Hemholtz Centre for Infection Research (HZI)

www.helmholtz-hzi.de

GERMANY

specialist screening site

BRAUNSCHWEIG

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THE PEOPLE

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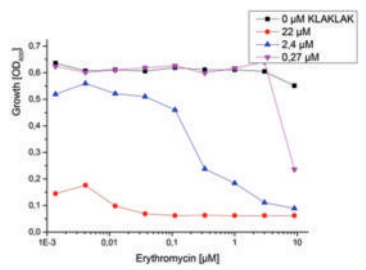
THE PROJECTS

Identification of sensitizers of Escherichia coli to macrolide antibiotics

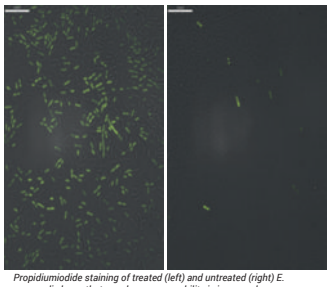
Scope: From assay development via medium scale screening to first mode of action studies.

Scientific and technical features: Several known antibiotics, such as erythromycin or fusidic acid, are only active against Gram-positive bacteria, because they either cannot penetrate the outer membrane of Gram-negative bacteria or are exported from the cytosol via export pump systems, such as ArcAB-TolC. Erythromycin has an IC50 in the µM range in the ΔTolC mutant, whereas the wildtype strain E. coli K12 is resistant to erythromycin. Thus, a phenotypic screening assay (384 well plate format) was established to discover compounds that sensitize the Gram-negative bacterium Escherichia coli to erythromycin. Approximately 9000 compounds were tested in the primary screening campaign, covering the proprietary HZI natural products, repurposing libraries, proprietary academic collection, and commercial synthetic compounds. Following IC50- determinations of positives, the mode of action of the most potent compounds was studied.

Key outcomes: Several compounds were identified that led to growth inhibition of E. coli K12 when combined with erythromycin or fusidic acid in µM concentrations. The secondary assays showed inhibition of the efflux system, but also effects on the membrane integrity, at least at higher concentrations.



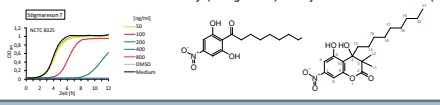
Effect of different concentrations of the sensitizer KLAKLAK on the susceptibility of Escherichia coli to erythromycin



Propidiumiodide staining of treated (left) and untreated (right) E. coli shows that membrane permeability is increased.

Antibiotics against dormant or persistent bacteria

Persistant populations are often associated with low growth and proliferation rates. 2700 myxobacterial extracts were screened for inhibitors of viability (not growth) of Mycobacterium bovis (BCG)

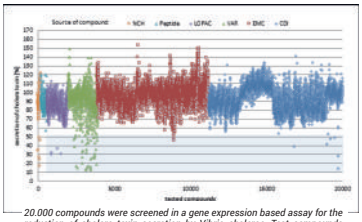


2 new groups of secondary metabolites were identified with activity also against Staphylococcus aureus.

Blocking the pathogenicity of Vibrio cholerae

Scope: Development of an assay that genetically links Vibrio cholerae's master virulence regulator aphA with the viability of the bacteria. Screening and mode of action analysis.

Scientific and technical features: AphA controls the secretion of the major virulence factors cholera toxin and toxin coregulated pilus. A plasmid with a kanamycin resistance (KmR) gene under the control of the aphA-like promoter was constructed and inserted into the bacteria. A screening assay was performed to identify compounds with inhibitory effect on the growth of V. cholerae in kanamycin-containing medium, but not in its absence. Of 20,000 compounds screened, six were identified to inhibit the pAKSB-induced KmR phenotype, and these compounds caused transcriptional inhibition of aphA. The compounds also caused a reduction of the secretion of cholera toxin and the expression of toxin coregulated pilus in vitro. The activity was confirmed in a suckling mouse model in vivo, which showed reduction of colonization by V. cholerae when co-administered with the most active compound. In addition to the virulence reducing substances, specific antimicrobial compounds against Vibrio cholerae were detected in a fluorescence based assay. A histidine kinase that is part of a particular two-component system was identified as the putative target by whole genome sequencing of resistant mutants in comparison with the wild type.

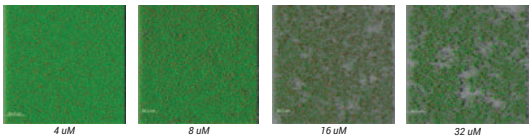


20.000 compounds were screened in a gene expression based assay for the reduction of cholera toxin secretion by Vibrio cholerae. Test compounds were employed at 20 µM concentration. Substances that reduced gene expression by >50% (area shaded in grey) were investigated further

Key outcomes: Identification of compounds with specific antimicrobial activity against V. cholera with a new mode of action, and of compounds that potently (1digit uM) reduce the secretion of the pathogenicity factors cholera toxin and toxin coregulated pilus.

Inhibitors of biofilm formation

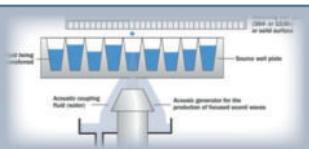
25.000 compounds were screened for the selective reduction of biofilm formation by S. aureus (joint project with Sanofi)



Biofilm structure after its formation in the presence of different concentrations of compound HZI13 as visualized by confocal laser fluorescence microscopy.

ESKAPE panel testing
Standard growth inhibition determinations (MIC assays) with bacteria from the ESKAPE panel are performed in an automated fashion for multiple internal and external partners in a continuous mode under S2 conditions. A counterscreen for cytotoxicity is performed simultanously against four cell lines.

THE HARDWARE



acoustic liquid handling

- Biological safety level S1-S3 facilities for screening of genetically modified and/or pathogenic organisms
- BSL3 screening facility equipped with Beckman Coulter Biomek FXP with Span 8, plate hotel, Multidrop despenser, incubator and fluorescence MTP reader for automated handling of large numbers of microtiter plates
- Echo® Liquid Handler from Labcyte to increase throughput, reduce compound consumption and increase accuracy of transferred low liquid volumes.
- For mechanistic studies: High resolution LC-MS, high content imaging, impedance spectroscopy, flow cytometry, sequencing, etc.
- Hit follow-up by medicinal chemistry & chemical biology

THE OUTPUT

1. *Bolger, G., Roy, S., Zapol'skii, V.A., Kaufmann, D.E., Schnürch, M., Mihovilovic, M.D., Nandy, R.K., Tegge, W.: "Targeting aphA: A new high throughput screening assay identifies compounds that reduce prime virulence factors of Vibrio cholerae"; J. Med. Microbiol. 2016, 65(7), 678-687*
2. *Kling, A; Lukat, P; Almeida, DV; Bauer, A; Fontaine, E; Sordello, S; Zaburannyi, N; ... Brönstrup, M;...Müller, R. Targeting DnaN for tuberculosis therapy using novel griselimycins Science (2015), 348(6239), 1106-12.*
3. *Koutsoudakis, G; Romero-Brey, I; Berger, C; Pérez-Vilaró, Gemma; M. Perin, Paula; Vondran, Florian W.R.; Kalesse, M; Harmrolfs, K; Müller, R; Martinez, JP.; Pietschmann, T; Bartenschlager, R; Bronstrup, M; Meyerhans, A; Diez, J. Soraphen A: a broad-spectrum antiviral natural product with potent anti-hepatitis C virus activity.J. Hepatology (2015), 63(4), 813-21.*
4. *Kempf, K; Kempf, O; Orozco, M; Bilitewski, U; Schobert, R: Synthesis and Structural Revision of the Fungal Tetramic Acid Metabolite Spiroscytalin, J. Org. Chem. 82 (2017) 7791–7795*
5. *Gilardi, A.; Bhamidimarri, S.P.; Brönstrup, M.; Bilitewski, U.; Marreddy, R.K.R.; Pos, K.M.; Benier, L.; Gribbon, P.; Winterhalter, M.; Windshügel, B.: Biophysical characterization of E. coli TolC interaction with the known blocker hexaaminnecobalt, BBA – General Subjects, 1861 (2017) 2702-9*

THE FUTURE

Collaborations:
Numerous universities and partners from EU Innovative Medicine Initiative (IMI)

Networks:
Deutsches Zentrum für Infektionsforschung (DZIF); Helmholtz Drug Discovery Initiative

Future plans:
Increased sample throughput through automation of protocols of even complex assays related to infection research

Added value:
The HZI is focussed on an indication of high medical need and offers state of the art technologies and know-how

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