LsrK kinase inhibitors as Quorum Sensing modulators

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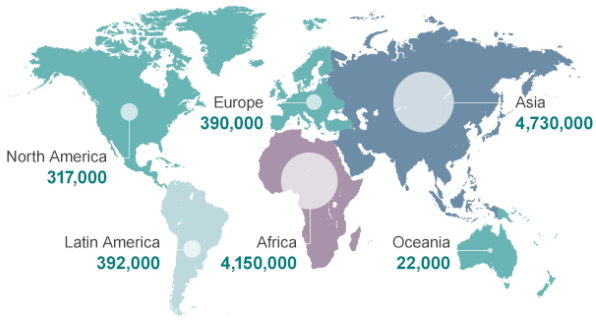
INTRODUCTION: Fighting antibiotic resistance

Antimicrobial resistance is posing a continuously-rising threat to global health. Each year in the EU, **multidrug-resistant bacterial infections cause the death of ca. 25.000 patients**, with further healthcare costs and productivity losses estimated to be at least EUR 1.5 billion. As shown in the picture, the estimated deaths attributed to antimicrobial resistance every year by 2050 will be over **10 million**[1]. Therefore, there is an urgent need for a better antibiotic stewardship and for the discovery and development of new drugs to fight against **Gram-negative** bacteria. The **INTEGRATE** project has assembled a team of 10 beneficiaries from eight EU member states, encompassing both academic and non-academic sectors, to form a consortium committed to train **Early Stage Researchers (ESRs)** in the discovery and preclinical validation of novel Gram-negative antibacterial agents and antibacterial targets.

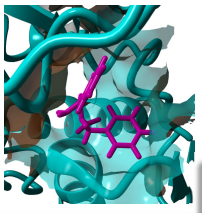
The ESRs are exposed to every aspect of the antimicrobial discovery process, ranging from target identification and validation, through organic synthesis, *in silico* design and compound screening, to mode-of-action and possible resistance mechanisms. The INTEGRATE **training framework** is built on an innovative research project aimed at targeting important but non-essential gene products as an effective means of **reducing bacterial fitness**, thereby facilitating clearance of the pathogen by the host immune system. It is now accepted that identification of **novel drug targets and non-conventional mechanisms**, as well as the development of novel chemotypes, is central to the fight against bacteria generally and against Gram-negative bacteria in particular.



TARGETS: Innovative approaches

Sulfur assimilation inhibitors

Design, synthesis and biological evaluation of *S. Typhimurium* **SAT** (serine acetyl transferase) and **OASS** (O-acetylserine sulfidrilase) inhibitors. Their inhibition will lead to a reduced availability of cystein, the preferential bacterial source of sulfur for all sulfur-containing biological molecules (e.g. methionine, biotin). The picture shows the binding mode of compound UPAR 319 in the binding pocket of *S. Typhimurium* OASS (PDB ID: 1OAS).[2]



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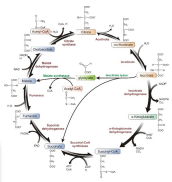
Glioxalate shunt

Characterization of the enzymology controlling carbon flux through the cytric acid cycle/**glyoxylate shunt** branchpoint of *P. aeruginosa*. Identification of potential inhibitors, determination of their mode of action and of potential resistance mechanism(s) which can reduce their potency. The glyoxylate shunt allows growth on acetate by providing a route from isocitrate to gluconeogenic precursors that bypasses the decarboxylative steps of the citric acid cycle. For these reasons it is widely considered to be one of the most important metabolic branchpoints in the whole of microbial metabolism.[3]



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ClpP inhibitors

Identification and optimization of covalent *E. coli* **ClpP** (caseinolytic peptidase ATP-dependent proteolytic subunit) inhibitors using *in silico* and *in vitro* screening approaches. ClpP plays a key role in controlling bacterial protein turnover and is the main enzyme involved in protein homeostasis by removing damaged, denatured and aberrantly folded proteins that are harmful to the cell. The figure shows the co-crystal structure of *E.coli* ClpP with a covalent inhibitor (PDB ID: 2FZS).[4]

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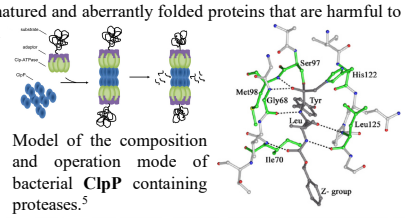
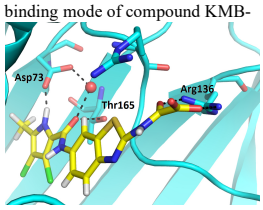


Model of the composition and operation mode of bacterial **ClpP** containing proteases.[5]

GyrB inhibitors and GyrB/ParE dual inhibitors

Design, synthesis and evaluation of structurally novel *E. coli*/*S. aureus* **GyrB** (DNA gyrase B) inhibitors and DNA gyrase B/topoisomerase IV (**GyrB/ParE**) dual inhibitors with *in vitro* antibacterial activity. Gyrase plays an essential role in the cell by maintaining DNA in a negatively-supercoiled conformation. The picture shows the binding mode of compound KMB-21 in the binding pocket of *E. coli* DNA gyrase B (PDB ID: 4DUH).[6]



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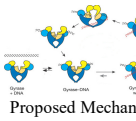
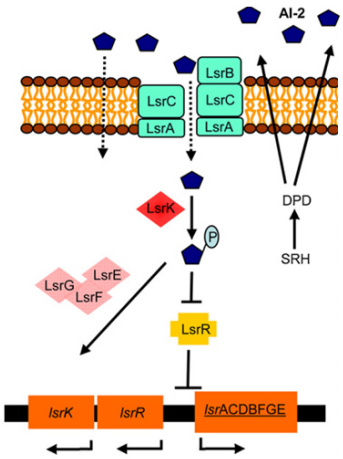
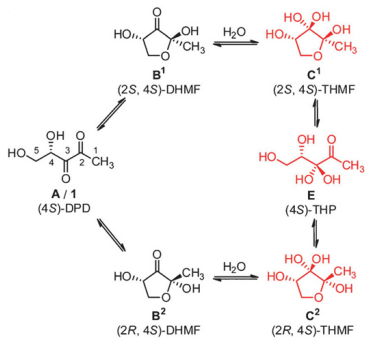


Proposed Mechanism of DNA supercoiling by **DNA gyrase**.[7]

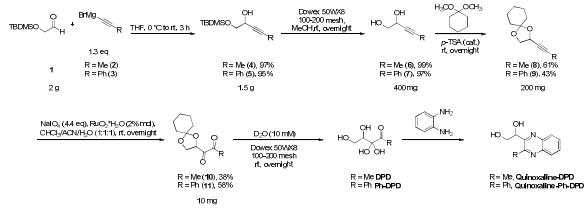
TAROS'CONTRIBUTION: LsrK kinase inhibitors

Bacteria communicate with each other through a complex system of small molecules. Once these molecules have reached a certain concentration, they activate gene expression leading to the production of molecules that will allow bacteria to start a "population dependent behavior" (i.e. **Quorum Sensing**, QS).[8,9]
LsrK kinase, phosphorylating one of these small molecules (i.e. **DPD**, 4,5-dihydroxy,2,3-pentanedione), is responsible for QS activation. As shown in the picture, DPD exists as an equilibrium of a linear and two cyclic structures (in a ratio 2:1:1).[10] This makes its synthesis very challenging.

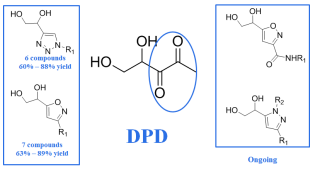




We have successfully developed a new, high yielding five steps synthesis of DPD as a racemic mixture. In a similar manner, phenyl-DPD (Ph-DPD) was synthesized as a negative control to be used in the development of the biological assay.



Using a ligand based approach and complementing literature reported DPD-analogues, the diketo moiety of DPD was replaced with two different heteroaromatic rings. Six 1,4-disubstituted triazoles and seven 3,5-disubstituted isoxazoles were synthesized for further biological evaluation. The synthesis of other two sets of DPD-analogues (1,3,5-trisubstituted pyrazoles and 3,5-disubstituted isoxazole bearing an amide functional group at position 3) is currently ongoing. The compounds will be further characterized at a functional cellular level in collaboration with the University of Helsinki and Cambridge. These novel tool compounds will serve to address the practical relevance of LsrK kinase inhibition to QS and *E. coli* survival.



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This work is supported by the Initial Training Network, funded by the H2020 Marie Curie Actions of the European Commission under Grant Agreement 642620