)
- **Limitations of current inhibitors**
  - Natural: lipophilicity, hepatotoxicity, only mycobacteria
  - Synthetic: weak or no antibacterial activity



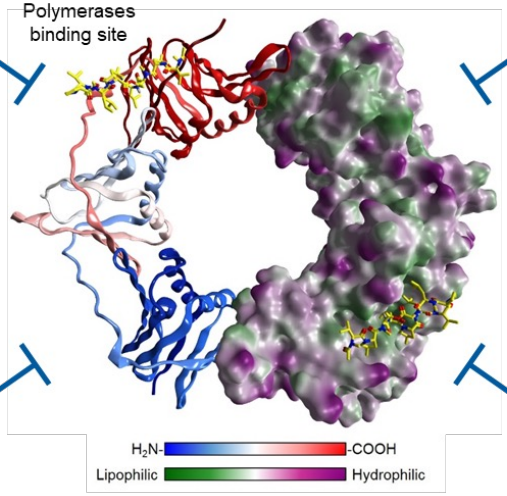
A. Kling, P. Lukat et al, *Science* **2015**, *348*, 1106–1112.

Strategies for novel inhibitors

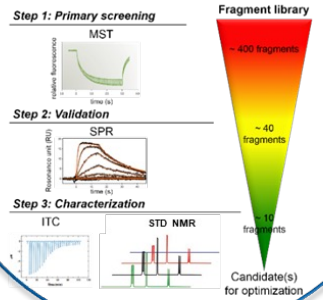
Target-guided synthesis (DCC & KTGS)



β-sliding clamp (DnaN)



Fragment screening

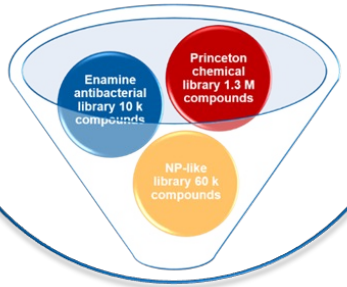


Walid Elgaher

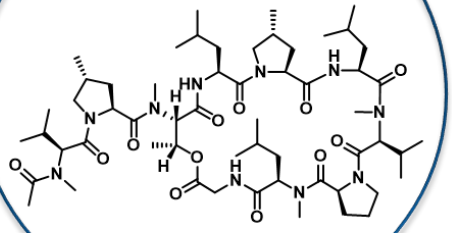
Federica Mancini

Uladzislau Hapko

Structure- & ligand-based virtual screening



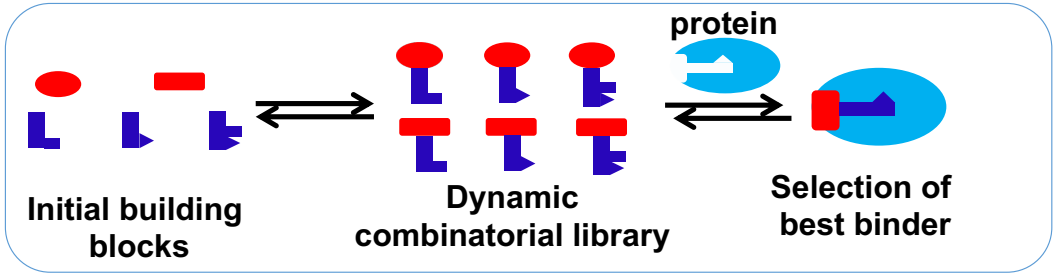
Total & semi-synthesis



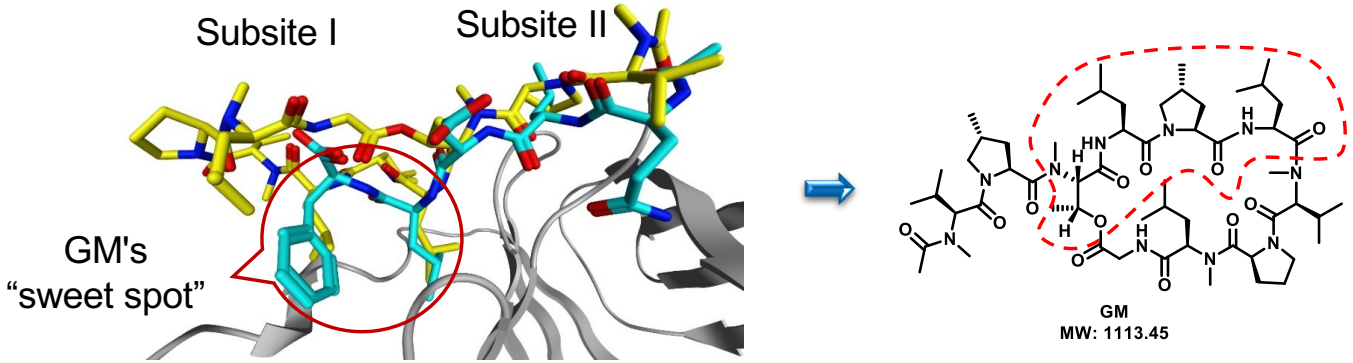
GM

1. 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.

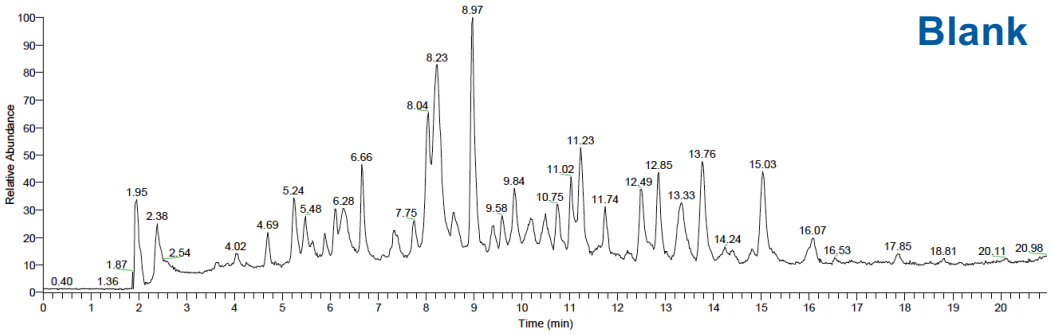


Overlay of GM (yellow) and the universal clamp-binding motif [AcQLDLF] (cyan) with DnaN (grey)

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

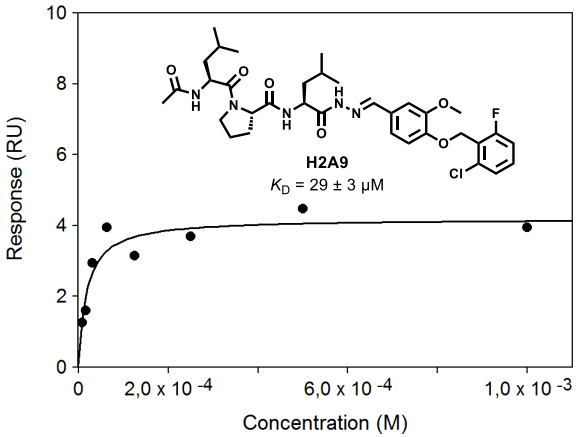
1. Dynamic combinatorial chemistry (DCC)

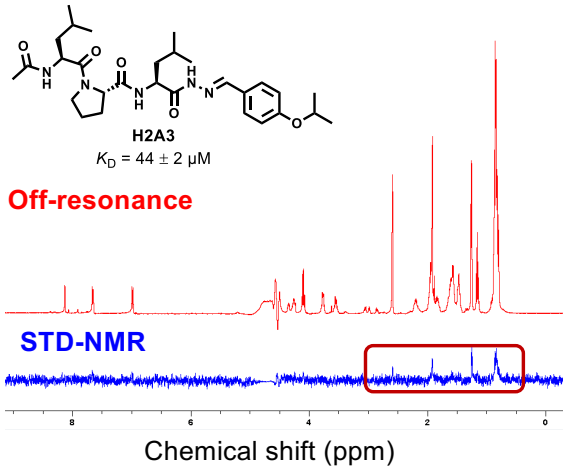
DCL analysis and hit identification



- Low micromolar affinity
- Potent antitubercular activity with no cytotoxicity
- Broadened spectrum
- Hit optimization is ongoing

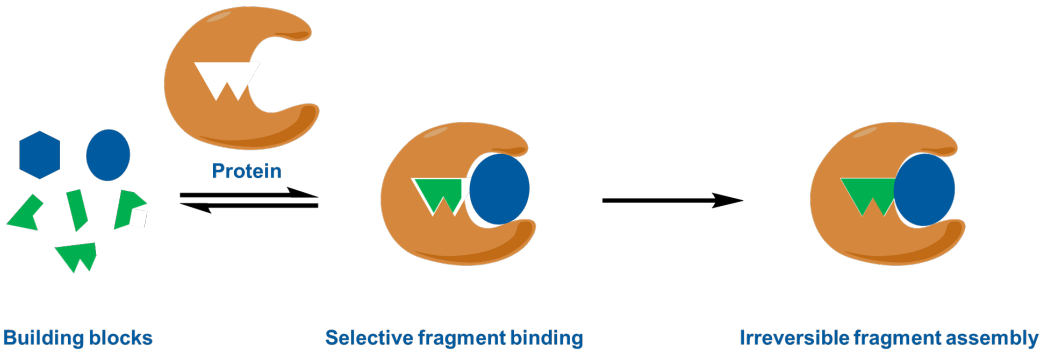
Hit validation





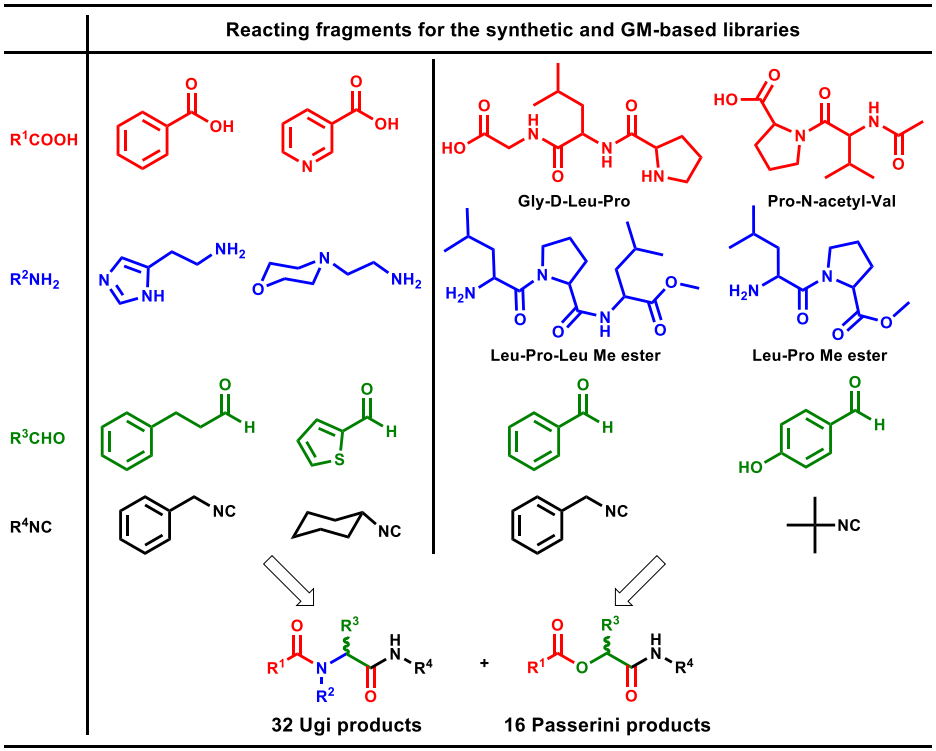
2. Kinetic target-guided synthesis (KTGS)

- Protein-templated **irreversible** reaction, *e.g.*, alkylation, 1,3-dipolar cycloaddition



- New chemical space: Ugi 4-component reaction

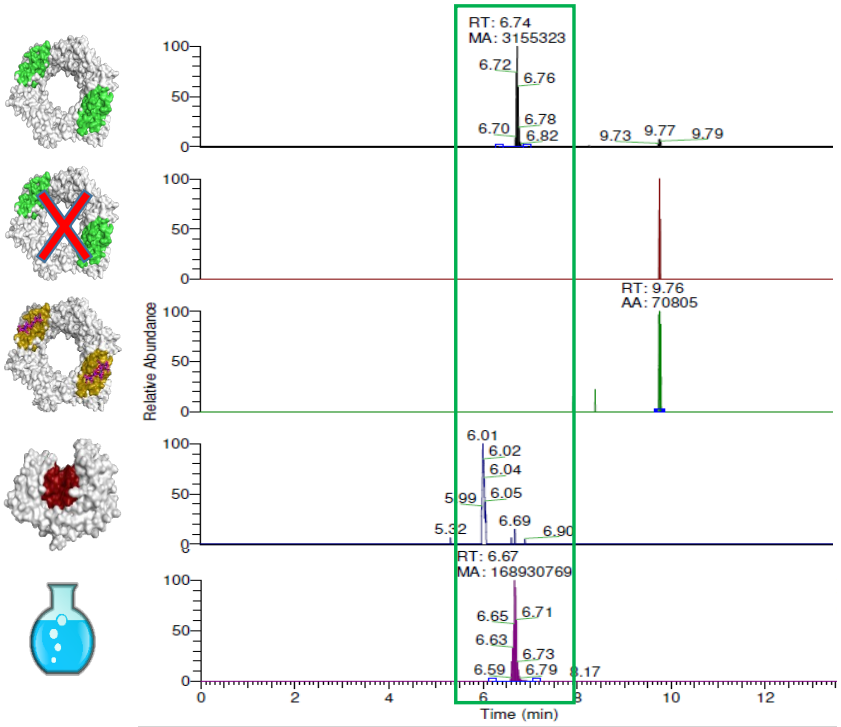
Design of two KTGS libraries

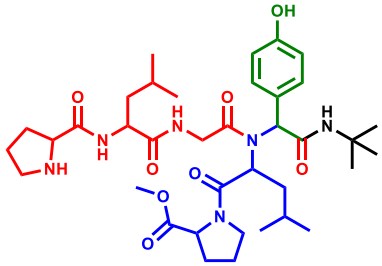


F. Mancini, *et al. Chem. Eur. J.* **2020**, *26*, 14585–14593.

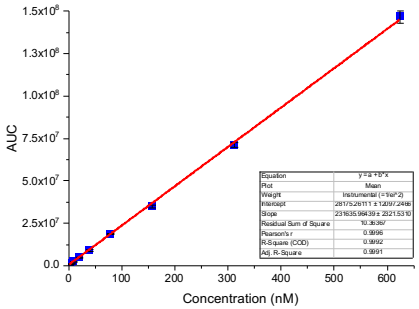
F. Mancini, *et al*. (in preparation).

2. KTGS: hit detection and validation

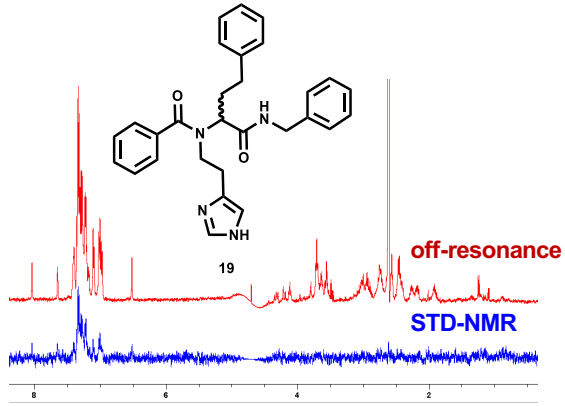




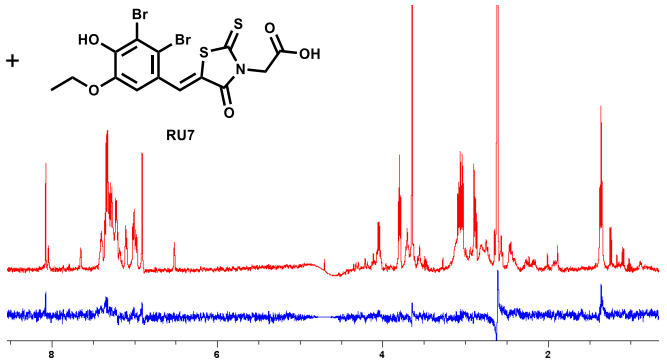
Ugi-4CR hit



Yield of KTGS: 1%



STD-NMR of **19**

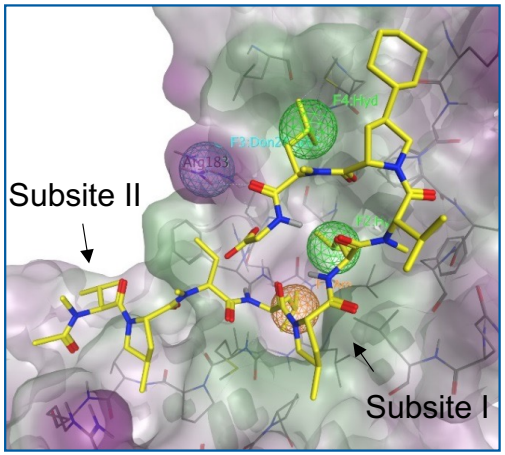


STD-NMR of **19** and **RU7** mixture

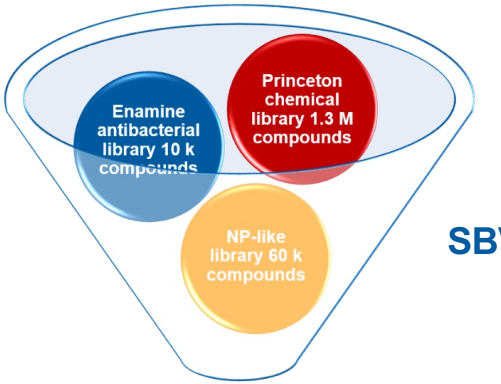
➡ New inhibitors with confirmed binding site and moderate antibacterial activity

F. Mancini, *et al. Chem. Eur. J.* **2020**, *26*, 14585–14593; F. Mancini, *et al.* (in preparation).

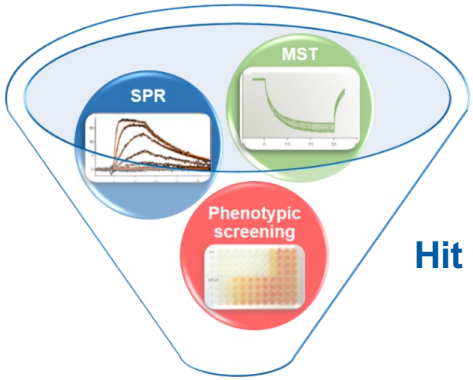
4. Structure-based virtual screening (SBVS)



Mtb DnaN–CGM cocrystal* & SB Ph4 query



SBVS



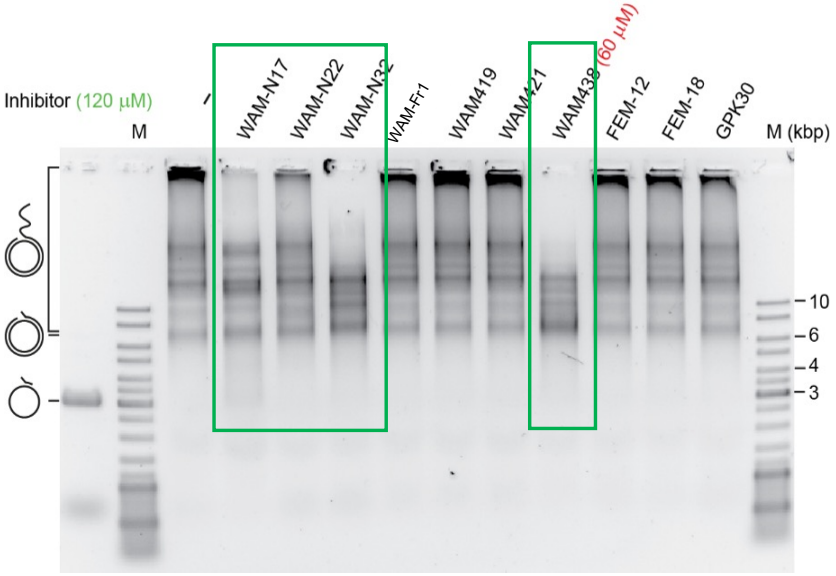
Hit validation

Validated hits

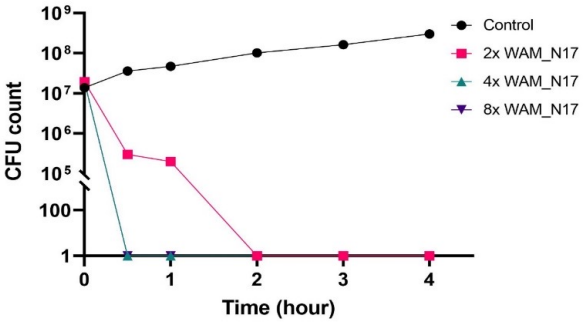
Bacterial strain / cell line	Affinity / MIC / IC_{50} (µM)
M. smegmatis DnaN	50
M. tuberculosis H37Rv	32
M. marinum	32
M. smegmatis mc^2155	4
M. smegmatis, GM^R	16
S. aureus	8
S. pneumoniae	16
E. faecium VRE51559	8–16
E. coli ΔacrB	8
E. coli K12	55
E. coli DSM-1116	128
A. baumannii	64
K. pneumoniae DSM-30104	64
P. aeruginosa PA14	128
HepG2	>111

A. Kling, P. Lukat et al., *Science* **2015**, *348*, 1106–1112.
W. A. M. Elgaher et al. (patent application and manuscripts in preparation).

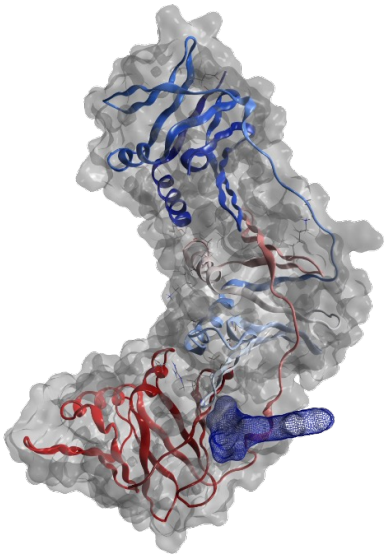
4. SBVS: mode of action and hit optimisation



E. coli DNA replication assay



Time-to-kill assay in *S. aureus*



Crystal structure of *R. typhi* DnaN–**WAM-N17** complex at 2.2 Å

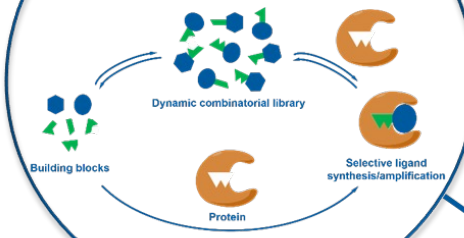
- Novel chemotype
- Low micromolar affinity and IC_{50} values
- Broad antibacterial activity (MDR pathogens)
- Rapid bactericidal effect with no cytotoxicity
- Crystal structure and SAR (85 derivatives)

with Nicholas Dixon

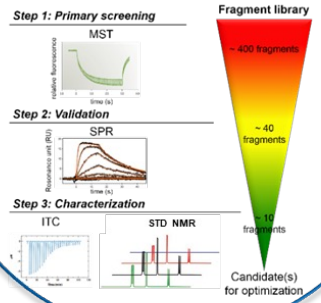
A. Kling, P. Lukat et al., *Science* **2015**, *348*, 1106–1112.
W. A. M. Elgaher et al. (patent application and manuscripts in preparation).

Conclusions – DnaN

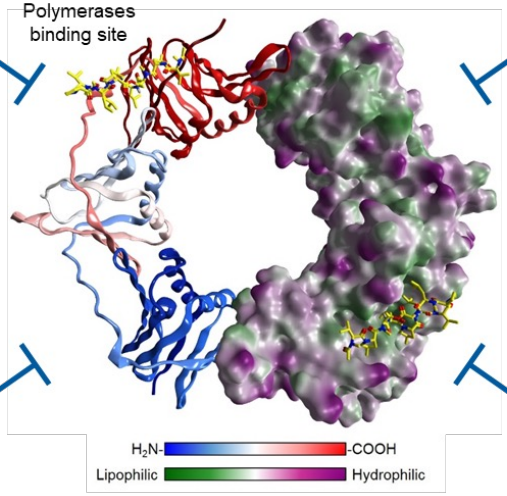
Target-guided synthesis (DCC & KTGS)

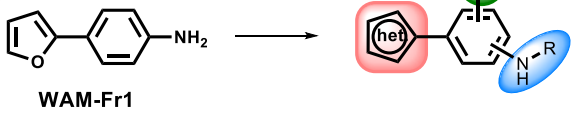


Fragment screening



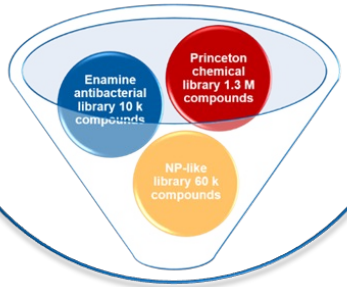
β-sliding clamp (DnaN)



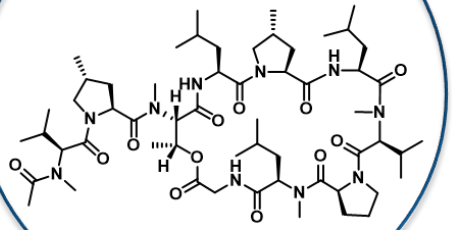


WAM-Fr1

Structure- & ligand-based virtual screening

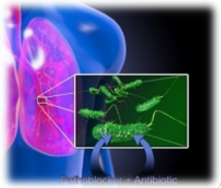


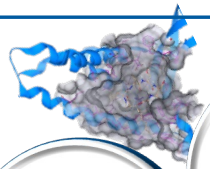
Total & semi-synthesis



GM

Projects at the department *Drug Design and Optimisation*



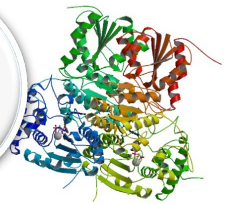


LspA
IC_{50}: 47 nM
Target strain(s):
PA, EC

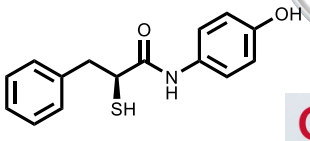
PqsR
IC_{50}: 5 & 30 nM
(PqsR & Pyocyanin)
Target strain(s): PA

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

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



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



LasB
IC_{50}: 1.5 nM
Target strain(s):
PA

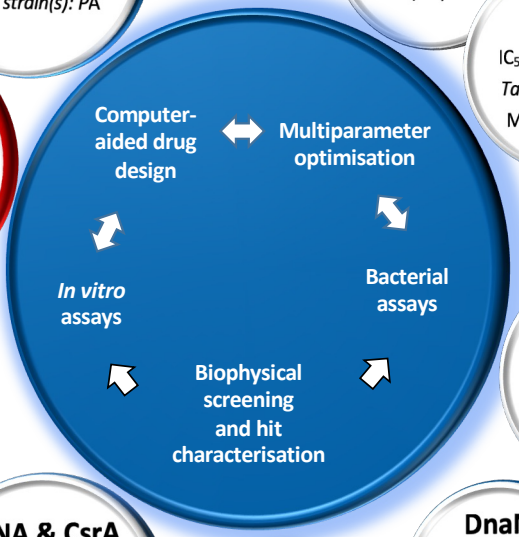
CARB-X
Combating Antibiotic-Resistant Bacteria

IspE
IC_{50} (PF): 170 nM
Target strain(s):
MT, PF, EC, AB

F protein
IC_{50}: 40 nM
Target strain(s):
RSV

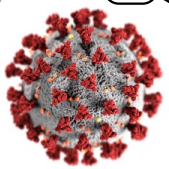
Nsp10/14
K_D: 17 µM
Target strain(s):
SARS-CoV-2

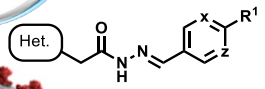
Nsp12
IC_{50} (RdRp): 1 µM
Target strain(s):
SARS-CoV-2



Mß-lact
IC_{50} (VIM-1):
80 nM
Target strain(s):
PA, KP, EnC

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



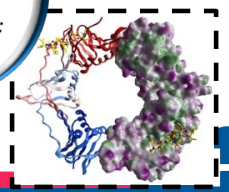


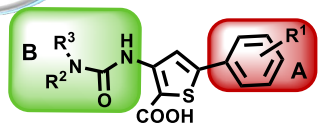
LecA/B
IC_{50}: 10.8 nM
Target strain(s):
PA

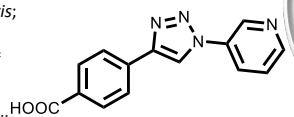
LANA & CsrA
IC_{50}: 17 µM
Target strain(s):
broad spectrum
PA, YT, SA...

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

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

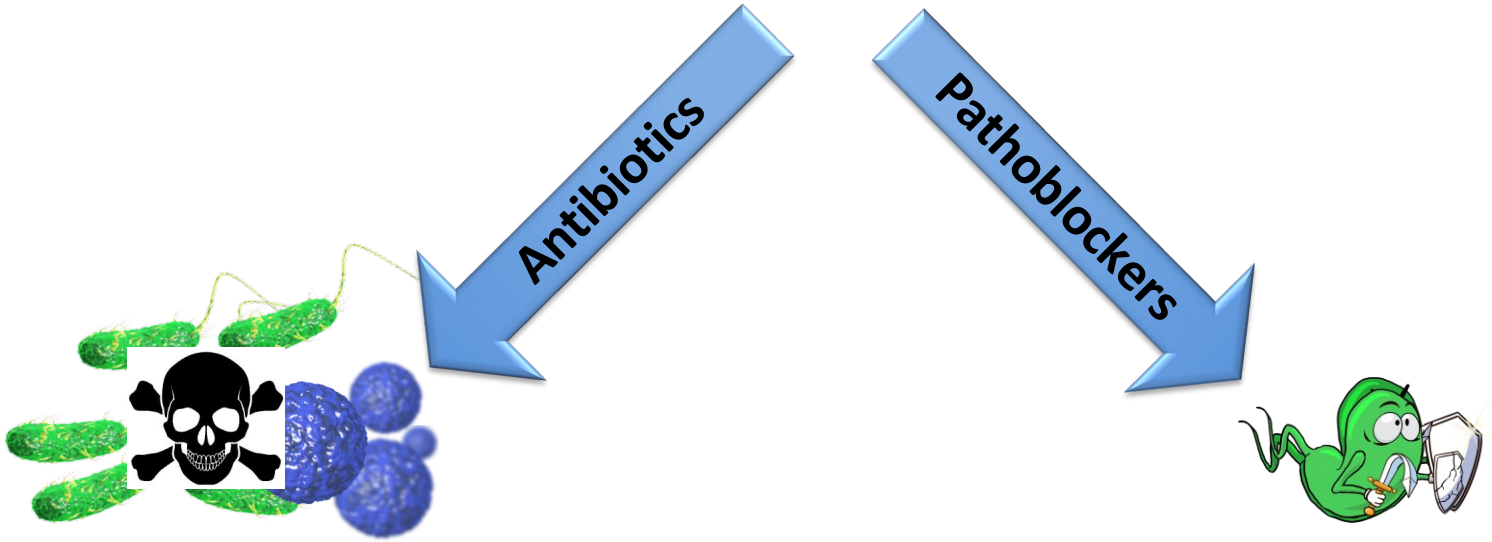






PA = *Pseudomonas aeruginosa*; CH = *Clostridium histolyticum*; MT = *Mycobacterium tuberculosis*; MS = *Mycobacterium smegmatis*; TB = *Trypanosoma brucei;* SA = *Staphylococcus aureus*; PF = *Plasmodium falciparum*; YT = *Yersinia pseudotuberculosis;* PF = *Plasmodium falciparum*; SP = *Streptococcus 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

Pathoblocker approach



bacteriostatic/
bactericidal effects

- prone to resistance development

do not kill bacteria but disable virulence/
pathogenicity traits

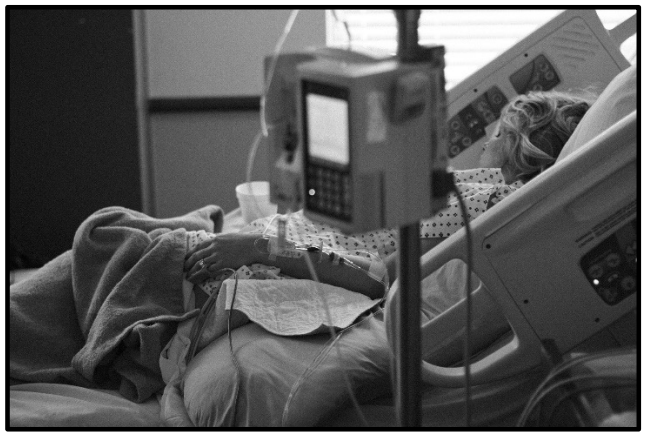
- low selection pressure towards resistance
- preserve commensal bacteria

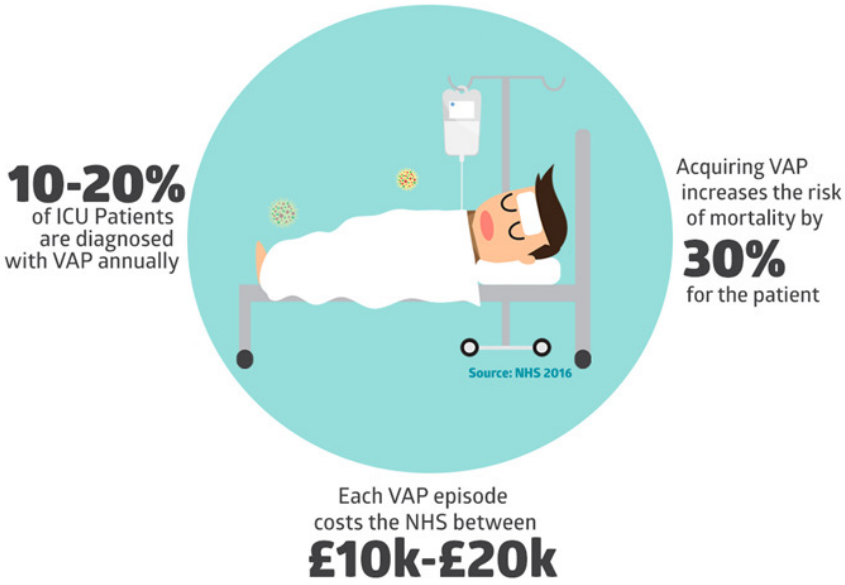
PoC: *Clostridium difficile* Toxin B (Bezlotoxumab, Merck, Sharp & Dohme); approved in 2017

Target disease

Hospital-acquired pneumonia (HAP) and ventilator-associated bacterial pneumonia (VAP) caused by *Pseudomonas aeruginosa*

- 86% of nosocomial infections associated with mechanical ventilation[1]





HAP and VAP represent a serious clinical concern

- High mortality (>20%) in spite of antibiotic therapy
- High number of cases (300k cases per year in the US)
- High healthcare burden

!!! Development of novel drugs urgently needed !!!

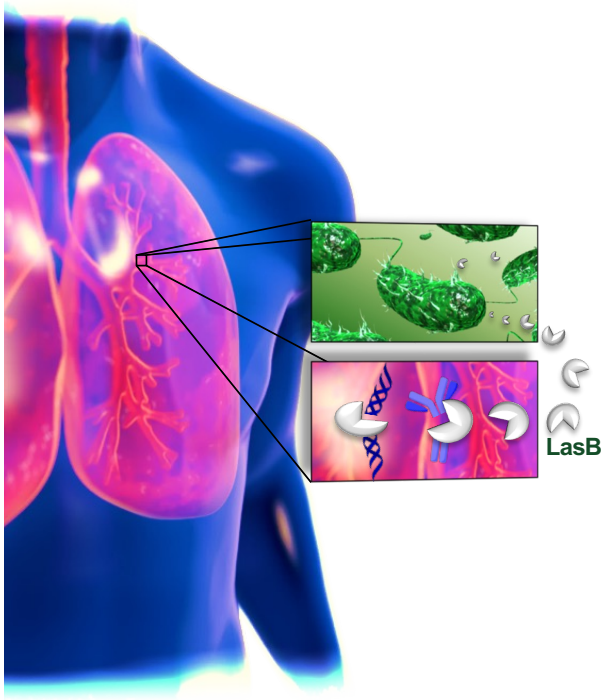
[1] Koenig and Truwit, 2006, Clin Microbiol Rev, doi:10.1128/CMR.00051-05

TPP: pneumonia therapy (HAP / VAP)

Product characteristic	Acceptance Criteria
Indication for use	Definitive adjunctive therapy of HAP / VAP due to confirmed *P. aeruginosa*
Target population	Hospitalized risk patients with documented *P. aeruginosa* pneumonia, when de-escalating from empirical antibiotic treatment regime. Trials may be confined to HAP or VAP. A convincing demonstration of efficacy in VAP could support an indication that includes HAP (~30% of patients as a minimum should have VAP). Patients with HAP should have been hospitalized for at least 48 hours before onset of the first signs or symptoms or these should occur within 7 days of hospital discharge. Patients with VAP should have received mechanical ventilation via an endotracheal or nasotracheal tube for at least 48 hours.
Point of care diagnostic	Detected LasB, e.g. multiplex PCR for pathogen detection, detection of LasB antigens / mRNA
Active pharmaceutical ingredient (API)	Small-molecule LasB inhibitor
Contraindication	Antagonism with SOC (Piperacillin/Tazobactam, Cefepim, Imipenem, Meropenem, Ceftazidim)
Safety	Benefit > risk
Measures of efficacy	Reduced 28d all-cause mortality; reduced risk of *P. aeruginosa* bacteremia
Unmet medical need	High mortality
Dose regimen	tbd, compliant with SoC antibiotic therapy
Route of administration	intravenously
Target validation	Subject to further target validation (Zupetic J et al. 2021, PMID: 33878342)

Pseudomonas aeruginosa LasB as target

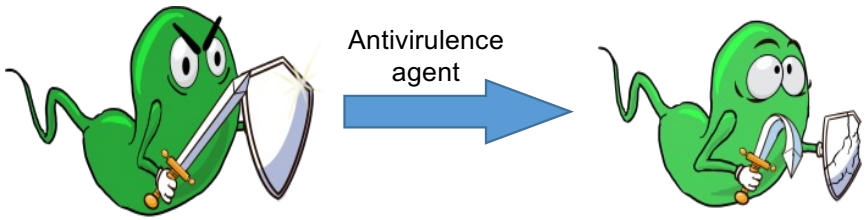
- *P. aeruginosa* classified as highest ("critical") priority by the WHO
- Causes lung infections, *e.g.* in HAP/VAP patients



Elastase (LasB)

- Secreted zinc-metalloprotease
- Degrades elastin and collagen (→ tissue penetration)
- Degrades components of the host immune system (→ immune evasion)
- Involved in biofilm formation

Targeting bacterial virulence to disarm *P. aeruginosa* rather than kill it



- LasB-expressing *P. a.* PAO1 more virulent in mice than Δ*lasB*-PAO1[1]
- LasB activity associated with increased 30-day mortality in IC patients

[1] Bastaert F *et al.*, **2018**, *Front Immunol.* 9, 1675. doi: 10.3389/fimmu.2018.01675.
[2] Zupetic J *et al.*, **2021**, *Chest* 160, 1624-1633. doi: 10.1016/j.chest.2021.04.015

Hit-identification strategies

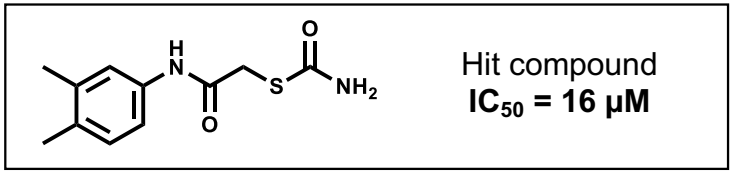
Target: LasB

- **Rational design and synthesis of novel inhibitors**
- **Protein-templated strategies: DCC and KTGS**
- **Fragment-based drug design**
- **Development of new biological assays**

New LasB inhibitors

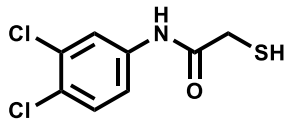
Screening of a focused protease inhibitor library
(*TimTec ActiTarg-P library* with 1192 compounds)

Functional FRET-based *in vitro* assay



Hit compound
IC_{50} = 16 µM

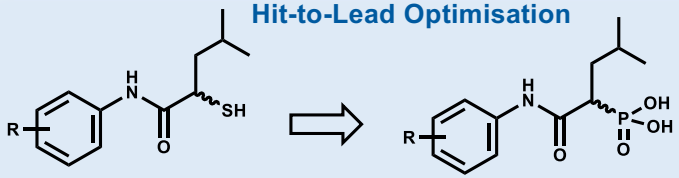
MedChem Optimisation
Further experiments (X-ray)



Thiol as active compound
IC_{50} = 7 µM[1]

Further MedChem Optimisation

Hit-to-Lead Optimisation

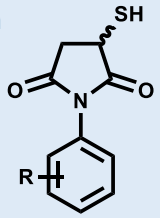


16-fold improved IC_{50}[3]

>100-fold improved IC_{50}[3]

Rigidification

2-fold improved IC_{50}[2]

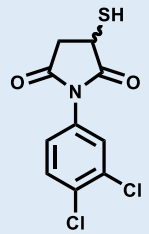


[1]: A. M. Kany *et al., ACS Infect. Dis.* **2018**
[2]: J. Konstantinovic *et al., J. Med. Chem.* **2020**
[3]: C. Kaya *et al., PCT/EP2021/073381*
C. Kaya *ei al., Angew. Chem. Int. Ed.* **2022**
C. Kaya *et al., ACS Inf. Dis.* **2022**
J. Konstantinovic *et al., manuscript in prep.*

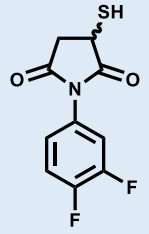
N-Aryl-3-mercaptosuccinimides as inhibitors of LasB

Medchem optimisation (35 derivatives):

- ☑ High selectivity over most of the MMPs and other off-targets
- ☑ No signs of cytotoxicity against human cell lines
- ☑ Improved chemical stability



29
IC_{50} (LasB) = 3.4 ± 0.2 µM



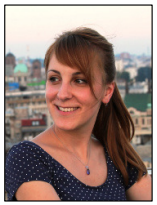
31
IC_{50} (LasB) = 3.5 ± 0.1 µM

		29
% inh @ 100 µM	**MMP-1**	n.i.
	MMP-2	38.7 ± 32.1
	MMP-3	n.i.
	MMP-7	n.i.
	MMP-8	83.9 ± 8.2
	MMP-14	n.i.
IC_{50} (µM)	**HDAC-3**	>100
	HDAC-8	>100
	TACE	5.2 ± 0.6

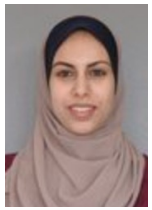
n.i. = <10% inhibition

		29
LD_{50} (µM)	**HepG2**	>100
	HEK293	>100
	A549	>100

Zebrafish facility at HIPS



Jelena Konstantinovic



Alaa Alhayek

Andreas Kany

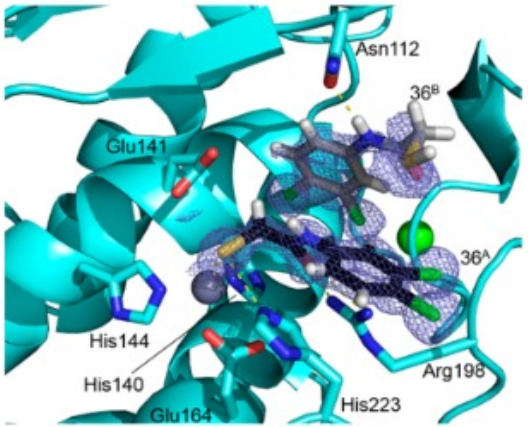
Jörg Haupenthal

Zebrafish toxicity
MTC = 100 µM

- ☑ Compound **31**: 10x less toxic in zebrafish compared to published LasB inhibitor (MTC = 10 µM)

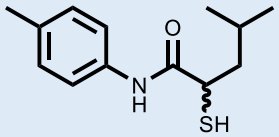
J. Konstantinovic *et al.*, *J. Med. Chem.* **2020**

Rational fragment growing and optimisation



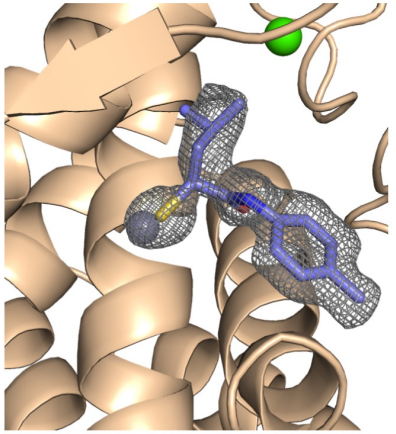
Co-crystal structure of the initial hit with LasB

Rational design



16-fold improved IC_{50}

55 derivatives



Co-crystal structure of the optimised hit with LasB

- Successful fragment merging/linking
- 16x improved activity compared to our published inhibitor (**IC_{50} = 0.40 ± 0.13 µM**)
- High selectivity over MMPs and other off-targets
- No signs of cytotoxicity against human cell lines

% 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.

n.i. = <10% inhibition

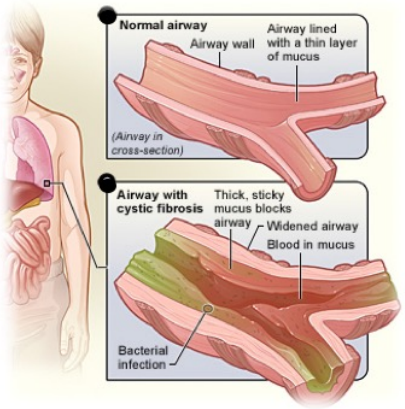
	LD_{50} (µM)
HepG2	>100
A549	>100

with J. Köhnke

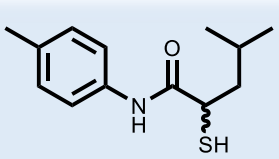
C. Kaya *et al., Angew. Chem. Int. Ed.* **2022**
C. Kaya *et al. PCT/EP2021/073381*

Activity of new inhibitors in cell-based assay and *in vivo*

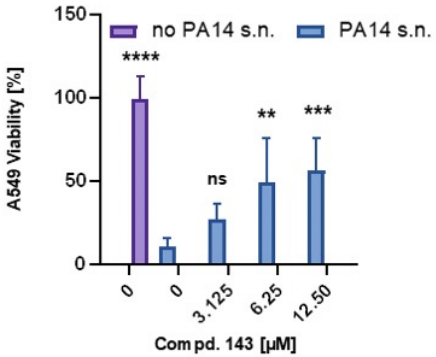
Effect on lung cell line (A549)

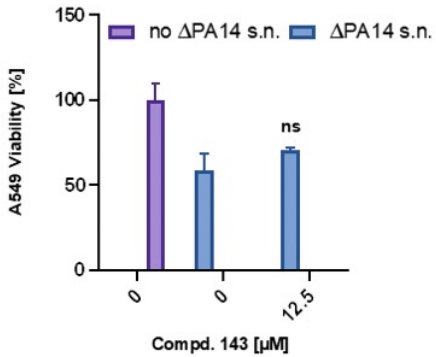


Graphic taken from the National Heart Lung and Blood Institute (NIH)

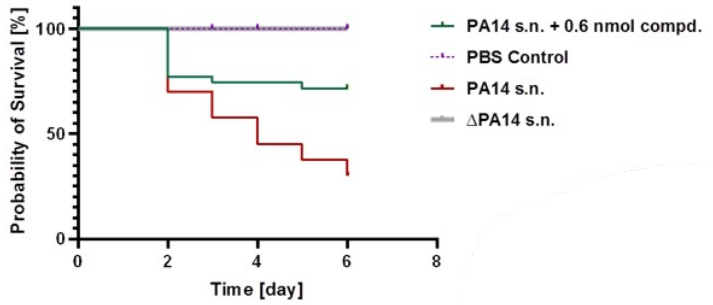


HIPS-5447



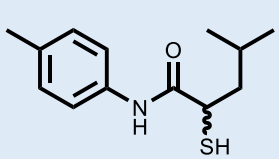


Enhanced survival rate of *Galleria mellonella* larvae

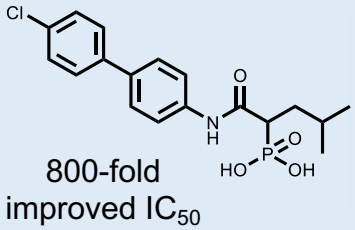


C. Kaya *et al., Angew. Chem.* Int. Ed. **2022**

Hit-to-lead optimisation



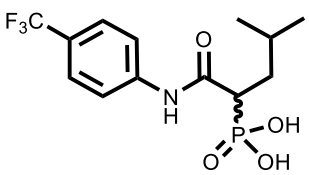
16-fold
improved IC_{50}



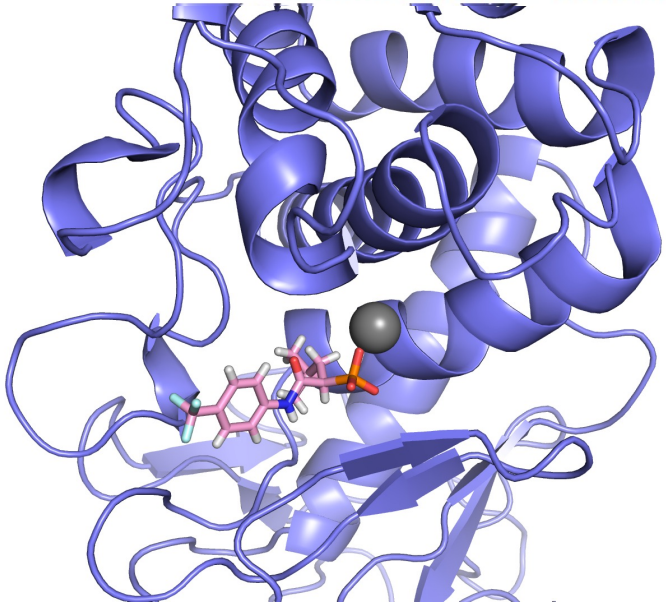
800-fold
improved IC_{50}

>70 derivatives

- ✅ 800x improved activity compared to our published inhibitor (**IC_{50} = 7.8 ± 0.3 nM**)
- ✅ High selectivity over MMPs and other off-targets
- ✅ Improved selectivity over TACE and COX-1 (problematic for the previous class)
- ✅ No signs of cytotoxicity against human cell lines
- ✅ Metabolic stability in mouse liver S9 >120 min

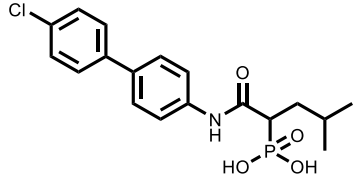


HIPS-5787
IC_{50} = 26.3 ± 7.6 nM



Co-crystal structure of the new hit with LasB

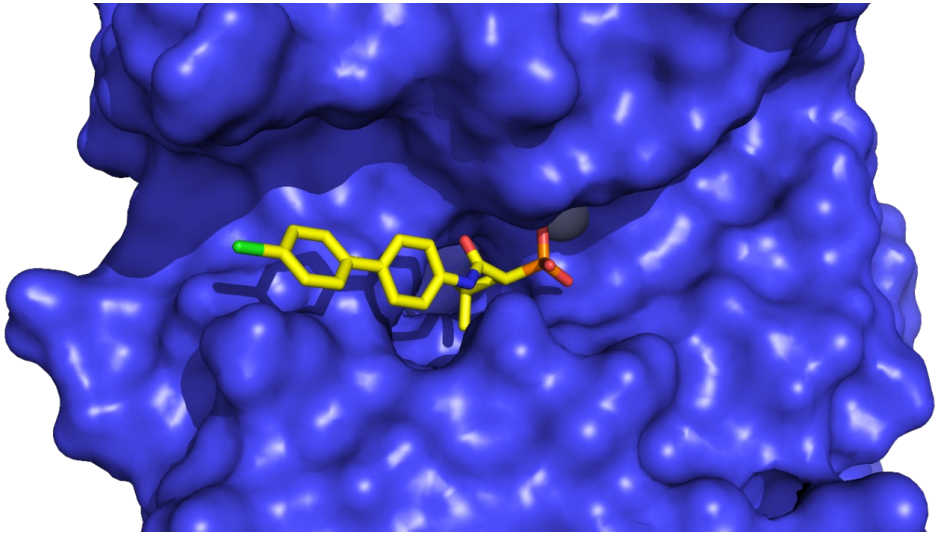
Rational design

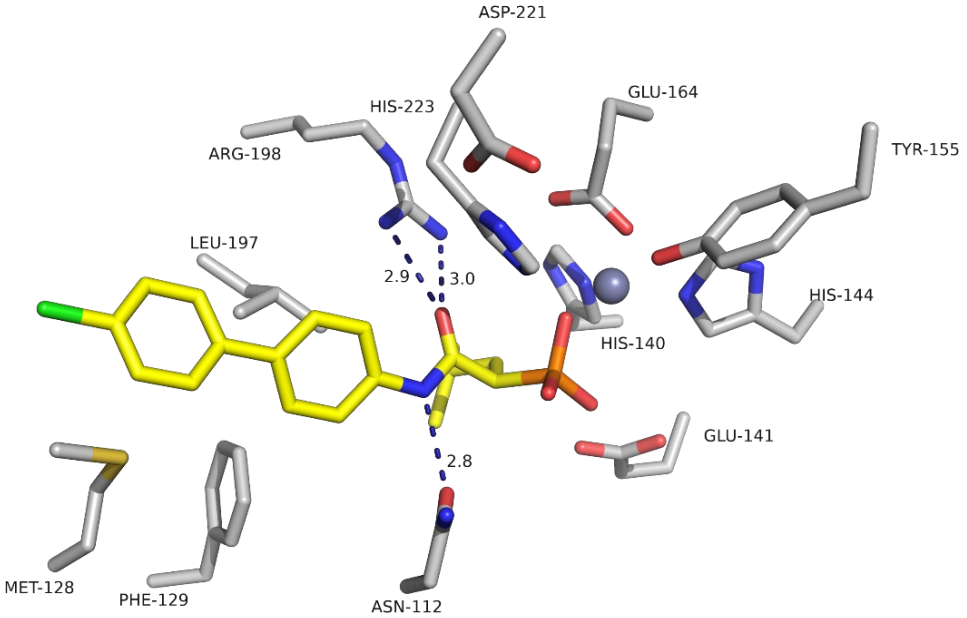


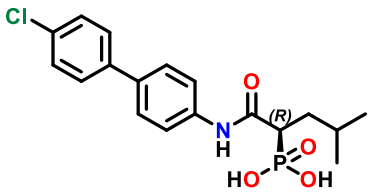
HIPS-5900
IC_{50} = 7.8 ± 0.3 nM

unpublished results

Co-crystal structure – HIPS-5900







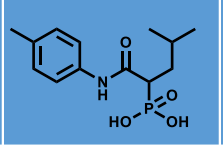
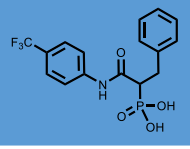
HIPS-5900
IC_{50} = 8.5 ± 0.5 nM

Benzene rings allows for additional hydrophobic interactions

Resolution: 1.60 Å

unpublished results

Target lead profile (TLP) guiding the hit-to-lead activities

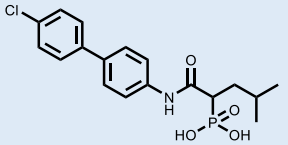
Criteria	TLP	HIPS-5704 	HIPS-5894 	HIPS-6271	HIPS-6466
Physicochemical Properties					
Molecular Weight [Da]	<500	285	373	407	412
Solubility [µM]	>100	>200	>200	>200	>200
LogD$_{7.4}$ chromatographic	-0.5 to 0.5	-0.64	0.04	0.2	-0.04
In vitro Activity					
LasB inhibition *in vitro*, IC$_{50}$ [nM]	<200	97	102	15	5.0
LasB inh. in presence of pulmonary surfactant (1%) [nM]	<3x IC$_{50}$	120	193	39	11.7
Pulmonary A549 cell assay, IC$_{50}$ [µM]	<2	0.4-0.7	n.d.	n.d.	n.d.
Antibacterial activity against PA14, MIC [µM]	>100	>100	>100	>100	>100
In vitro and *in vivo* ADME/T					
Activity toward MMPs 1-3, IC$_{50}$ [µM]	>50	>100	>100	>100	>100
Activity toward ADAM17, IC$_{50}$ [µM]	>50	>100	>100	>100	>100
Activity toward COX1, IC$_{50}$ [µM]	>50	>100	>100	n.d.	n.d.
Metabolic stability in mouse liver S9 t$_{1/2}$ [min]	>30	>120	>120	>120	>120
Metabolic stability in human liver S9 t$_{1/2}$ [min]	>30	>120	>120	n.d.	>120
Stability in mouse plasma t$_{1/2}$ [min]	>30	>150	>150	>150	>150
Plasma protein binding, mouse plasma [%]	80 to 95	91.8	89.9	95.0	88.3
Cytotoxicity toward A549, HepG2, HEK293 IC$_{50}$ [µM]	>50	>100	>100	>100	>100
Preliminary *in vivo* PK (IV route)	ELF >100 ng/mL a 2 mg/kg dosing IV (5 h)	968	814	5,627	3,292
Ex vivo Target Engagement					
Pig lung homogenate model IC$_{50}$ [µM]	<2	0.385	0.355	0.119	0.176
In vivo Target Engagement and Profiling					
Neutropenic lung inf. model with *P.a.*, subcut. route	Effective at 30 mg/kg in combination with levofloxacin	n.d.	n.d.	n.d.	Effective

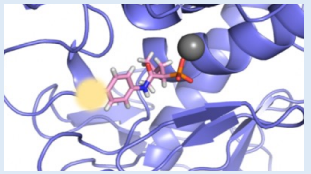
Results are given for representative inhibitors. Green flag: fulfills TLP criteria; yellow: TLP criteria missed up to 10-fold; red: TLP criteria missed >10-fold. N.d. = not determined.

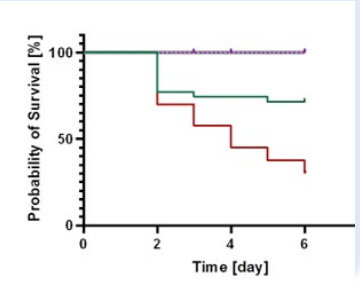
Summary – LasB

- Highly potent LasB (+MBL) inhibitors in various chemical classes
- No inhibition of relevant human off-targets
- Co-crystal structures for various LasB inhibitors available
- Excellent *in vitro* ADME-T profile
- Very promising retention of compounds in the lung and ELF
- Promising *in vivo* PD data (*Galleria mellonella* and mouse models)

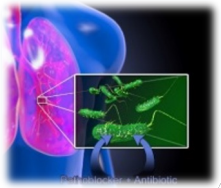
→ Excellent starting point for lead optimisation

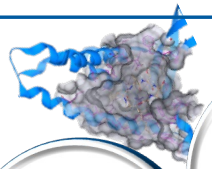






Conclusions



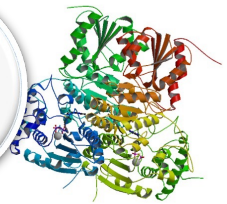


LspA
IC_{50}: 47 nM
Target strain(s):
PA, EC

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

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

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



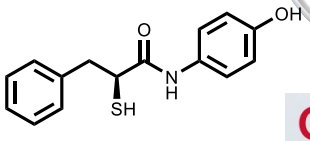
Col H/Q1
IC_{50}: 10 nM
Target strain(s):
CH, BC

LasB
IC_{50}: 1.5 nM
Target strain(s):
PA

IspE
IC_{50} (PF): 170 nM
Target strain(s):
MT, PF, EC, AB

F protein
IC_{50}: 40 nM
Target strain(s):
RSV

Nsp10/14
K_D: 17 µM
Target strain(s):
SARS-CoV-2

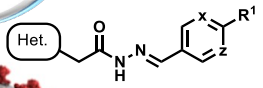


CARB-X
Combating Antibiotic-Resistant Bacteria

- Target-based anti-infective drug discovery
- Multiple hit-identification strategies
- Multiparameter optimisation

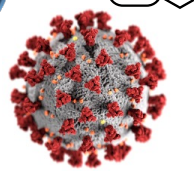
→ Promising hits/leads

Nsp12
IC_{50} (RdRp): 1 µM
Target strain(s):
SARS-CoV-2



Mß-lact
IC_{50} (VIM-1):
80 nM
Target strain(s):
PA, KP, EnC

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

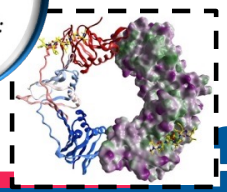


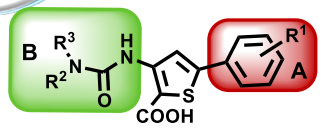
LecA/B
IC_{50}: 10.8 nM
Target strain(s):
PA

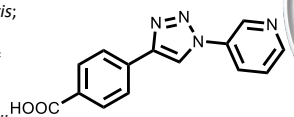
LANA & CsrA
IC_{50}: 17 µM
Target strain(s):
broad spectrum
PA, YT, SA…

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

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







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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