EURESTOP GENERAL MEETING (Grant Period 2)
21-22 March 2024 Roma (Italy)

Organized in collaboration with Sapienza University of Rome and UnitelmaSapienza

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Bruno Botta
Francesca Ghirga
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Organizing Committee:

Mattia Mori
Francesca Ghirga
Susanna Piva
Antonella Cerreto
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Thursday 21 March

LINK for REMOTE CONNECTION (click here)

08:30 – 9:00 **Registration of participants** **Building CU019 Room "A. Giuliano"**

09:00-09:30 Welcome by Sapienza University Rector (Antonella Polimeni), EURESTOP Chair (Mattia Mori), and UnitelmaSapienza Rector (Bruno Botta)
Building CU034 Room 8

Session #1. Chairs: Maria Luisa Mangoni and Maria Daniela Silva Building CU034 Room 8

09:30-10:10 **Paolo Visca**, Roma Tre University (Italy): **Bacterial iron metabolism; turning basic physiology into antibacterial drug development**.

10:10-10:30 **Markus Kalesse**, Leibniz University Hannover (Germany): **Acanthodoral as potentially new antibiotic to fight bacterial resistance.**

10:30-10:50 **Vasile Parvulescu**, University of Bucharest (Romania): **Prostaglandin derivatives synthesized in heterogeneous catalysis following green routes.**

10:50-11:40 Coffee break **Building CU019**

Session #2. Chairs: Maria Amparo Faustino and Amar Osmanovic Building CU019 Room "A. Giuliano"

11:40-12:00 **Raffaele Saladino**, University of Tuscia (Italy): **Nanostructured multi-enzyme cascade reactions in the synthesis of antimicrobial scaffolds.**

12:00-12:20 **Dieter Schinzer**, Otto-von-Guericke-Universität-Magdeburg (Germany): **Transforming natural product synthesis into therapeutic products.**

12:20-12:40 **Janez Ilaš**, University of Ljubljana (Slovenia): **Exploring the chemical space of benzothiazole-based DNA Gyrase B Inhibitors.**

12:40-13:00 **Marianna Damian**, Biotage Sweden AB (Sweden): **Parallel Peptide Workflow: Biotage technologies for peptide drug discovery.**

13:00-14:20 Lunch break

Session #3. Chairs: Younes Smani and Nadejda Neronova Building CU019 Room "A. Giuliano"

14:20-14:40 **Aubin Pitiot**, Luxembourg Institute of Health (Luxembourg): **Complement-activating Multimeric immunotherapeutic compleXes (CoMiX) elicit killing of *Pseudomonas aeruginosa* and prevent biofilm formation.**

14:40-15:00 **Alvaro G. Temprano**, University of Salamanca (Spain): **Molecular dynamics simulations of the interaction between antibacterial small molecules and the *Staphylococcus aureus* lipid bilayer.**

15:00-15:20 **Tomislav Meštrović**, University of Washington (USA) and University centre Varaždin (Croatia): **Mapping the impact of bacterial antimicrobial resistance in Central Europe: a cross-country comparison of key pathogens and pathogen-drug combinations.**

15:20-15:40 **Alessandra Bragonzi**, IRCCS San Raffaele Scientific Institute (Italy): **Preclinical mouse models for assessing drug efficacy in respiratory bacterial infection and inflammation.**

15:40-16:00 **Stephen J. Fey**, CelVivo Aps (Denmark): **Determination of drug-resistance using 3d cell culture.**

***16:00-17:00 poster session & coffee break + GROUP PICTURE* Building CU019**

17:00-18:00 **MC meeting** (reserved to MC members/delegates) **LINK for REMOTE CONNECTION (click here) Building CU019 Room "A. Giuliano"**

20:00 **Social dinner**

Friday 22 March

LINK for REMOTE CONNECTION (click here)

Session #4. Chairs: Cristina Nativi and Mikayel Ginovyan Building CU019 Room "A. Giuliano"

09:00-09:40 **Gian Maria Rossolini**, University of Florence (Italy): **Antimicrobial resistance: evolutionary complexity and changing needs in clinical practice**.

09:40-10:00 **Maria Amparo Ferreira Faustino**, University of Aveiro (Portugal): **An insight on photodynamic inactivation to mitigate microbial resistance.**

10:00-10:20 **Gaetano Angelici**, University of Pisa (Italy): **Design and synthesis of a new functionalizable visible-light photoswitch for antimicrobial applications.**

10:20-10:40 **Xavier Just-Baringo**, Universitat de Barcelona (Spain): **Photoswitchable antimicrobial peptides: towards a general method for solid phase synthesis of visible-light-operated peptides.**

***10:40-11:40 Poster session & coffee break* Building CU019**

Session #5. Chairs: Carole Devaux and Rossella Aronne Building CU019 Room "A. Giuliano"

11:40-12:00 **Younes Smani**, University of Pablo de Olavide (Spain): **Intracellular trafficking of Acinetobacter baumannii in host cells requires activation of Transcriptional Factor EB and phospholipase A_2.**

12:00-12:20 **M. Carmen Galan**, University of Bristol (United Kingdom): **Small molecule G-quadruplex ligands are antibacterial candidates for Gram-negative bacteria.**

12:20-12:40 **Fanni Judák**, Hungarian Research Network (Hungary): **Competition-driven evolution and targeted antibiotic production to fight multidrug-resistant bacteria.**

12:40-13:00 **Didem Şen Karaman**, Izmir Katip Çelebi University (Turkey): **Evolving nanoparticles aided strategies against bacterial infections.**

13:00-14:20 Lunch break

Session #6. Chairs: Simone Carradori and Demokrat Nuha Building CU019 Room "A. Giuliano"

14:20-14:40 **Ryszard Ostaszewski**, Polish Academy of Sciences (Poland): **Enzymatic synthesis of coumarin aminophosphonates peptidomimetics as novel antimicrobial agents**.

14:40-15:00 **Ismail Ocsoy**, Erciyes University (Turkey): **Natural pH Indicator incorporated-assays for rapid and colorimetric detection of carbapenem resistance in Acinetobacter.**

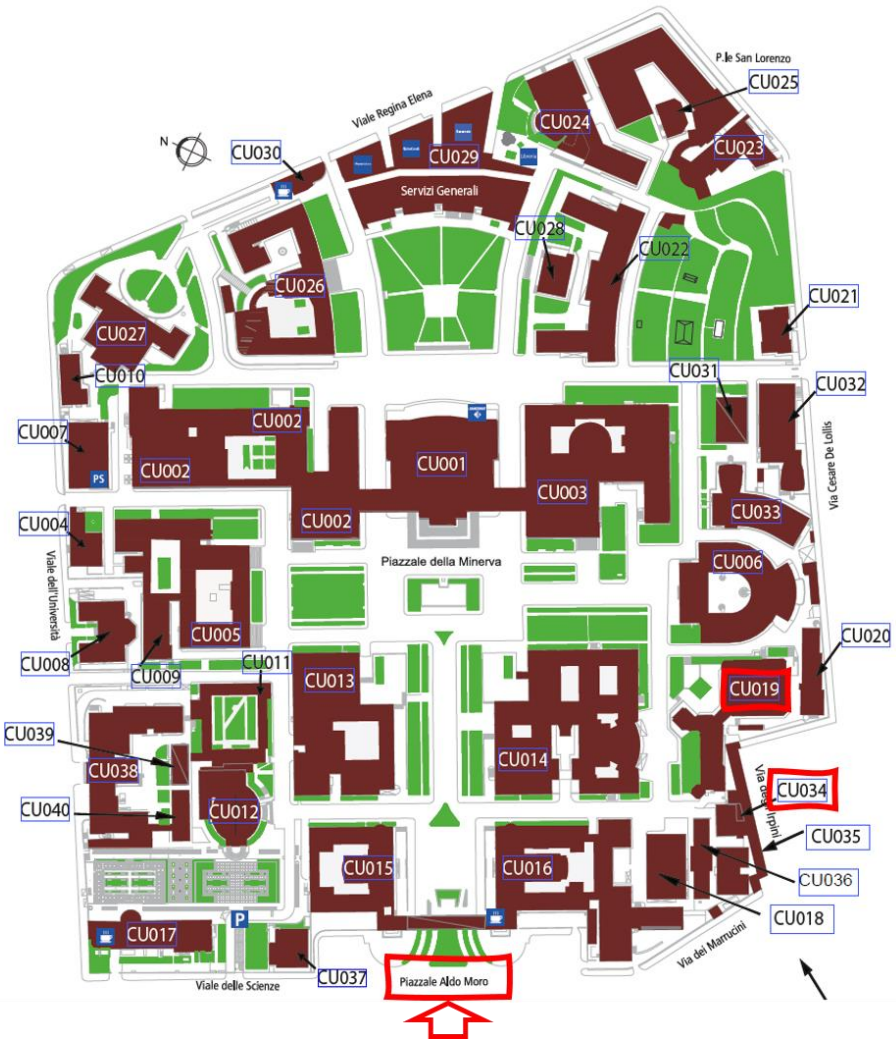
15:00-15:20 **Anna Kedziora**, University of Wroclaw (Poland): **Silver nanoformulations as antibacterial agents: mode of action and impact on the development of bacterial resistance.**

15:20-15:40 **Maria Daniela Silva**, University of Minho (Portugal): **Bacteriophages effective against stationary cells: promising agents against antibiotic tolerant bacteria.**

15:40-16:00 **Srđan Bjedov**, University of Novi Sad (Serbia): **Antibiotic potential of steroidal and triterpenoid compounds.**

16:00-16:20 **Entela Haloci**, University of Medicine Tirane (Albania): **Symmetric synthesis of polysulfure derivatives.**

16:20-16:30 Conclusions and next events/calls/grants **Building CU019 Room "A. Giuliano"**



Piazzale Aldo Moro 5: main entrance
Building CU034 Room 8: opening and meeting session #1
Building CU019 Room A. Giuliano: registration desk and meeting sessions #2-#6

ABSTRACTS OF TALKS
(in order of presentation)

Bacterial iron metabolism; turning basic physiology into antibacterial drug development

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Iron is an essential nutrient for almost all forms of life, but it is poorly available due to low solubility and entrapment by iron-binding (macro)molecules. Therefore, iron must be transported across the membrane(s) through specific active transport systems. Bacteria, fungi, and some plants have evolved the ability to produce, release, and uptake high-affinity iron-binding small molecules called siderophores, belonging to different chemical classes (1). Despite species-specific chemical diversity, siderophore biogenesis, transport, and regulation are governed by similar rules in prokaryotes; they are produced and taken up only when the cytoplasmic iron concentration is too low to sustain the cellular metabolism. Repression under conditions of iron sufficiency is a dogma of iron uptake regulation since too much iron would be lethal due to oxidative stress. However, positive control of iron uptake is also observed, especially in species that can prey upon different exogenous iron carriers, so exogenous carriers are sensed, and cognate transporters are expressed only when a given carrier is available (2). In bacterial pathogens, iron scarcity is also an environmental signal of their transition into the host, where iron-binding proteins contribute to innate immunity by depriving the invading pathogen of an essential metal. It is now clear that sensing low iron levels in vivo serves as an inducive stimulus for the expression of some virulence factors by bacterial pathogens, and that both nutrition and virulence are closely linked to bacterial iron uptake capabilities and metabolism (3). Therefore, both iron uptake systems and iron metabolism have become attractive targets for the development of novel antimicrobials against multidrug-resistant critical pathogens. Recent advances in the discovery of novel antimicrobials like iron chelators, iron mimetics (4), and siderophore-antibiotic conjugates (5) provide a paradigmatic example of how basic knowledge of bacterial physiology can be turned into promising antibacterial strategies.

References

1. Miethke M, Marahiel MA. Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev. 2007;71:413-51.
2. Llamas MA, Imperi F, Visca P, Lamont IL. Cell-surface signaling in *Pseudomonas*: stress responses, iron transport, and pathogenicity. FEMS Microbiol Rev. 2014 38:569-97.
3. Cassat JE, Skaar EP. Iron in infection and immunity. Cell Host Microbe. 2013;13:509-519.
4. Bonchi C, Imperi F, Minandri F, Visca P, Frangipani E. Repurposing of gallium-based drugs for antibacterial therapy. Biofactors. 2014;40:303-12.
5. Rayner B, Verderosa AD, Ferro V, Blaskovich MAT. Siderophore conjugates to combat antibiotic-resistant bacteria. RSC Med Chem. 2023;14:800-822.

Acanthodoral As potentially New Antibiotic To Fight Bacterial Resistance

Alina Eggert[a], Karl T. Schuppe[a], Hazel L. S. Fuchs[b], Mark Brönstrup[a,b] and **Markus Kalesse*[a]**
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[b] *Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig (Germany)*

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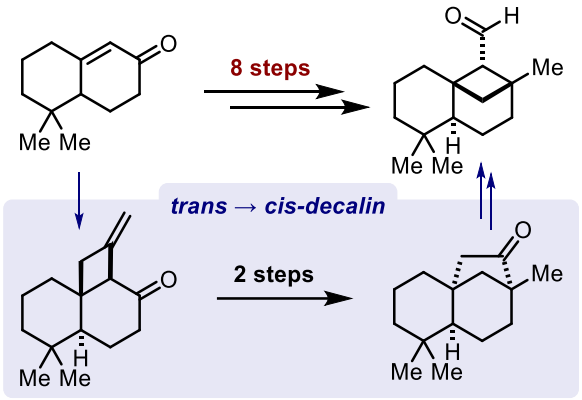
We will present our synthesis[1] of (±)-acanthodoral, a sesquiterpenoid derived from the marine nudibranch *Acanthodoris nanaimoensis*. Our approach utilizes a three-step sequence involving a Sm(II)-induced 1,2-rearrangement and a semipinacol reaction to convert a relaxed trans-decalin framework into its cis-decalin-skeleton. The synthesis could be accomplished in 14 steps (4.2% yield) from a commercially available carboxylic acid. Finally, the endgame reported by Koreeda and co-workers[2] was followed and remained unchanged to obtain the targeted compound.



The resulting analogs of acanthodoral were subjected to biological testings and identified the pivotal pharmacophore. All compounds were tested against a selection of bacteria and fungi, namely: *Saccharomyces pombe, Mucor hiemalis, Candida albicans, Bacillus subtilis and Staphylococcus aureus.*

References

1. A. Eggert, K. T. Schuppe, H. L. S. Fuchs, M. Brönstrup, M. Kalesse, *Org. Lett.* **2024**, https://doi.org/10.1021/acs.orglett.3c03717
2. L. Zhang, M. Koreeda, *Org. Lett*. **2004**, *6*, 537–540.

Prostaglandin derivatives synthesized in heterogeneous catalysis following green routes

Simona Coman[1], Florea Cocu[2], Iunia Podolean[1], Madalina Tudorache[1], Bogdan Cojocaru[1], Octavian Pavel[1], Camelia Bala[3], **Vasile I. Parvulescu[1]**

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2. *Institute of Pharmaceutical Chemistry, Bucharest, Romania*
3. *University of Bucharest, Department of analytical and physical chemistry, Bucharest, Romania*

Prostaglandins are lipid autacoids that have been found in almost every humans and other animal tissues (A). They are enzymatically derived from the fatty arachidonic acid [1] representing a subclass of the eicosanoids and prostanoid class of fatty acid derivatives. These incorporate 20 carbon atoms, also including a 5-carbon ring. From the medicinal point of view, the prostaglandins act as hormone-like substances with influence upon several bodily functions including inflammation, pain and uterine contractions [1]. Due to their specific properties and the large request, the medicine uses synthetic forms of prostaglandins to treat several diseases including an attenuation of the bactericidal effects of kanamycin or ampicillin in Staphylococcus aureus, as well as the methicillin-resistant S. To date, most of their syntheses were carried out with either bio- or homogeneous catalysts [2].

Based on this state of art the aim of this work focused the synthesis of various prostaglandin derivatives following a heterogeneous catalytic approach with a series of advantages as the recyclability and stability of the catalyst, and the replacement of the expensive catalysts with stable green high-surface area carbon- and porous-inorganic catalysts. Figure 1 shows an example of a synthesis of a F prostaglandin intermediate substrate (A) and the catalytic performances in this synthesis (B)

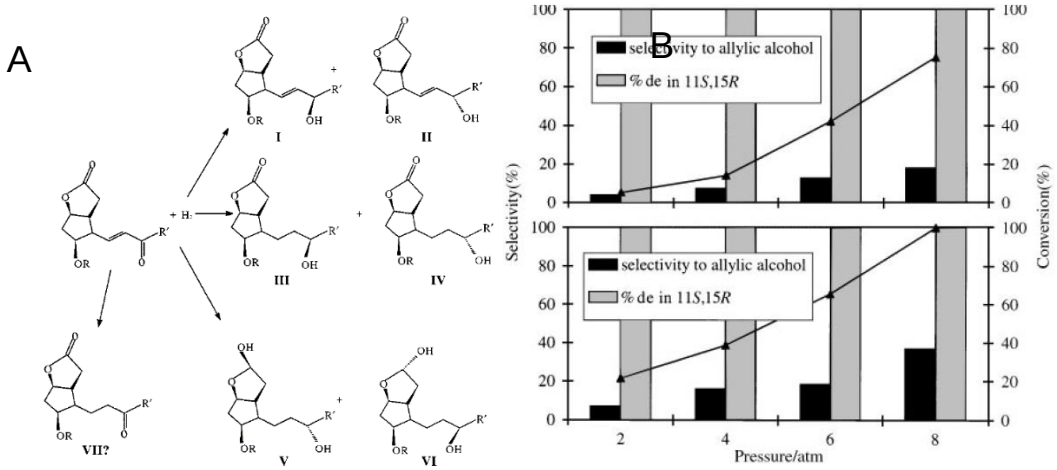

Figure 1. A: Routes in hydrogenation of the F prostaglandin intermediate**; B:** Catalytic behavior of **(a)** 0.13 wt% Ru-MCM-41, (b) 0.67 wt% Ru-MCM-41; solvent methanol, room temp., 2h).

References

1. E. Ricciotti, G.A. Fitzgerald, *Arteriosclerosis, Thrombosis, and Vascular Biology*. **31** (2011) 986–1000.
2. J.-Y. Cai, I. Takashi, *Prostaglandins Leukot. Essent*. 133 (2018)16-22.

Nanostructured multi-enzyme cascade reactions in the synthesis of antimicrobial scaffolds

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We describe here the possibility to combine multi-enzyme cascade of lipase and tyrosinase immobilized on lignin nano-platforms[1] (Figure, panel A) and multi-component chemistry in a unique reactive framework to yield privileged scaffolds useful in the synthesis of antimicrobial compounds, such as benzoxazines and flavanones. Highly functionalized bi- and tri-cyclic benzoxazines were synthesized through generation in-situ of electrophilic quinones from appropriate phenol esters by cascade lipase and tyrosinase catalysis,[2] followed by internal nucleophilic 1,6-Michael addition and subsequent intramolecular lactonization and aromatization processes (Figure, panel B).[3] Likewise, the preparation of flavanones was obtained from aromatic compounds by promiscuous aldolase activity of a tandem of two lipases followed by unexpected intramolecular oxa-Michael addition (Figure, panel C). Pummerer's ketone derivatives were synthesized by a similar approach involving oxidative coupling processes.[4] Overall, the immobilization of the multi-enzyme cascade on electroactive lignin nano-platform improved the efficacy of the transformation as a consequence of substrate channelling effects under mild reaction conditions and excellent atom economy and chemo-selectivity, providing new entry for sustainable organic synthesis.[5]

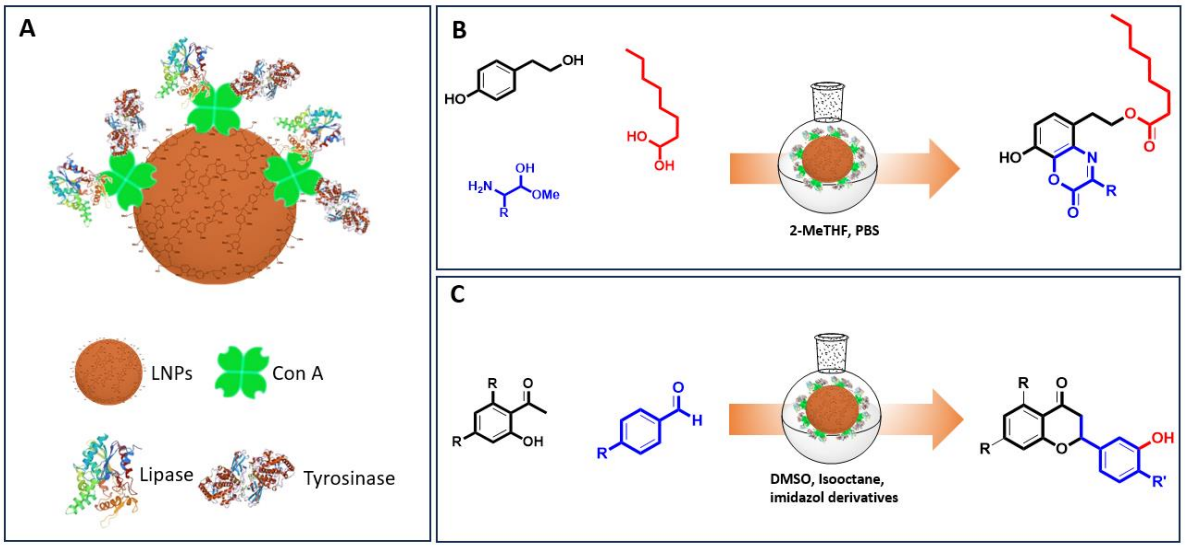

Figure. Nanostructured multi-enzyme cascade reactions. A) Lignin-based nano-reactors. B) Multicomponent synthesis of benzoxazines. C) Promiscuous synthesis of flavanones.

References

1. R. Saladino et al ChemCatChem 2023, 15, e202300533.
2. R. Saladino et al ChemCatChem 2022, 14, e202200.
3. R. Saladino et al J. Org. Chem. 2024, 89, 4, 2343–2350.
4. R. Saladino et al Eur. J. Org. Chem. 2023, 26(32),e202300356Zzz.
5. R. Saladino et al RSC Advances 2020, 10(48), pp. 29031-29042.

Transforming Natural Product Synthesis into Therapeutic Products

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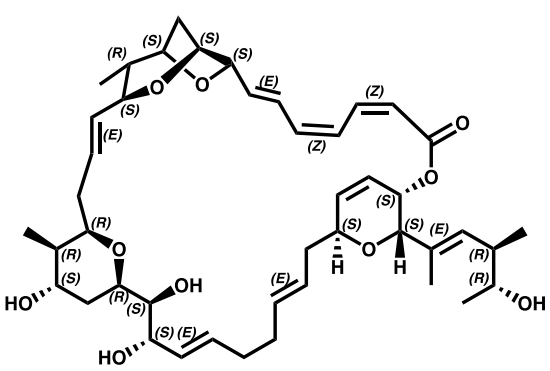
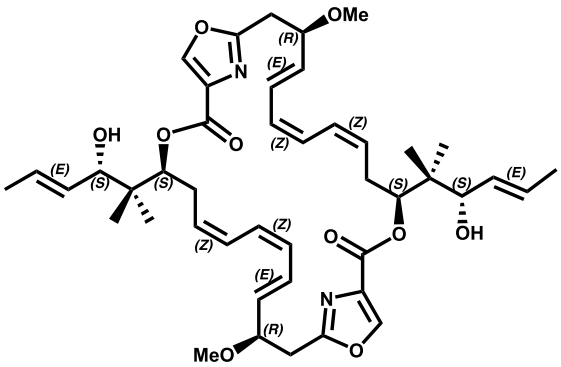
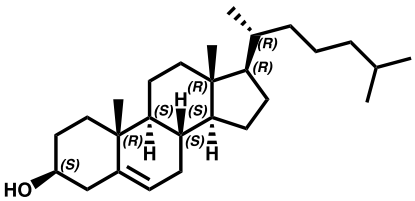


(+)-Neosorangicin A



(-)-Disorazole C_1



(-)-Cholesterol

The lecture will focus on complex natural product synthesis based on three compounds: (-)-disorazole C_1 a powerful antitumor-active compound, (+)-neosorangicin A a new type of antibiotic, and finally (-)-cholesterol as part of the lipid cocktail for modern mRNA-based Corona vaccines.

Exploring the Chemical Space of Benzothiazole-Based DNA Gyrase B Inhibitors

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Bacterial topoisomerases, a class of enzymes that orchestrate alterations in the topology of DNA, are recognized as clinically validated targets for antibacterial drugs. These enzymes play a pivotal role in the DNA replication and transcription, ensuring the smooth functioning of these vital biological processes. Two key players in this arena are DNA gyrase and topoisomerase IV, both of which belong to the type IIA topoisomerases family. Despite their distinct names, these two enzymes share a high degree of structural and functional similarities. Both DNA gyrase and topoisomerase IV are heterotetrameric enzymes. The DNA gyrase is a quartet of two GyrA and two GyrB subunits, while topoisomerase IV is a quartet of two ParC and two ParE subunits. The primary function of DNA gyrase and topoisomerase IV is to catalyse the transient break and reunion of the DNA double strand. This process is crucial for winding and unwinding the DNA molecule during its replication or transcription.

We designed and synthesized a series of inhibitors of the bacterial enzymes DNA gyrase and DNA topoisomerase IV, based on our recently published benzothiazole-based inhibitor bearing an oxalyl moiety. To improve the antibacterial activity and retain potent enzymatic activity, we systematically explored the chemical space. Several strategies of modification were followed: varying substituents on the pyrrole carboxamide moiety, alteration of the central scaffold, including variation of substitution position and, most importantly, modification of the oxalyl moiety. Compounds with acidic, basic, and neutral properties were synthesized. To understand the mechanism of action and binding mode, we have obtained a crystal structure of compound 16a, bearing a primary amino group, in complex with the N-terminal domain of E. coli gyrase B (24 kDa) (PDB: 6YD9).

References

1. Skok Ž, Barančoková M, Benek O, et al. Exploring the Chemical Space of Benzothiazole-Based DNA Gyrase B Inhibitors. *ACS Med Chem Lett*. **2020**;11(12):2433-2440. doi:10.1021/acsmedchemlett.0c00416
2. Durcik M, Cotman AE, Toplak Ž, et al. New Dual Inhibitors of Bacterial Topoisomerases with Broad-Spectrum Antibacterial Activity and In Vivo Efficacy against Vancomycin-Intermediate *Staphylococcus aureus*. *J Med Chem*. **2023**;66(6):3968-3994. doi:10.1021/acs.jmedchem.2c01905
3. Cotman AE, Durcik M, Benedetto Tiz D, et al. Discovery and Hit-to-Lead Optimization of Benzothiazole Scaffold-Based DNA Gyrase Inhibitors with Potent Activity against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *J Med Chem*. **2023**;66(2):1380-1425. doi:10.1021/acs.jmedchem.2c01597

Parallel Peptide Workflow: Biotage Technologies for Peptide Drug Discovery

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Peptides based therapeutics have been evolving rapidly in the last four decades and have diversified from traditional endogenous human peptides to a plethora of structural and chemical modifications, screening technologies, providing a rich peptide therapeutic landscape including over 100 peptide-based therapeutics.[1, 2, 3]

Fast synthesis of peptide libraries to scan for new biological targets and hit identification becomes a requirement in the drug discovery process and our efforts were towards providing solutions for the entire hight-throughput workflow, not only synthesis but also purification – a clear bottleneck in reaching the final product of sufficient purity for screening and validation assays.
We introduce herewith the first automated parallel 96-well plate peptide purification system which completes the peptide workflow for researches and enables a faster development of drug modalities (Fig. 1).[4]

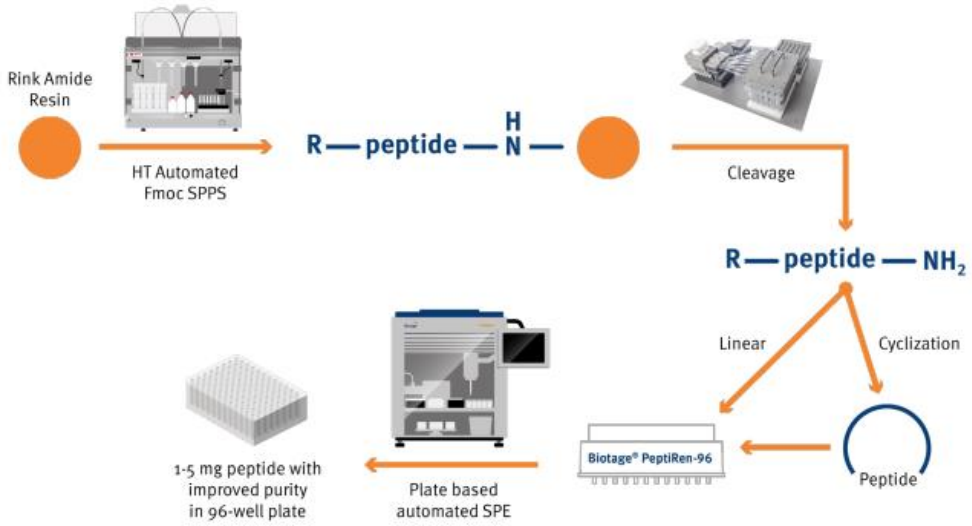

Fig. 1. Biotage High-Throughput Automated Parallel Peptide Synthesis Workflow

References

1. Nature Reviews Drug discovery 2021, 20, 309-325
2. Drug Discovery Today 2021, 26, 6, 1409-14-19
3. ACS Med. Chem. Lett. 2022, 13, 11, 1691–1698
4. Parallel Peptide Processing (biotage.com)

Complement-activating Multimeric immunotherapeutic compleXes (CoMiX) elicit killing of *Pseudomonas aeruginosa* and prevent biofilm formation

Pitiot A[a], Brandus B[a], Iserentant G[a], Mely Y[b], Richeirt L[b], Rassam P[b], Fouquenet D[c], Briard B[c], Si-Tahar M[c], Desoubeaux G[c], Devaux C[a]

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Bacterial respiratory infections, both acute and chronic, represent major threats to human health, especially due to the increase of resistance's mechanisms against everyday care treatment (antibiotics). *Pseudomonas aeruginosa* is one of such opportunistic pathogens causing a variety of life-threatening infections in individuals with compromised immune defences. Since the development of biotherapies, therapeutic Abs and Abs-based constructs have proven their efficacy in the treatment and the prevention of multiple infections. This efficacy relies on their ability to target specifically a pathogen and induce global immune responses.

As a novel antibody-based approach, we developed Complement-activating Multimeric immunotherapeutic compleXes (CoMiX), to target and kill the bacterium, combining a single-chain variable fragment, recognizing the Psl-exopolysaccharide of *Pseudomonas aeruginosa*, and variable effector functions through the oligomerization scaffold of the C4b-binding protein. Among those functions, the Fc-region (CoMiX-Fc) activates the classical complement pathway whereas the Factor H-related protein 1 (CoMiX-FHR1) activates the alternative complement pathway.

We have shown that CoMiX, in the presence of human serum, significantly increased C3b and C5b9 deposition ($p<0.001$) on both reference and clinical bacterial strains. Using confocal microscopy, we observed C3b staining at the surface of the bacterial cell that was associated with an early opsonization of bacteria and bacterial cell lysis, especially when incubated with CoMiX-FHR1. Supplementing 10% human serum with 3 µg CoMiX-Fc or CoMiX-FHR1 resulted in a 50% inhibition of PAO1-Luc growth for six hours as well as a ~30% reduction in the number of Colony Forming Units (CFUs) after 24h of culture, compared to serum alone without both CoMiX. The killing of the bacteria by CoMiX was facilitated with the use of amikacin, a standard of care antibiotic. During chronic infection, bacterial cells can form biofilms limiting the action of treatments. Thus, we investigated the action of the CoMiX in this context, and we showed the reduction of the biofilm formation on abiotic surfaces with the use of CoMiX-Fc. Finally, in a mouse model of acute *Pseudomonas aeruginosa* lung infection, the delivery in the lungs of Comix-Fc, non-immunogenic to lung epithelial cells, was able to reduce the bacterial load and the mortality in a first set of mice, compared to the control group.

CoMiX act as bacteriostatic agents in presence of complement *in vitro* and we showed preliminary effects *in vivo* in an acute model of lung infection. This opens novel perspectives on the design of anti-infective molecules to treat respiratory infections.

MOLECULAR DYNAMICS SIMULATIONS OF THE INTERACTION BETWEEN ANTIBACTERIAL SMALL MOLECULES AND THE *STAPHYLOCOCCUS AUREUS* LIPID BILAYER

Alvaro G. Temprano[a], Luigi Cutarella[b], Mattia Mori[b]

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Background/Aim: Bacterial resistance to antimicrobial drugs poses an immense threat to human health, with numerous clinically relevant organisms swiftly evolving towards multidrug and even pandrug-resistant strains. This global proliferation presents a pressing Public Health concern (1). The escalating incidence of infections linked to these resistant bacteria results in elevated rates of mortality and morbidity, highlighting the urgent need for novel antibacterial therapeutics. In this study, we employed molecular dynamics (MD) simulations to explore the mechanism of action of antibacterial small molecules targeting the cytoplasmic membrane of *S. aureus*.

Method: The CHARMM-GUI (2,3) Membrane Builder tool was used to build the lipid bilayer *S. aureus* membrane, while MD simulations were performed with AMBER18 using the CHARMM36m38 force field (4). Two independent MD replicas of 500 ns were run for each membrane/small molecule complex. The interaction of *S. aureus* membrane with two molecules endowed with opposite bioactivity profile and high chemical similarity was simulated.

Results: MD results of the bioactive hit 1 revealed a noticeable tendency for the molecule to bind tightly to the *S. aureus* membrane model. Analysis of the ligand mass density distribution throughout MD simulations indicates that 1 becomes fully integrated into the membrane from 70 ns onwards until the end of the simulation. In contrast, the inactive compound 2 exhibits less stable interactions with the phospholipid bilayer. Mass density analysis reveals a peak in ligand concentration approximately 30 Å from the bilayer mass center, suggesting that the compound primarily resides outside the membrane. These findings lead us to hypothesize that the membrane of *S. aureus* likely serves as the primary site of action for 1, aligning with experimental observations. Furthermore, our results imply that slight alterations in chemical structure could significantly influence the interaction with the membrane and the bioactivity profile of congeneric small molecules.

Conclusion: MD simulations are profitable tools in the study of small molecule interaction with the *S. aureus* membrane, which can be further used in lead optimization of antibacterial compounds.

References

1) Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial Antibiotic Resistance: The Most Critical Pathogens. Pathogens (Basel, Switzerland), 10(10), 1310.

2) Jo, S., Kim, T., Iyer, V. G., & Im, W. (2008). CHARMM-GUI: a web-based graphical user interface for CHARMM. Journal of computational chemistry, 29(11), 1859–1865.

3) Li, Y., Liu, J., & Gumbart, J. C. (2021). Preparing Membrane Proteins for Simulation Using CHARMM-GUI. Methods in molecular biology (Clifton, N.J.), 2302, 237–251.

4) Lee, J., Hitzenberger, M., Rieger, M., Kern, N. R., Zacharias, M., & Im, W. (2020). CHARMM-GUI supports the Amber force fields. The Journal of chemical physics, 153(3), 035103.

Mapping the impact of bacterial antimicrobial resistance in Central Europe: a cross-country comparison of key pathogens and pathogen-drug combinations

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Antimicrobial resistance (AMR) presents a critical global health challenge, impacting countries in diverse ways. Our objective was to provide comprehensive pre-COVID estimates of the burden of bacterial AMR in Central Europe, facilitating future comparisons. We extensively evaluated the impact of AMR in terms of deaths and disability-adjusted life-years (DALYs) for 23 bacterial pathogens and 88 pathogen-drug combinations across Central European countries (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Hungary, Montenegro, North Macedonia, Poland, Romania, Serbia, Slovakia and Slovenia). Two counterfactual scenarios were employed: one focusing on deaths directly caused by AMR, considering a situation where drug-resistant pathogens are replaced with susceptible ones, and the other examining deaths associated with AMR, envisioning a scenario where drug-resistant infections do not occur at all. Data were sourced from research hospitals, surveillance networks and infection databases maintained by private laboratories and medical technology companies. Cross-validation of models was conducted to assess predictive validity. The study revealed 18,957 (95% UI 11,959–28,528) deaths and 391,439 (244,481–590,771) DALYs attributable to AMR, along with 77,621 (49,319–115,279) deaths and 1,602,089 (1,007,362–2,378,397) DALYs associated with AMR in Central Europe. *Escherichia coli* (*E. coli*) emerged as a primary pathogen in the region, with 20,582 deaths associated with AMR and 4,812 deaths attributable to AMR. While *E. coli* was the leading pathogen in all examined countries, significant variations were observed in the rankings of other pathogens. Methicillin-resistant *Staphylococcus aureus* was the predominant pathogen-drug combination for deaths attributable to AMR in nine out of the thirteen countries, whereas aminopenicillin-resistant *E. coli* predominated in eleven countries for deaths associated with AMR. A positive correlation was noted between mortality rates related to AMR and antibiotic consumption rates. Overall, the findings emphasize the importance of tailored interventions to mitigate the impact of bacterial AMR and safeguard public health in Central Europe.

References

Meštrović T, Robles Aguilar G, Swetschinski LR, Ikuta KS, Gray AP, Weaver ND, et al. The burden of bacterial antimicrobial resistance in the WHO European region in 2019: a cross-country systematic analysis. Lancet Public Health. 2022;7(11):e897-e913. doi: 10.1016/S2468-2667(22)00225-0.

Preclinical mouse models for assessing drug efficacy in respiratory bacterial infection and inflammation

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One of the biggest challenges faced in translational studies is a more accurate reproduction of human diseases in *in vivo* models and to move forward with experimentation of new therapeutics. Mouse models simulating acute and chronic infections, along with lipopolysaccharide (LPS)-induced pulmonary inflammation, are key assets in translational studies for human pulmonary diseases. Despite inherent limitations, these models serve as valuable resources, mimicking both the initial and progressive bronchopulmonary infections and damage observed in diverse patient population, including those in hospital settings, individuals with cystic fibrosis (CF), or Chronic Obstructive Pulmonary Disease (COPD). Moreover, LPS-based models exhibit a noteworthy correlation with the lesions seen in COVID-induced acute respiratory distress syndrome. The Cystic Fibrosis animal Core (CFaCore) Facility offers these models within a preclinical platform designed to study pathological processes and evaluate novel therapies aimed at reducing infection or inflammation [1]. Our goal is to meet the demand for animal models, protocols, and endpoints to improve the predictive value and clinical relevance of drug testing, aligning with recommendations from the European Respiratory Society task force [2]. By profiling bacterial and host responses, we defined multiple end points that are critical parameters to validate the efficacy of anti-bacterial and anti-inflammatory treatments. Recently, we introduced an integrated platform called flexiVent (Scireq) that combines a mechanical ventilator with measurements of respiratory mechanics. Our results in a preclinical platform underline the importance of carefully selecting the appropriate mouse model (bacteria strains or LPS stimulation) and treatment regimen (anti-inflammatory or anti-bacteria drugs) for the disease under investigation to optimise drug testing [3,4]. Overall, CFaCore is expected to make a significant contribution to advancing knowledge on pathogenesis and improving translational studies in the field of pulmonary infection.

References

1 Cigana, C. et al. Antibiotic efficacy varies based on the infection model and treatment regimen for Pseudomonas aeruginosa. Eur Respir J 55, doi:10.1183/13993003.02456-2018 (2020).

2 Bonniaud, P. et al. Optimising experimental research in respiratory diseases: an ERS statement. Eur Respir J 51, doi:10.1183/13993003.02133-2017 (2018).

3 Cigana, C. et al. Efficacy of the Novel Antibiotic POL7001 in Preclinical Models of Pseudomonas aeruginosa Pneumonia. Antimicrobial agents and chemotherapy 60, 4991-5000, doi:10.1128/AAC.00390-16 (2016).

4 Bragonzi, A. Fighting back: peptidomimetics as a new weapon in the battle against antibiotic resistance. Science translational medicine 2, 21ps29, doi:10.1126/scitranslmed.3000889 (2010).

DETERMINATION OF DRUG-RESISTANCE USING 3D CELL CULTURE

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3D cell culture is rapidly becoming the tool of choice to mimic efficacy and toxicity *in vivo*. There now exists a wide variety of technologies and microorganism-eukaryotic models of infection that can be used to investigate novel compounds in the race to mitigate the approaching drug resistance crisis.
The 3D cell culture technology relies on the fact that when eukaryotic cells are given the chance to interact extensively, they recover physiological processes that they normally exhibit *in vivo*. This can have a profound effect on how they respond to the microorganisms and subsequently how the microorganism-eukaryotic model responds to novel compound/drug treatment.
Even with access to primary human cells (e.g. from eSC or ipSC or even patient derived) there are compounding factors that blur the true response. Common confusing factors are: 3D culture-induced stress, the 'immaturity' of the cells present and the lack of an immunological component.
Despite these limitations, microorganism-eukaryotic 3D models now exist for many infectious diseases – whether caused by **parasites**: *Plasmodium berghei* (malaria), Cryptosporidium parvum (AIDS patients); **yeasts**: *Candida albicans* (Candidiasis); **bacteria**: pathogenic E. coli (diarrhoea, vomiting), *Helicobacter pylori* (ulcer), *Shigella flexneri* (shigellosis), *Enterococcus faecalis* (bladder); *Salmonella enterica* (typhoid fever) and *S. typhimurium* (food poisoning), *Campylobacter jejuni* (food poisoning), *Bacteroides caccae* (inflammatory bowel disease); or **viruses**: Norovirus (stomach bug), SARS-CoV-2 (Covid-19), Zika virus (conjunctivitis, microcephaly), influenza ('flu), Respiratory Syncytial Virus (lung), *Mycobacterium tuberculosis* (tuberculosis), Hepatitis B virus (hepatitis), Japanese Encephalitis virus (encephalitis) and Epstein Barr virus (infectious mononucleosis).
While no system perfectly mimics the *in vivo* response, several can provide valuable data, illuminating the infection-response-treatment triad. This presentation will highlight strengths and pitfalls of the technology as well as illustrate a few of the models that have been successfully used.

References

1. Krzysztof Wrzesinski & Stephen J. Fey. Metabolic Reprogramming and the Recovery of Physiological Functionality in 3D Cultures in Micro-Bioreactors. Bioengineering 2018, 5, 22; doi: 10.3390/ bioengineering5010022.
2. Marianne Ø Poulsen, Sujata G Dastidar, Debalina Sinha Roy, Shauroseni Palchoudhuri, Jette E.H. Kristiansen & Stephen J Fey. A Double-Edged Sword: Thioxanthenes Act on Both the Mind and the Microbiome. Molecules. 2021 Dec 29;27(1):196. doi: 10.3390/molecules27010196.
3. Marianne Ø. Poulsen, Janne K. Klitgaard, Jørn B. Christensen, Birgitte H. Kallipolitis, Glenn W. Kaatz, Per Plenge, Stephen J. Fey & Jette E. Kristiansen. Comparison of Antibacterial Activity of (–) Thioridazine and Racemic Thioridazine in Staphylococcus aureus. Am J. Bioavailab Bioequiv. 2018;1(1): 001-009
4. Jette E.H. Kristiansen & Stephen J Fey. The Accepted "Clinical Interaction Model": A Special Case of Reality. J. Bioequiv Availab 2017, 9:3. doi: 10.4172/jbb.1000335
5. Hendrik Keyzer, Stephen J. Fey, Barry Thornton & Jette E. Kristiansen. Molar ratios of therapeutic water-soluble phenothiazine•water-insoluble phospholipid adducts reveal a Fibonacci correlation and a putative link for structure–activity relationships. RSC Adv., 2015, 5, 20865. doi: 10.1039/c4ra16551a

Antimicrobial resistance: evolutionary complexity and changing needs in clinical practice

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Antimicrobial resistance (AMR) remains one of the leading public health concerns, due to its relevant burden on morbidity, mortality, quality of life and healthcare-associated costs on a global scale. AMR evolution depends on the interplay of several factors, and contrasting AMR requires a multi-tiered approach, including surveillance, discovery and development of new antimicrobial agents and strategies, antimicrobial and diagnostic stewardship, and infection control and prevention policies, in a one-health perspective. After a relatively long period of dearth, several novel antimicrobial agents are entering clinical practice, changing concepts and paradigms in antimicrobial and diagnostic stewardship as well as the evolutionary trajectories of resistance in major bacterial pathogens. Examples will be discussed, to highlight the changing epidemiology of AMR among major bacterial pathogens in different clinical settings, and the emerging and changing needs in the field of antimicrobial chemotherapy.

An insight on photodynamic inactivation to mitigate microbial resistance

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Photodynamic therapeutic approaches are based on the production of reactive oxygen species (ROS) as a result of the activation of drugs named photosensitizers by visible light in the presence of dioxygen. The cytotoxic oxygen species produced from that combined action can lead to cell death, after a spatial-temporal interaction with biomolecules present on the biological structures. This type of approach is already used in clinical practice to destroy tumoral cells (PhotoDynamic Therapy) and more recently to inactivate microorganisms (MO) and viruses. In fact, antimicrobial photodynamic therapy (aPDT) has emerged as an alternative to treat diseases and to prevent the development of antibiotic resistance by pathogenic bacteria, virus particles including SARS-CoV2 or parasites.
Given aPDT is a non-selective approach, with a multitarget action, it is recognized that through this mechanism of inactivation, it is very improbable that MO could develop resistances. Furthermore, sensitive and resistant strains are efficiently inactivated by aPDT.
Herein, it will be discussed important achievements obtained in aPDT context using different types of photosensitizers, namely porphyrin derivatives.

Acknowledgments and funding:
Thanks are due to the University of Aveiro and FCT/MCTES for the financial support to LAQV-Requimte (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020, DOI 10.54499/UIDP/50006/2020, UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020); CESAM (UID/AMB/50017/2020 and UIDB/50017/2020 + UIDP/50017/2020) and the FCT project PORP2PS (EXPL/QUI-QOR/0586/2021). C.J.D. C.V. thanks FCT for their doctoral grants SFRH/BD/150676/2020 and SFRH/BD/150358/2019. NMM Moura thanks FCT for his funding through program DL 57/2016 – Norma transitória (CDL-CTTRI-048-88-ARH/2018). Thanks are also to Cost Action EURESTOP CA21145

References

1. Mesquita M.Q., Dias C.J., Neves M.G.P.M.S., Almeida A., Faustino M.A.F., *Molecules*, **2018**, 23(10), 2424; http://dx.doi.org/10.3390/molecules23102424
2. Almeida A., Faustino M.A.F., Neves M.G.P.M.S., *Antibiotics* **2020**, 9, 320; http://dx.doi.org/10.3390/antibiotics9060320
3. Bartolomeu M., Monteiro C.J.P., Fontes M., Neves M.G.P.M.S., Faustino M.A.F., Almeida A., *Science of the Total Environment*, **2023**, 860, 160427, http://dx.doi.org/10.1016/j.scitotenv.2022.160427
4. Vieira C., Santos A., Mesquita M.Q., Gomes A.T.P.C., Neves M.G.P.M.S., Faustino M.A.F., Almeida A. s *Journal Porphyrins and Phthalocyanines*, **2019**, 23(4-5)534-545; http://dx.doi.org/10.1142/S1088424619500408
5. Branco T.M., Valério N.C., Jesus V.I.R., Dias C.J., Neves M.G.P.M.S., Faustino M.A.F., Almeida A.. *Photodiagnosis and Photodynamic Therapy*, **2018**, 21, 285-293; http://dx.doi.org/10.1016/j.pdpdt.2018.01.001

Design and synthesis of a new functionalizable visible-light photoswitch for antimicrobial applications

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Visible-light photoswitches are an interesting class of molecules that holds significant potential in the field of photopharmacology. In this work, we present the rational design and synthesis of a new photoswitch with appropriate photochemical properties for photopharmacological applications. Simulations preceded synthesis, guiding and optimizing time and resources. Initially, a computational screening was performed in which the absorption properties of thirty-six stilbene-based molecules with substituents of various natures were calculated. The synthesis of the selected molecule, that absorbs at wavelengths close to the biological window, was carried out through an innovative and convergent procedure that allowed to obtain the compound with a good yield. The new photoswitch was designed to be functionalizable through an ester bond with bioactive molecules, like for example antibiotics.

For the study of the photochemical properties of our molecule, we needed to perform photoisomerization reactions, in well controlled conditions. Therefore, we realized and deposited the patent, of a new device with unique modular characteristics to host different analytical supports.

Photoswitchable Antimicrobial Peptides: Towards a General Method for Solid Phase Synthesis of Visible-Light-Operated Peptides

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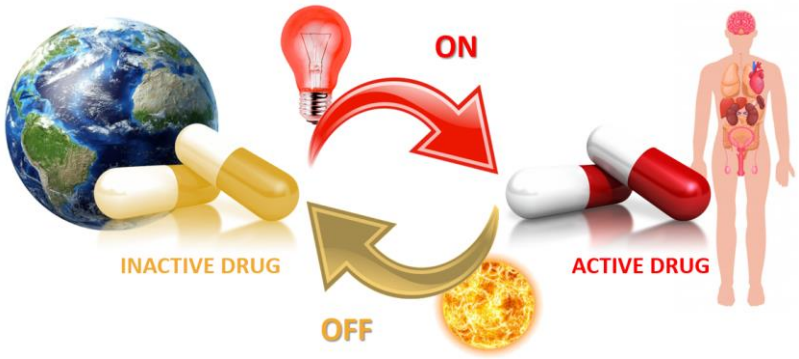
Photopharmacology has recently appeared as a unique way of turning drugs on and off using light. ***Switching drugs off after their therapeutic use renders them inactive to avoid off-target effects and reducing the chances of resistance appearing as their accumulation does not increase their evolutionary pressure.***[1] Many approaches to photoswitchable drugs rely on the use of azobenzenes, which require the use of harmful UV light for their activation. However, these can be modified to cause a red-shift that allows ***activation with visible light that does not harm tissues and can penetrate deeper than shorter wavelengths***.

In our laboratory, we have recently developed the first photoswitchable antimicrobial peptides fully operated with visible light,[2] allowing us to control the activity of the compounds with harmless illumination and enabling deactivation by simple exposure to sunlight. For instance, we found that linear analogues of tyrocidine A granted the best photocontrol of their antimicrobial activity, leading to compounds whose ***activity against Acinetobacter baumannii or Streptococus pyogenes can be turned on and off at will***.[2]



More recently, we have developed a method to access highly coveted tetra-*ortho*-methoxylated azobenzene photoswitches whose synthesis is very elusive. ***This new methods allows us to access state-of-the-art photoswitches that will lead to visible-light-operated peptides using the well-stablished protocols of Solid Phase Peptide Synthesis (SPPS)***.[3]

References

1. Kobauri, P.; Dekker, F. J.; Szy-manski, W.; Feringa, B. L. *Angew. Chem. Int. Ed.* **2023**, *62*, e202300681.
2. Just-Baringo, X.; Yeste-Vázquez, A.; Moreno-Morales, J.; Ballesté-Delpierre, C.; Vila, J.; Giralt, E. *Chem. Eur. J.* **2021**, *27*, 12987.
3. Ruiz-Soriano, A.; Lamelza, L.; Pizzamiglio, E.; Just-Baringo, X. *ChemRxiv* **2023**. DOI: 10.26434/chemrxiv-2023-dr111.

Intracellular trafficking of *Acinetobacter baumannii* in host cells requires activation of Transcriptional Factor EB and phospholipase A_2

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Adhesion represents an initial and crucial stage in *Acinetobacter baumannii* infections. However, the mechanism of entry and persistence within host cells remains unclear and requires further understanding.

In this study, we observed a progressive and significant increase in Transcirptional Factor EB (TFEB) expression in human lung epithelial cells (A549) infected with the ATCC17978 strain, reaching a 180% increment at 6 hours compared to control non-infected cells. Bacterial invasion in A549 cells was reduced by 64% in TFEB-deficient cells, and increased by 150% in TFEB-overexpressing cells compared to control non-infected cells; however, no difference was observed in bacterial adhesion in both cell types. Furthermore, infected cells exhibited lysosome biogenesis and activation of autophagy, as evidenced by increased LC3BII expression. Additionally, pretreatment of A549 cells with autophagosome inhibitors such as bafilomycin, pepstatin, and wortmannin resulted in reduced bacterial invasion. Interestingly, treatment with TFEB siRNA and autophagosome inhibitors significantly reduced bacterial invasion compared to untreated A549 cells. Finally, treatment of Hela cells with specific inhibitors of cPLA2 and iPLA2 (BEL and MAFP) reduced the expression and translocation of TFEB into the nucleus.

These findings contribute to a better understanding of the TFEB-dependent endosome/lysosome and autophagosome/lysosome systems in the pathogenesis of *A. baumannii*. Targeting this network "node" may offer a novel therapeutic approach for treating *A. baumannii* infections by modulating autophagy/lysosome function, potentially leading to the development of a new class of antimicrobial therapeutics targeting host factors.

Small molecule G-quadruplex ligands are antibacterial candidates for Gram-negative bacteria

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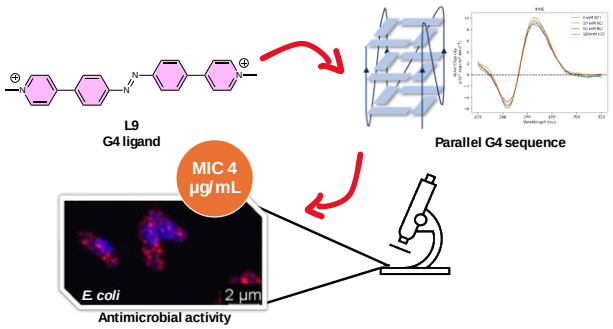
G-quadruplexes oligonucleotides (G4) are a fascinating class of nucleic acid structures formed from the self-association of guanine-rich sequences. The prevalence of G4s, along with the growing realization of their importance in human disease progression (e.g. cancer, diabetes and neurogenerative diseases), and involvement in cellular processes in a wider range of organisms including plants, parasites, fungi, protozoa, bacteria and viruses; has excited considerable interest in their potential as therapeutic targets.[1,2]

Antimicrobial resistance (AMR) is a 21st century global public health emergency. With the continuing weakness of the antibacterial pipeline and decades of empirical antibiotic overuse, once readily treatable infections now cause increasing morbidity and mortality, making the search for alternative agents urgent. Therefore, there is a great need for novel strategies to tackle antimicrobial resistance, in particular in Gram-negative species such as *Escherichia coli* that cause opportunistic infections of already compromised patients.

During this lecture, I will describe examples of photoresponsive ligands for G4 DNA regulations developed within our research group. I will also discuss recent results where we identify G4 ligands with promising antibacterial activity (MIC values ≤ 4 mg/ml) against multi-drug resistant *E. coli*.[3]



References

1. J. Ramos-Soriani and M. C. Galan, J. Am. Chem. Soc. Au **2021**, 1, 10, 1516.

2. a) M. P. O'Hagan, S. Haldar, M. Duchi, T. A. A. Oliver, A. J. Mulholland, J. C. Moralres, M. C. Galan, *Angew. Chem. Int. Ed.*, **2019**, 58, 4334-4338. b) M. P. O'Hagan, S. Haldar, J. C. Morales, A. J. Mulholland* and M. C. Galan* Chem. Sci. **2021**, 12, 1415

3. Spencer, J.; Galan, M. C., et al. Small molecule G-quadruplex ligands are antibacterial candidates for Gram-negative bacteria. *bioRxiv* **2024**, 2022.09.01.506212.

Competition-driven evolution and targeted antibiotic production to fight multidrug-resistant bacteria

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Developing new, effective antimicrobial agents to treat infections caused by multi-drug resistant bacteria poses a significant challenge within the drug development pipeline. Identifying effective, resistance-free compounds in the early stages of development is crucial due to the considerable economic investments required to bring a new antibiotic to market. The phylum *Actenomycetota* comprises very diverse bacteria with a high metabolic versatility. These bacteria have been the source of most of the antibiotic compounds still in use nowadays. However, more recently, different genomics and metabolomics studies revealed that *Actenomycetota* have the genomic potential for covering a much larger chemical space than what is known. Many microbial biosynthetic pathways that would be active in the wild as a response to environmental stimuli, and as result of years of coevolution of the species in a particular environment, are silent under most laboratory conditions. In this study, we introduce a novel competition-based approach to identify and characterize new antimicrobials exploring the potential of such ecological competition in the context of antibiotic-producing bacteria and human pathogens. Our method consists of evolving antibiotic-producing bacteria in the presence of drug-resistant pathogens over an extended period, aiming to induce antibiotic production specifically targeting antibiotic-resistant pathogens. By directing antibiotic production towards antibiotic-resistant pathogens and their mechanisms of resistance, our method presents an innovative approach to identifying novel and effective candidates for treating infections caused by drug-resistant pathogens. This strategy has the potential to accelerate the early stages of the antibiotic development pipeline, and may lead to the implementation of a new high-throughput drug-discovery platform.

Evolving nanoparticles aided strategies against bacterial infections

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Different nanostructures with polymeric/inorganic constitutes offer advantages in reducing acute toxicity, overcoming the challenges of the conventional antibacterial therapies, antibiotic resistance resistance and reducing costs in comparison to conventional antibiotics.[1] Most of the studies to date have focused on inorganic NP containing silver, gold copper, zinc, titanium oxide, and cerium oxide as antibacterial agents. 2These materials can inherently exert antibacterial activity. These strategies enable the bacterial attack on many fronts, making it significantly more difficult for microbes to develop resistance. The physically acting NP can further be combined with chemical strategies to provide multifunctionality i.e. loading of drugs to the same nanosystems or used in combinatorial therapy, thereby increasing their effect. Among the existing NP, mesoporous silica nanoparticles (MSN) can provide multifunctionality (i.e. inherently therapeutic, drug delivery, targeted delivery, precision in dosing) due to its modular design options.[3] In our research activities we have developed different MSN based antibacterial nanostructure designs by altering the composition of nanoparticles possessing antibacterial core-shell structure, loaded antibacterial agents, antibacterial polymeric layer and/or antimicrobial peptides coatings as well.[4,5] Research findings on the designed MSN based nano antibiotic aided antibacterial/antibiofilm treatments revealed that performed macromolecules accommodation on MSN boosts the antibacterial activity by reducing the required treatment dosage and improving MSN penetration through the biofilm matrix or bacterial cell interactions.

References

Sahlgren C.et al., “Advanced Healthcare Materials 6, 21 (2017): 1700258.
Wang, L.et al. International Journal of Nanomedicine 12 (2017): 1227–49.
Şen Karaman D. et al. Therapeutic Delivery 9,4 (March 2018): 241–44.
Pamukcu A. et al. Journal of Biomedical Materials Research Part B, Applied Biomaterials (June 2022)
Pamukçu A. et al 2023 Nano Futures 7 025007

Enzymatic Synthesis of Coumarin Aminophosphonates Peptidomimetics as Novel Antimicrobial Agents.

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Escherichia coli is a gram-negative bacteria which assists as a diverse microorganism in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. This normally harmless commensal can easily become a highly adaptable pathogen that causes serious diseases, such as gastroenteritis and extraintestinal infections of the urinary tract, bloodstream, and central nervous system [1]. Multi-drug resistance of E. coli to antimicrobial medicines, especially in developing countries, is considered one of the main causes of an ineffectiveness in the treatment of infectious diseases [2]. According to World Health Organization (WHO) reports, multi-drug-resistant pathogens are among the biggest challenges in the treatment of bacterial infections worldwide, causing 10 million deaths a year by 2050. As a result of these premises and due to the high degree of antimicrobial resistance, it is necessary to develop effective antimicrobial drugs to treat bacterial resistance.

A new protocol was designed for the synthesis of antimicrobial peptidomimetics containing coumarin scaffolds through enzyme-promoted Kabachnik- Fields. The products were provided with an excellent yield (up to 92%) under mild, solvent and metal-free conditions. The structure–activity relationship revealed that an inhibitory activity of synthesized compounds is strongly related to the type of the substituents located on the phenyl ring [3]. Based on obtained results further studies have shown that coumarin-based peptidomimetics are non-selective and act efficiently against various Gram-positive and Gram-negative pathogens, which is of great importance for hospitalised patients [4]. The results of preliminary exploration of coumarin α-amino dimethyl phosphonate peptidomimetics as novel antimicrobial agents will be presented. The basic features of the structure responsible for the observed biological activity will be discuss.

Acknowledgments
We gratefully acknowledge the financial support from the National Science Centre, Poland, under the research project Opus no 2019/33/B/ST4/01118 and 2023/49/B/NZ7/02469; the European Community COST 21145 EURSTOP action for providing a stimulating environment that led to this collaborative research.

References

1. Kaper, J.; Nataro, J.; Mobley, H. *Nat. Rev. Microbiol*. **2004**, *2*, 123–140.
2. Erb, A.; Stürmer, T.; Marre, R.; Brenner, H. *Eur. J. Clin. Microbiol. Infect. Dis.* **2007**, *26*, 83–90.
3. Koszelewski, D.; Kowalczyk, P.; Brodzka, A; Hrunyk, A.; Kramkowski, J..K.; Ostaszewski, R. *Int. J. Mol. Sci.* **2023**, *24*, 7609.
4. Kowalczyk, P.; Koszelewski, D.; Brodzka, A.; Kramkowski, J.K.; Ostaszewski, R. *Int. J. Mol. Sci.* **2023**, *24*, 14886.

Natural pH Indicator Incorporated-Assays for Rapid and Colorimetric Detection of Carbapenem Resistance in Acinetobacter

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Multidrug resistant microorganisms (MDR) pose a significant threat to public health around the world. Unconscious and frequent use of antibiotics and long hospital stays are the main factors that trigger the development of new resistance. *Acinetobacter* has an important place in the Gram-negative bacteria (GNB) family, which is a hospital- and community acquired resistant microorganism. These carbapenem-resistant bacteria are becoming an increasingly important problem all over the world. Herein, we have prepared anthocyanin molecules (obtained from red cabbage (*Brassica oleracea*) and used as a natural pH indicator) incorporated-diagnostic tests with liquid, agar and microfluidic chip forms, which contain both significantly economical and biocompatible content. The detection time has been-reduced to 1.5-2 hours with the phenotypic antibiotic sensitivity tests we developed. Thus, these diagnostic test are innovative, economical, accurate and provide fast results. In addition to that these phenotypic antibiotic susceptibility tests will also reduce the workload of laboratories with their ease of application and evaluation. Our goal is to achieve the production and marketing of anthocyanin-based rapid and economical phenotypic antibiotic susceptibility tests in liquid form, agar and microfluidic chip forms.

References

1. J Clin Microbiol. 53, 9, 2015, 3003–3008. https://doi.org/10.1128/jcm.00977-15
2. Talanta Volume 219, 2020, 121292. https://doi.org/10.1016/j.talanta.2020.121292
3. Analytica Chimica Acta Volume 1128, 2020, 80-89. https://doi.org/10.1016/j.aca.2020.06.005
4. Anal. Chem. 93, 15, 202, 6246–6253. https://doi.org/10.1021/acs.analchem.1c00663
5. Sci Rep 13, 2056, 2023, 1-10. https://doi.org/10.1038/s41598-023-28604-1

Silver nanoformulations as antibacterial agents: mode of action and impact on the development of bacterial resistance

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The main goal of my research is to analyze the antibacterial mode of action of silver nanoformulations and the development of bacterial resistance to these forms and antibiotics.

The specific objectives of my research include analysis of the antimicrobial activity of silver nanoformulations against Gram-positive and Gram-negative bacteria, analysis of the prevalence of silver resistance genes (*sil*) among bacteria strains, assessment of the consequences of the exposure of bacteria to silver nanoformulations by determining phenotypic and genetic changes in bacteria cell. I compared the antibacterial mode of action of Ag^+ silver ions and selected silver nanoformulations (including strains with exogenous and endogenous silver resistance, mutants lacking the selected outer membrane proteins OmpA, OmpC, OmpF, CusS) by determining the minimum growth inhibitory concentration (MIC) of silver ions and nanoformulations against different bacterial phenotypes, detection of free oxygen radicals, microscopic observation of the interaction of ions and silver nanoformulations with bacterial cell, observation of changes in albumin structure under the influence of ions and silver nanoformulations, comparison of the path of the Ag^+ silver ion and Ag^0 silver metal through the internal channel of OmpF and OmpC proteins using computational chemistry methods and bioinformatic analysis.

Overall, nanosilver has shown broad-spectrum antibacterial activity against various bacterial species. However, it is important to note that bacteria can develop resistance to nanosilver over time and it strongly depends on the physico-chemical properties of silver nanoformulations. Consequently, it is essential to continue research on nanosilver and its long-term effects for effective use in antimicrobial applications.

Further research plans and searching for new collaborations include: examining the transcriptome of selected bacterial strains after contact with silver nanoformulations showing a potentially different mode of action, analysis of molecular changes within the external structures of bacterial cells, such as the outer membrane or the motility system and effectiveness of specific and non-specific immunity against phenotypic strains of bacteria as a result of exposure to silver nanoformulations.

References:

1. A. Kędziora, et al. Similarities and differences between silver ions and silver in nanoforms as antibacterial agents. IJMS 19, 2 (2018), 444, s. 2-17 https://doi.org/10.3390/ijms19020444
2. A. Kędziora, et al. Silver nanoforms as a therapeutic agent for killing *Escherichia coli* and certain ESKAPE pathogens. Current Microbiology 73, 1 (2016), s. 139-147 DOI 10.1007/s00284-016-1034-8
3. A. Kędziora, et al. Consequences of long-term bacteria's exposure to silver nanoformulations with different physico-chemical properties. IJM 15 (2020), s. 199-213 https://doi.org/10.2147/IJN.S208838
4. A. Kędziora, et al. Comparison of antibacterial mode of action of silver ions and silver nanoformulations with different physico-chemical properties: experimental and computational studies. Frontiers in Microbiology 12:659614 (2021), s. 1-12 doi: 10.3389/fmicb.2021.659614
5. A. Kędziora, et al. How bacteria change after exposure to silver nanoformulations: analysis of the genome and outer membrane proteome. Pathogens 10, 817 (2021), s. 1-14, doi.org/10.3390/pathogens10070817

Bacteriophages effective against stationary cells: promising agents against antibiotic tolerant bacteria

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Bacteriophages (phages), the viruses that specifically infect and kill bacteria, are ubiquitous in the environment. Most phage-host interaction studies are performed with exponentially growing cells. In nature, however, this is not the primary pattern of growth, with bacteria often surviving in the stationary phase. These bacterial cells are typically in a slower or non-dividing state, with low metabolic activity. As phages use the metabolic machinery of the host bacteria to replicate, their efficacy against stationary cells is usually limited. Likewise, stationary cells can tolerate high doses of antibiotics, since the cellular processes commonly attacked by them are tuned down. Nonetheless, some phages have a unique ability to replicate in stationary cells. Previously, we showed that the *Staphylococcus epidermidis* phage SEP1 has this rare characteristic, significantly reducing the numbers of stationary cells [1]. More recently, the *Pseudomonas aeruginosa* phage Paride was shown to be able to replicate and lyse stationary cells in a deep dormant state [2]. In the present study, using RNA-seq, we investigated the transcriptomic profiles of both exponential and stationary cells infected with SEP1 phage to have an enlightened understanding of this phenomenon. SEP1 gradually took over the transcriptional machinery of the host in both conditions, although slowly in stationary cells. A DNA modification system was used by exponential cells, and later by stationary cells, as a defence against SEP1 infection. However, upregulation of the restriction endonuclease gene, needed for phage DNA cleavage, was not observed, with SEP1 being able to successfully replicate. In stationary cells, SEP1 was shown to activate numerous metabolic and biosynthetic processes crucial to the completion of its lifecycle, with 894 and 1319 genes upregulated at 15- and 30-min post-infection, respectively. Phages effective against stationary cells, such as SEP1, are promising agents to treat recalcitrant infections, particularly those caused by bacteria with an increased tolerance to antibiotics. Moreover, they can be used to “awake” stationary cells, by activating their metabolic and biosynthetic activity, and consequently to resensitize them to antibiotics.

References

1. Melo LDR, França A, Brandão A, Sillankorva S, Cerca N, Azeredo J. Assessment of Sep1virus interaction with stationary cultures by transcriptional and flow cytometry studies. FEMS Microbiol Ecol. 2018; 94: 1–8.
2. Maffei E, Woischnig A-K, Burkolter MR, Heyer Y, Humolli D, Thürkauf N, et al. Phage Paride can kill dormant, antibiotic-tolerant cells of Pseudomonas aeruginosa by direct lytic replication. Nat Commun. 2024; 15:175.

Antibiotic Potential of Steroidal and Triterpenoid Compounds

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In addressing the critical challenge of antibiotic resistance, this presentation will delve into the promising realm of steroidal and triterpenoid compounds as novel antibacterial agents. Drawing from recent research, we'll explore the advancements in fusidic acid derivatives,[1] highlighting their improved efficacy against resistant Staphylococcus aureus strains, including MRSA. The discussion will extend to innovative cationic steroid antibiotics,[2] showcasing their unique bactericidal mechanisms and potential for enhanced membrane selectivity. Furthermore, the untapped potential of pentacyclic triterpenoids will be examined,[3] emphasizing their novel targets within bacterial cells and their promising antistaphylococcal activities. Additionally, we'll delve into the microbiome-modulating activity of bile acids and the potent antibacterial effects of bile acid oligomers against Gram-positive bacteria, highlighting new avenues in antimicrobial drug development and strategies to combat antibiotic resistance. This review aims to illuminate the path toward harnessing these compounds in the fight against the escalating threat of drug-resistant bacteria.

References

1. https://doi.org/10.1021/acsinfecdis.0c00869
2. https://doi.org/10.1111/j.1574-6968.2002.tb11448.x
3. https://doi.org/10.1016/j.phymed.2019.152933
4. https://doi.org/10.1080/19490976.2020.1732268
5. https://doi.org/10.1021/acs.jmedchem.8b01433

SYMMETRIC SYNTHESIS OF POLYSULFURE DERIVATIVES

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Organic polysulfides from garlic have been the focus of attention for many years due to their biological activities, including antibacterial, antifungal, antiviral, phytoprotective and antiproliferative properties. These compounds have attracted medical interest recently as chemo preventive and chemotherapeutic agents as a promising source of new cancer agents. Among the various polysulfides studied, trisulfides and tetrasulfides occupy a prominent position as these are the most active and stable compounds. They are ubiquitous in nature, including first plants of the genus Allium, Scorodophleus zenkeri, Shiitake mushrooms, etc. Polysulfides can be divided into symmetric and asymmetric, based on the method of synthesis. The main goal of this work was the synthesis of symmetrical polysulfides, mostly the introduction of different alkyl groups in the trisulfide bond. Specifically, the study is directed towards the synthesis of an asymmetric trisulfide (APTS) and with alkylated derivatives of cysteine (S-propyl-L-cysteine). After synthesizing, the products were to identified, purified and analysed using some of the instrumental methods of analysis.

Chemical reagents and the analytical analyses were provided by Sigma-Aldrich, Munich and Saarland University. Purification was performed by column chromatography using silica gel (50-200 µm diameter). Thin-layer chromatography was performed on silica-coated aluminium plates (Merck, silica gel 60 F254). NMR spectra were recorded on a Bruker Avance 500. The measurement frequency was 500 MHz for 1H-NMR and 125 MHz for 13C-NMR. LC-MS was performed on a Bischoff Lambda 1000 UV/VIS at 275 nm using a YMCC 18 Pro column and methanol/water (85:15) as the mobile phase at a flow rate of 1.0 ml/min. S-propyl-L-cysteine had the highest yield (99%) followed by dibenzyl trisulfide (94%). Alkyl radicals that were successfully introduced into the trisulfide bond ranged from three carbon atoms to eight carbon atoms. The methyl and ethyl group did not give a result and the cycloalkyl and aromatic radicals also did not show a result.

References

1. Reiter, J.; Hubbers, A.M.; Albrecht, F.; Leichert, L.I.O.; Slusarenko, A.J. Allicin, a natural antimicrobial defence substance from garlic, inhibits DNA gyrase activity in bacteria. Int. J. Med. Microbiol. 2020, 310, 151359.
2. Gruhlke, M.C.H.; Antelmann, H.; Bernhardt, J.; Kloubert, V.; Rink, L.; Slusarenko, A.J. The human allicin-proteome: S-thioallylation of proteins by the garlic defence substance allicin and its biological effects. Free Radic. Biol. Med. 2019, 131, 144– 153, doi:10.1016/j.freeradbiomed.2018.11.022.
3. Reiter, J.; Borlinghaus, J.; Dörner, P.; Schröder, W.; Gruhlke, M.C.; Klaas, M.; Slusarenko, A.J. Investigation of the deposition behaviour and antibacterial effectivity of allicin aerosols and vapour using a lung model. Exp. Ther. Med. 2020, 19, 1541– 1549, doi:10.3892/etm.2019.8387.
4. Dörner, P.; Müller, P.M.; Reiter, J.; Gruhlke, M.C.; Slusarenko, A.J.; Schröder, W.; Klaas, M. Feasibility study of a surface- coated lung model to quantify active agent deposition for preclinical studies. Clin. Biomech. 2020, 76, 10529, doi:10.1016/j.clinbiomech.2020.105029.

ABSTRACTS OF POSTERS
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Chemical Cartography: Mapping the G-quadruplexes

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G-quadruplexes (G4s) are non-canonical secondary structures of DNA implicated in various biological processes. G4s have been initially identified as anti-cancer targets due to their crucial role in telomerase functioning and play a key role in cellular replication across different organisms including viruses, bacteria and yeasts.

Given that G4s are heavily involved in bacterial replication and proliferation, they have recently emerged as intriguing targets for antibiotic development[1]. However, the lack of G4 structural data and information on druggable sites makes the discovery of bacterial G4-stabilizing agents a huge challenge. Computational methods offer promising new paths for target exploration. In this work, Molecular Dynamics simulations are used to map the chemical space of bacterial G4s using small molecular probes. This process can be almost entirely automated, from probe selection and parametrization to trajectory analysis. This protocol leverages both 1D and 3D structural information, yielding noteworthy data on probe-target interactions, including the identification of privileged interaction sites, interactions stability, and linear interaction energy. Such methodology proves invaluable in identifying G4 hotspots and ligands interaction, and discerning the specific molecules and pharmacophores that engage with the target G4.

References

1. Neidle, S. Quadruplex Nucleic Acids as Novel Therapeutic Targets. J. Med. Chem. 2016, 59, 13, 5987–6011Yyy

Tamoxifen and chalcone derivatives with antimicrobial activity

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In the present days, the abundant use of antibiotics for human or animal therapy leads to the development of microbial resistance to multiple drugs. As a consequence, the infections by multidrug-resistant (MDR) bacteria represent an increased and severe problem for the global public health.[1] The urgency to provide new and alternative drugs for controlling antibiotic-resistant pathogens is of significant importance. In this scenario, Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) resistant bacteria came to our attention for the identification and development of new scaffolds with MDR activity.

Two different classes of synthetic compounds, tamoxifen and chalcone derivatives (Figure 1), well-known for their wide range of pharmacological activities,[2-4] were tested as potential antimicrobial agents in combination with already known antibiotics like colistin and erythromycin.

Three tamoxifen derivatives displayed MIC values between 0.125 and 2 µg/mL in combination with colistin, against colistin-resistant *Acinetobacter baumannii*, and four chalcone derivatives with MIC values between 2.7 and 30 µM in combination with colistin, against colistin-resistant *Pseudomonas aeruginosa*. Finally, two chalcone derivatives showed a synergy effect with erythromycin against *Staphylococcus aureus*, in concentrations ranging from 0.7 to 26 µM.

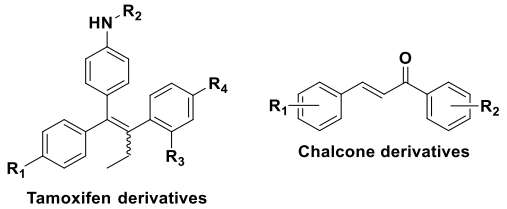

Figure1. General structure of the tested tamoxifen and chalcone derivatives.

References

1. Nikaido, H. Multidrug Resistance in Bacteria. *Annu Rev Biochem.* **2009**, *78*, 119–146.
2. Miró-Canturri, A. Repurposing of the tamoxifen metabolites to combat infections by multidrug-resistant gram-negative bacilli. *Antibiotics* **2021**, *10*, 336.
3. Dhaliwal, J. S. *et al.* Pharmacotherapeutics Applications and Chemistry of Chalcone Derivatives. *Molecules* **2022**, *27*, 7062.
4. Man, Xu. *et al.* Chalcone derivatives and their antibacterial activities: Current development. Bioorg. Chem. **2019**, *91*, 103133.

BUG WARS: The Rise of G-Quadruplex

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G-quadruplexes (GQs) are non-canonical four-stranded structural motifs formed by guanine-rich sequences identified in both DNA and RNA playing a wide range of pivotal biological functions in human and non-human cells. The most variable portions of the GQs are the loops-connecting the G-rich tracts, which can have different lengths and compositions[1].
Antimicrobial resistance (AMR) is an underrated problem that has been underestimated for decades promoted by the abuse and misuse of antibiotics. As a result, public health systems are facing enormous costs for the treatment of nosocomial infections due to AMR bacteria. In particular, the World Health Organization (WHO) has identified six highly virulent bacteria (E.S.K.A.P.E.) as the main causes of infections worldwide[2].
In recent years, a few attempts were made to discover and develop new clinically relevant antibiotics mostly by developing close chemical derivatives of existing antimicrobial drugs, which represents a short-term solution as the AMR was already established. Hence, we are unable to efficiently treat multi-drugs resistance (MDR) bacterial infections and are unprepared for a potential AMR bacteria outbreak[3].
In this context, "the G-Q-eat ESKAPE" project aims to use a multidisciplinary approach combining computational models with biophysical tools to: *i*) identify new compounds able to interact within the groove of bacterial GQs for the treatment of microbial infections overcoming AMR; *ii*) develop a comprehensive platform for effective and rapid virtual screening as a first resource in emergency upon pathogen outbreak.

References

[1] Monsen RC, Trent JO, Chaires JB. G-quadruplex DNA: A Longer Story. Acc Chem Res. 2022 Nov 15;55(22):3242-3252.
[2] Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther. 2013 Mar;11(3):297-308.
[3] Theuretzbacher U, Outterson K, Engel A, Karlén A. The global preclinical antibacterial pipeline. Nat Rev Microbiol. 2020 May;18(5):275-285.

Acknowledgement

S.C. has received funding for this study from the EU's Horizon Europe research and innovation program under HORIZON-MSCA-2022-PF-01, grant agreement no. 101106871 (The G-Q-reat ESKAPE).

ATOMISTIC SIMULATIONS OF BACTERIAL MEMBRANES TO UNRAVEL MECHANISMS OF SMALL MOLECULES INTERNALIZATION

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Antimicrobial resistance (AMR) poses a serious threat to global public health in the 21st century. The escalating need to develop new antimicrobial treatments capable of successfully addressing multi-resistant pathogens has prompted the scientific community to explore innovative approaches to combat AMR. The bacterial cell membrane has emerged as a key molecular component for the efficacy of drugs, and in the genesis of antibiotic resistance, gaining significant interest as a potential target for the development of novel antimicrobials.
The complexity of gram-positive and gram-negative bacterial membranes poses a challenge at the molecular level. In particular, in gram-negative bacteria since there is an additional degree of complexity due to the presence of an outer layer. Recently, the use of computational modeling has unveiled fundamental aspects regarding the structure and organization of bacterial cell membranes. Through atomistic simulations in this study, we aim to elucidate the interaction and internalization of small molecules into bacterial membranes. Specifically, we focused our efforts on the interaction of the antibacterial drug clofoctol and the membrane of *S. aureus*. The same approach based on MD simulations was used to explore the internalization of colistin into the membrane of *P. Aeruginosa* may vary depending on its protonation state. Both of these inquiries can provide valuable insights into key interactions between small molecules and bacteria membranes, thereby contributing to study the interaction between drugs with bacterial membranes.

DNA G-QUADRUPLEXES AS NEW FRONTIERS IN ANTIMICROBIAL DRUG DISCOVERY

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Antimicrobial resistance (AMR) poses a serious global threat to public health, compromising the effectiveness of prevention and treatment of bacterial infections. The lack of new antibiotics and pharmaceutical research focused on analogs of existing drugs underscore the urgent need for new therapeutic targets to escape AMR.
DNA G-quadruplexes (G4s) emerge as promising targets for their prevalence in crucial genomic regions, and their roles in gene expression. The advent of Next-Generation Sequencing (NGS) has facilitated rapid acquisition of genomic sequences, triggering the exploration of G4s as potential therapeutic targets. Despite extensive studies on human G4s, their potential as therapeutic targets in bacteria remains mostly underexplored.
In this study, molecular dynamics (MD) simulations validated the structure of TET22, a G4 present in the pathogen *Pseudomonas aeruginosa*, enabling virtual screening and molecular docking for the identification of potential ligands. The successful validation of TET22 structure coupled with the identification of TET22 candidate binders highlights a promising path to develop innovative antimicrobial agents.
Experimental validation is undergoing to support computational findings.

Role of the stringent response in the interaction of *Pseudomonas aeruginosa* with airway epithelial cells

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Pseudomonas aeruginosa is classically defined as an extracellular opportunistic human pathogen. However, several studies have shown that *P. aeruginosa* is internalized and survives inside not specialized phagocytic cells [1,2]. Although intracellular survival of *P. aeruginosa* could be an important strategy to evade the host immune system and escape antibiotic activity, it remains poorly characterized.

The stringent response (SR) is a global regulatory pathway controlling the expression of virulence-related genes. In Gram-negative bacteria including *P. aeruginosa*, SR is based on the combined action of the (p)ppGpp second messenger and of the DksA protein [3-5]. However, little is known about the role played by SR in *P. aeruginosa* intracellular lifestyle.

We developed a model of infection to study the interaction between *P. aeruginosa* and airway epithelial cells, and investigated the impact of SR on *P. aeruginosa* intracellular survival.

A549 lung carcinoma epithelial cells were infected with the *P. aeruginosa* PAO1 strain or its isogenic mutants unable to produce (p)ppGpp or DksA or both. All strains constitutively expressed the *gfp* reporter gene. Bacterial association with infected cells was studied for up to 24 hours by CFU count, confocal microscopy, and flow cytometry.

Our results showed that *P. aeruginosa* can penetrate inside A549 cells and survive for up to 24 hours after infection, even if the number of bacteria associated to each cell decreased over time for all the tested strains. The mutants unable to produce DksA or (p)ppGpp showed decreased persistence in A549 cells relative to the parental strain, with the mutant unable to produce both factors displaying the less persistent phenotype.

Overall, our findings suggest that both DksA and (p)ppGpp play an important role in *P. aeruginosa* intracellular survival, and the *in vitro* model here developed lays the foundation for future studies aimed at characterizing *P. aeruginosa* intracellular infection.

References

1. Del Mar Cendra M, Torrents E (2020) *Virulence* 11:862-876.
2. Kember M, Grandy S, Raudonis R, Cheng Z (2022) *Pathogens* 11:220.
3. Dalebroux ZD, Svensson SL, Gaynor EC, Swanson MS (2010) *Microbiol Mol Biol Rev* 74:171-199.
4. Pletzer D, Blimkie TM, Wolfmeier H, Li Y, Baghela A, Lee AHY, Falsafi R, Hancock REW (2020) *mSystems* 5:e00495-20.
5. Fortuna A, Bähre H, Visca P, Rampioni G, Leoni L (2021) *Environ Microbiol* 23:5487-5504.

Adapting IgG separation techniques for small plasma pools in developing antibody-based therapeutics against antibiotic-resistant staphylococcal infections

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The escalating challenge of antibiotic resistance poses a critical threat to global health, requiring novel therapeutic strategies as traditional antibiotics increasingly fail to act efficiently [1]. Vaccination is an effective approach, often considered a potential solution, however, its application against some of the most challenging multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* MRSA, has been unsuccessful so far [2]. In contrast, passive immunotherapy offers a distinct advantage over active immunization by providing an immediate supply of high-titer specific antibodies targeted against particular pathogens or their toxins. Although its effect is short-term, it is sufficient to overcome ongoing infections [1]. The overall goal of our research project is the development of hyperimmunoglobulin products for the management of infectious diseases caused by resistant staphylococcal strains. Within the scope of this project, we have developed appropriate methods for the separation of the Immunoglobulin G (IgG) fraction from small plasma pools, as it is a key step in the development of hyperimmunoglobulin products. We adapted the classical Kohn cold ethanol fractionation method for small-scale fractionation, supplemented with additional purification steps to satisfy European Pharmacopoeia standards. Small human blood plasma pools were used for the experimental production of IgG preparations in three independent replicates. Based on the obtained data, the yield of IgG was 4.5±0.2 g/L, an excellent result compared to the yields achieved by the largest plasma fractionation centers using both the Cohn and/or chromatography methods. The quality and safety of the separated IgG preparation were tested with both *in vitro* and *in vivo* methods. The results confirmed that the separated IgG protein composition, molecular characteristics, stability, and other parameters adhere to the requirements of the European Pharmacopoeia. Our future work will involve immunizing animals with advanced methodologies from Western countries and comparing these with traditional techniques long established in post-Soviet regions to produce an hyperimmunoglobulin product. Upon completion of the project, we expect to successfully develop and produce IgG products targeting MRSA toxins.

References

1. Seixas AMM, Sousa SA, Leitão JH. Antibody-Based Immunotherapies as a Tool for Tackling Multidrug-Resistant Bacterial Infections. Vaccines (Basel). MDPI; 2022.
2. Han X, Ortines R, Mukherjee I, et al. Hyperimmune Targeting Staphylococcal Toxins Effectively Protect Against USA 300 MRSA Infection in Mouse Bacteremia and Pneumonia Models. Front Immunol. 2022;13.

Selection of Plant-Derived Antimicrobial Peptides against *Salmonella* Typhi Using Informational Spectrum Method

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The escalating threat of antibiotic resistance necessitates innovative approaches to combat bacterial infections. Antimicrobial peptides (AMPs) have shown promise in addressing the challenge of antimicrobial resistance (1). Recent study has highlighted the effectiveness of tomato-derived antimicrobial peptides (tdAMPs) against foodborne pathogens, particularly *Salmonella* enterica serovar Typhi (*S*. Typhi), a deadly human-specific pathogen responsible for typhoid fever (2). These tdAMPs have demonstrated potent antimicrobial properties by disrupting bacterial membranes, with proven efficacy against drug-resistant strains of *S*. Typhi.

In our research, we utilized the Informational Spectrum Method (ISM) (3,4), a virtual spectroscopy technique for analyzing biological molecules, to examine the effectiveness of tdAMPs against *S*. Typhi. We established a criterion for selecting antimicrobial peptides with potential activity against this pathogen. From the DBAASP antimicrobial peptide database (5), we identified three plant-derived peptides, ranging from 17 to 50 amino acids, that met the ISM criterion. These peptides are proposed as promising candidates for further experimental validation in the fight against *S*. Typhi.

References

1. Lewies, A., Du Plessis, L. H., & Wentzel, J. F. (2019). Antimicrobial peptides: the Achilles' heel of antibiotic resistance?. Probiotics and antimicrobial proteins, 11, 370-381.
2. Kwon, R. S., Lee, G. Y., Lee, S., & Song, J. (2024). Antimicrobial properties of tomato juice and peptides against typhoidal Salmonella. Microbiology Spectrum, e03102-23.
3. Veljkovic, N.; Glisic, S.; Perovic, V.; Veljkovic, V. The role of long range intermolecular interactions in discovery of new drugs. Exp. Opin. Drug Discov. 2011, 6, 1263.
4. Veljkovic, V.; Veljkovic, N.; Este, J.; Huther, A.; Dietrich, U. Application of the EIIP/ISM bioinformatics concept in development of new drugs. Curr. Med. Chem. 2007, 14, 441. 5.
5. Pirtskhalava M, Amstrong AA, Grigolava M, Chubinidze M, Alimbarashvili E, Vishnepolsky B, Gabrielian A, Rosenthal A, Hurt DE, Tartakovsky M. DBAASP v3: database of antimicrobial/cytotoxic activity and structure of peptides as a resource for development of new therapeutics. Nucleic Acids Res. 2021 Jan 8;49(D1):D288-D297.

The synergistic activity of colistin and clofoctol against Gram-negative pathogens

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Despite its toxicity, colistin is used as a last resort antibiotic to treat infections caused by multi-drug resistant Gram-negative pathogens (1). Of note, colistin is commonly used for the treatment of acute and chronic lung infections in people with cystic fibrosis (CF). Consequently, colistin resistance is emerging in CF and other clinical contexts, calling for the development of colistin adjuvants.
Clofoctol is an FDA-approved synthetic antibiotic active against Gram-positive pathogens (2).
Recently, we reported that clofoctol restores colistin susceptibility in colistin-resistant clinical isolates of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii* (3). The mechanism of action of the colistin-clofoctol combination is currently unknown.
On this basis, the aims of this project are *i*) to expand the number of tested pathogens, including those frequently isolated from CF individuals, and *ii*) to unravel the mechanism of action of the colistin-clofoctol combination.
The efficacy of the colistin-clofoctol combination has been tested on a panel of CF clinical isolates including *Burkholderia cenocepacia*, *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia.* Results showed that clofoctol potentiates colistin activity also in these species, supporting the use of the colistin-clofoctol combination in CF therapy.
In parallel, the mechanism of action of the colistin-clofoctol combination has been investigated by *in silico* and wet lab approaches. Molecular dynamics studies suggested that colistin could help clofoctol cross the outer membrane of Gram-negative bacteria. To validate this model and identify possible molecular targets of clofoctol, *P. aeruginosa* mutants resistant to the colistin-clofoctol combination were evolved *in vitro* and their genomes were sequenced. Experiments are in progress to confirm the involvement of identified mutations in the resistance to the colistin-clofoctol combination.

References

1. Hamel M, Rolain JM, Baron SA (2021). *Microorganisms* 9:442. doi: 10.3390/microorganisms9020442.
2. Buogo A (1981). *G Ital Chemioter* 28:65-71.
3. Collalto D, Fortuna A, Visca P, Imperi F, Rampioni G, Leoni L (2023). *Microbiol Spectr* 11(2):e0427522. doi: 10.1128/spectrum.04275-22.

Investigation of *Mycobacterium tuberculosis* DNA-gyrase-fluoroquinolone complexes by molecular dynamics simulations

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Multidrug resistant (MDR) *Mycobacterium tuberculosis* (Mtb) poses a critical health burden to the society and fluoroquinolones have been used as successful agents against this pathogen. Unfortunately, resistance to fluoroquinolone therapeutics is increasing, thus posing the necessity for better understanding of the mechanistic aspects of their action and for finding alternatives to bypass Mtb MDR. In this study we report molecular dynamics (MD) simulations of the wild-type (WT) Mtb DNA-gyrase complexes and A90S fluoroquinolone-sensitized mutants (MUT) of three fluoroquinolones - moxifloxacin (Mox), gatifloxacin (Gat), and levofloxacin (Lev). The X-ray structures of the complexes were downloaded from PDB. The MD simulations were performed with Amber 18 (https://ambermd.org/) for 200 ns to evaluate the binding free energies (ΔG_{bind}, kcal/mol) and the strongest interactions of the studied compounds (two ligands per complex in the binding sites). The MD trajectories were further processed by K-means clustering algorithm with 100 clusters and coordinated RMSD distances. The average ΔG_{bind} of the central pose of each cluster was calculated using the Generalized Born method. The ranking of the drugs according to their ΔG_{bind} for the WT complex was: Mox > Gat > Lev. In addition, for WT Mox complex the highest number of contributing binding site residues was recorded. Notably, these results are in accordance with the reversibility assay data and observed clinical effects of the drugs [1]. For the A90S mutants Gat showed stronger binding compared to Mox, and Lev had the lowest ΔG_{bind}. As expected, in all complexes, Mg^{2+} had the highest contribution to ΔG_{bind} with some variations among the drugs and their ligands. Interactions with mutated Ser90 with comparable contributions, were observed in all complexes. Differences in ΔG_{bind} contributions of DNA nucleotides and amino acids in the binding sites were also recorded thus pointing to different interactions of the studied compounds with the DNA-gyrase complex. In conclusion, our results help to explain the experimentally observed activities against Mtb and clinical effects of the studied drugs, and could direct the design of quinolone-based anti-Mtb agents able to overcome the bacterial resistance.

The authors acknowledge the networking support by the COST Action CA21145 “European Network for diagnosis and treatment of antibiotic-resistant bacterial infections (EURESTOP)”; IL, PA, AD, TP and IT thank the National Science Fund of Bulgaria (grant KP-06-COST/3).

References

1. Blower et al., PNAS, 2016, 113 (7), 1706–1713.

Set up of a whole-cell biosensor-based screening system to identify molecules inhibiting *Pseudomonas aeruginosa* growth or pathogenicity in the lung of individuals with cystic fibrosis

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The success of a bacterial pathogen in establishing hard-to-eradicate infections strictly correlates to its ability to use the host environment as a growth medium, and to produce virulence factors and resists to the action of antibiotics inside the host. The opportunistic human pathogen *Pseudomonas aeruginosa* uses the airway sputum as a nutritional source during cystic fibrosis (CF) lung infection [1], and finely modulates the formation of antibiotic-resistant biofilms and virulence factors production in response to stimuli associated to the host environment through c-di-GMP and quorum sensing (QS) signaling systems [2,3]. The CF sputum has been characterized and reconstituted as a synthetic CF sputum medium (SCFM) [4]. Genes required for *P. aeruginosa* growth in SCFM are dispensable in standard laboratory media. Moreover, *P. aeruginosa* displays similar biofilm formation and QS activation during growth in the CF sputum and in SCFM [4,5]. Hence, unexplored molecular pathways are required for growth, virulence and biofilm formation in the CF sputum, and molecules inhibiting these pathways in SCFM have the potential to reduce *P. aeruginosa* load and pathogenicity in the CF lung. To identify these molecules, we will screen a library of FDA-approved drugs using an *ad hoc* engineered biosensor strain cultured in SCFM.

Here, we will present the generation and validation of a *P. aeruginosa*-based biosensor strain in which molecules hampering c-di-GMP or QS signaling systems decrease light or fluorescence emission, respectively. Interestingly, the known c-di-GMP and QS inhibitors sodium nitroprusside and niclosamide were more effective in reducing the biosensor activity in the standard medium Mueller Hinton Broth (MHB) than in SCFM, highlighting the necessity to identify new drugs that specifically inhibit *P. aeruginosa* biofilm formation and virulence in SCFM. Preliminary data collected during the screening of a library of more that 3,000 FDA-approved drugs using the biosensor strain grown in SCFM will be also presented.

References:

1. Malhotra *et al*. (2019) *Clin Microbiol Rev* 32:e00138-18.
2. Römling *et al.* (2013) *Microbiol Mol Biol Rev* 77:1-52.
3. Waters and Bassler (2005) *Annu Rev Cell Dev Biol* 21:319-346.
4. Palmer *et al*. (2007) *J Bacteriol* 189:8079-8087.
5. Turner *et al.* (2015) *Proc Natl Acad Sci USA* 112:4110-4115.

Aligning initiatives: suggested policy pathways by the American Society for Microbiology (ASM) resonate with the EU Council Recommendation and the WHO European Region roadmap in combating antimicrobial resistance crisis

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The American Society for Microbiology (ASM) recently released a set of policy recommendations aimed at addressing antimicrobial resistance (AMR), acknowledging its complexity as a significant public health concern and a matter of national security. In a nutshell, these guidelines emphasize how policymakers should prioritize science and empower microbiologists by supporting innovative research on antimicrobial resistance, streamlining approval processes for antimicrobials, strengthening the microbiology workforce, modernizing data systems, enhancing rapid detection methods, promoting stewardship programs for appropriate antimicrobial use, harmonizing domestic and global policies to bolster stewardship efforts and expand laboratory capacity in low- and middle-income countries, but also by collaborating with partner countries to develop a comprehensive global assessment of AMR (while concurrently offering technical assistance to researchers navigating international research frameworks). The recommendations further place a special emphasis on drug development, stating how there is a need to incentivize the development of antibiotics by implementing a subscription program designed to offer a reliable return on investments for the development of critically needed new antimicrobial drugs. There is also a big emphasis on One Health Framework, where ASM underscores the preparedness to collaborate with the US Congress, federal agencies and international governing bodies to establish a comprehensive strategy for addressing AMR from human, animal and environmental perspective. But the question arises: are they relevant for the European context as well? The majority of recommendations closely align with the European Union (EU) Council Recommendation on stepping up EU actions to combat antimicrobial resistance in a One Health approach. Similarly, they resonate with the action areas outlined in the Roadmap on antimicrobial resistance for the WHO European Region 2023–2030 (RC73) by the World Health Organization. The aforementioned Roadmap not only guides actions until 2030, but also incorporates advancements in antimicrobial resistance, such as heightened attention to environmental and social factors, digital innovation, and a greater emphasis on patient-centred approaches. These parallel efforts underscore the global consensus on the urgency of combating AMR and the collaborative approach needed to address this pressing issue effectively.

References

American Society for Microbiology. Policy Pathways to Combat the Global Crisis of Antimicrobial Resistance. Policy Paper, 2023. Available at: https://asm.org/getmedia/5f665383-881a-493d-ae05-04a960a25548/AMR-Policy-Paper-2023.pdf

The utility of the MICROBE Interactive Visualization Tool in comparing the burden of antimicrobial resistance across Europe

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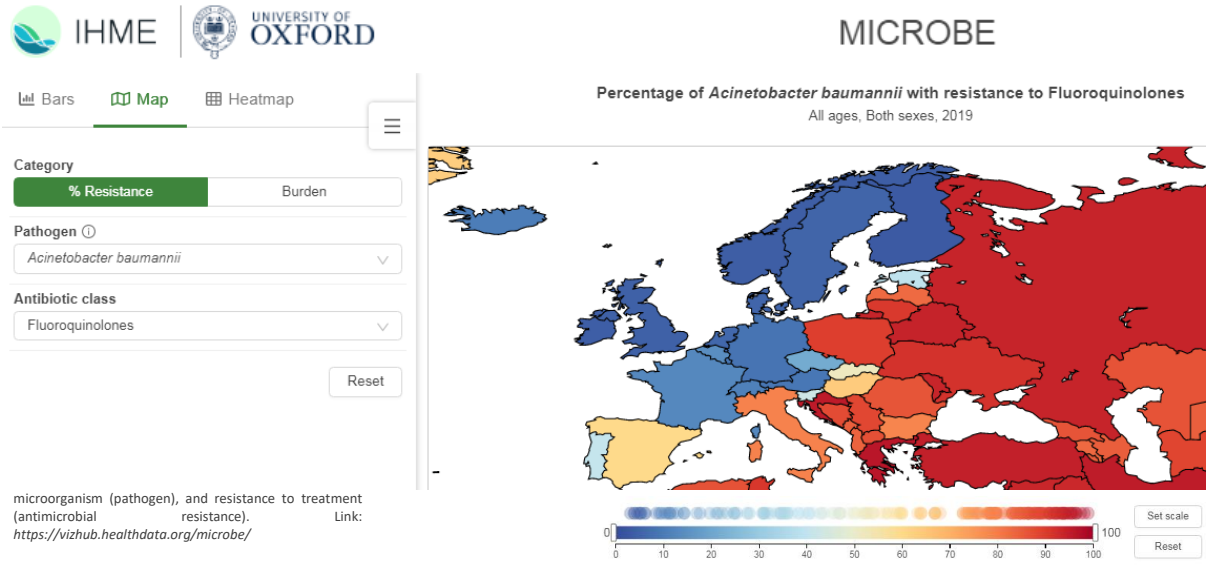
The analysis of bacterial antimicrobial resistance (AMR) burden in the WHO European Region indicated 133 thousand deaths caused by resistant bacterial pathogens, with more than half a million deaths associated with such pathogens. However, a comprehensive comparison among different countries of the region was previously lacking. Here, we aimed to introduce a tool facilitating comprehensive cross-country comparisons. The MICROBE (Measuring Infectious Causes and Resistance Outcomes for Burden Estimation) tool facilitates visualization of fatal/nonfatal health outcomes of infections, pathogens, and AMR across different regions and countries. It provides insights into burden and interrelationships among outcomes, exploring various health metrics by geography, age, and sex through bar visualization and global maps. For the burden estimation, input data includes 471 million individual records/isolates and 7585 study-location-years from surveillance systems, hospital databases, systematic literature reviews, and other sources. The modelling approach is comprised of five key components: infection-related deaths, proportion of infectious deaths due to specific syndromes, proportion of syndrome-related deaths attributed to particular pathogens, percentage of resistance among pathogens, and the increased risk of death associated with this resistance. Two counterfactual scenarios are employed: deaths attributable to and deaths associated with bacterial AMR. The tool can be used to evaluate stark differences in AMR burden across different countries – emphasizing the necessity for tailored interventions, informed policy decisions and enhanced AMR surveillance. The tool is available on the following link: *https://vizhub.healthdata.org/microbe/*



microorganism (pathogen), and resistance to treatment (antimicrobial resistance). Link: *https://vizhub.healthdata.org/microbe/*

New Carbohydrates-Based Candidates to Target ESKAPE Bacteria

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Antimicrobial resistance (AMR) is one of the most alarming phenomena in current medicine. Among different pathogens that developed AMR, of note are the bacteria of the ESKAPE group, composed by *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacters*. Indeed, these bacteria have developed effective strategies to evade the action of antibiotics, such as β-lactamases, drug efflux pumps or active sites modification,[1] thus, the research for an alternative therapy to antibiotics is a hot topic. One of the most promising strategy to target AMR bacteria is immune therapy. This consists in training the immune system of the host to react against molecules expressed on the surface of pathogens. Capsular polysaccharides (CPS) have a strongly conserved sequence, and this makes the repeating unit an eligible candidate for immune therapy. However, small sugars cannot trigger an immune response but multivalent presentation of the antigen or conjugation to immune active molecules, could enhance their immunogenicity. From the hydrolysis of the capsular polysaccharide of *Klebsiella pneumoniae*, we obtained the structure of the repeating unit and starting from it, we designed the synthesis of an analogue that could be conjugated to different entities. The results of this research will be presented in this communication.

References

1. De Oliveira D.M.P., Forde B.M, Kidd T.j.K., Harris P.N.A., Schembri M.A., Beatson S.A., Paterson D.L., Walker M.J., **2020**, *Clin Microbiol Rev*, 33(*3*).

Design and Synthesis of Innovative Oxadiazole Derivatives for the Treatment of Alzheimer's Disease

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Alzheimer's disease (AD) is a significant global health concern characterized by memory loss and cognitive decline, affecting millions of individuals worldwide. In this study, we explore the synthesis and evaluation of eleven novel compounds as potential anti-Alzheimer agents targeting cholinesterase and ß-secretase inhibition [1,2]. The compounds were assessed for their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using the modified Ellman method. Notably, compounds **6d**, **7a**, and **7e** exhibited substantial inhibition of AChE, with IC_{50} values of 0.120 µM, 0.039 µM, and 0.063 µM, respectively. Compound **7e** also showed promising inhibitory effects against BACE-1, a key enzyme involved in Aβ peptide production. Structural analysis revealed that compounds containing substituted benzothiazole and thiazole moieties displayed the most potent inhibitory activity [3]. Additionally, computational techniques including density functional theory (DFT), molecular docking, and molecular dynamics simulations were employed to elucidate the action mechanism and structure-activity relationship of the active compounds [4]. Compound **6d** exhibited enhanced electrophilic character, while compound **7e** demonstrated nucleophilic character, providing valuable insights into their biological activity [5]. Overall, this study highlights the potential of these synthesized derivatives as promising candidates for the treatment of Alzheimer's disease and underscores the importance of multifunctional small molecules in targeting key pathways implicated in AD progression.

References:

1. Asaf Evrim EVREN, Demokrat NUHA, Sam DAWBAA, Abdullah Burak KARADUMAN, Begüm Nurpelin SAĞLIK & Leyla YURTTAŞ (2023) Novel oxadiazole-thiadiazole derivatives: synthesis, biological evaluation, and in silico studies, Journal of Biomolecular Structure and Dynamics, https://doi.org/10.1080/07391102.2023.2247087.
2. Sam DAWBAA, Demokrat NUHA, Asaf Evrim EVREN, Meral YILMAZ CANKILIÇ, Leyla YURTTAŞ & Gülhan TURAN (2023): New oxadiazole/triazole derivatives with antimicrobial and antioxidant properties, Journal of Molecular Structure, https://doi.org/10.1016/j.molstruc.2023.135213.
3. Demokrat NUHA, Asaf Evrim EVREN, Özge KAPUSIZ, Ülküye Dudu GÜL, Nalan GUNDOGDU-KARABURUN, Ahmet Çagri KARABURUN & Halil BERBER (2023): Design, synthesis, and antimicrobial activity of novel coumarin derivatives: An in-silico and in-vitro study, Journal of Molecular Structure, https://doi.org/10.1016/j.molstruc.2022.134166.

Synthesis of Tetra-*ortho*-Methoxylated Azobenzene Photoswitches via Sequential Catalytic C–H Activation and Methoxylation

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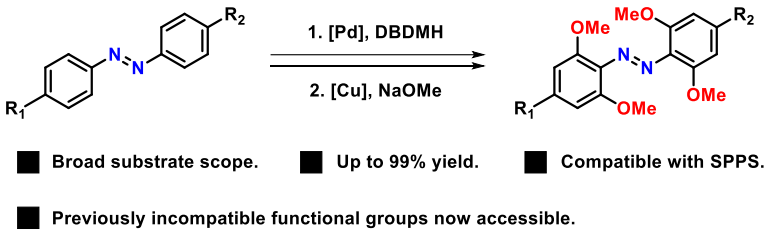
The azobenzene moiety stands as the preferred photoswitch in various applications within photopharmacology. However, its reliance on UV light isomerization limits its *in vivo* applications. The azobenzene scaffold can be modified to cause a red-shift, allowing ***activation with visible light that does not harm tissues and can penetrate deeper than shorter wavelengths***.[1] However, the syntheses available present several limitations, such as low yields or harsh conditions.

In our group, we have recently developed a method to access highly coveted tetra-*ortho*-methoxylated azobenzene photoswitches through sequential catalytic C-H bromination and subsequent methoxylation. This method not only demonstrates improved yields but also exhibits remarkable tolerance for diverse functional groups previously challenging to incorporate using other strategies.[2]



Furthermore, our investigation extends to the application of this tetra-*ortho*-methoxylated scaffold in Solid Phase Peptide Synthesis (SPPS), with a focus in the development of visible-light operated photoswitchable antimicrobial peptides.[3] Comparative analyses highlight its superior robustness over its chlorinated counterpart, showcasing its potential for SPPS applications. ***This new methodology allows us to access visible-light-operated peptides using the well-established protocols of SPPS in a more efficient and versatile approach***.

References

1. Wegener, M.; Hansen, M. J.; Drieseen, A. J. M.; Szymanski, W.; Feringa, B. L. J. Am. Chem. Soc. 2017, 139, 17979-17986.
2. Ruiz-Soriano, A.; Lamelza, L.; Pizzamiglio, E.; Just-Baringo, X. *ChemRxiv* **2023**. DOI: 10.26434/chemrxiv-2023-dr111.
3. Just-Baringo, X.; Yeste-Vázquez, A.; Moreno-Morales, J.; Ballesté-Delpierre, C.; Vila, J.; Giralt, E. *Chem. Eur. J.* **2021**, *27*, 12987.

Antimicrobial screening of Natural Sources for pharmaceutical applications

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The skin, being the body's largest organ, plays a crucial role as the primary defense against infections and injuries. The exploration of natural sources for biologically active agents has become pivotal in the pursuit of innovative, cost-effective, highly efficient, and safe molecules for treating various skin disorders. Examples such as: *Plectranthus* species, belonging to a genus with a rich history in traditional medicine, has garnered attention due to its antibacterial, and antifungal properties; Essential and vegetable oils derived from plants also exhibit antimicrobial properties, adding to potential therapeutic options; Honey, a natural substance produced by honeybees has been used for wound healing owing to its remarkable antimicrobial properties; The black soldier fly larvae possess oil biomass rich in fatty acids. These fatty acids enhance the larvae's potential as a valuable source of bioactive compounds with applications in skin health and pharmaceutical development.

The primary objective of this study was to investigate the antimicrobial potential of diverse natural sources. The well-diffusion method was employed against a collection of Gram-negative and Gram-positive bacteria, as well as yeast strains. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values were determined using the microdilution method, providing a comprehensive assessment of the antimicrobial efficacy of the investigated substances.

Results indicated that *Plectranthus* extracts demonstrated effective antibacterial activity against the tested Gram-positive bacterial strains. Among the tested oils, two displayed promising activity against Gram-positive bacteria, while three exhibited moderate activity against Gram-negative bacteria. Conversely, the honey samples did not show significant antimicrobial activity. Notably, the black soldier fly extracts demonstrated activity against both Gram-positive and Gram-negative bacteria, as well as yeast.

Ongoing research is now delving into the potential use of these natural sources in addressing antibiotic-resistant bacterial infections. The focus is to explore their efficacy as pharmaceutical products, paving the way for further understanding and application in the field of medical treatment.

References

1. Chambers, E.S., Vukmanovic-Stejic, M., 2020. Immunology. DOI:10.1111/imm.13152
2. Franco, A., *et. al.*, *Insects.* 2021, *13*, 41. DOI:10.3390/insects13010041.
3. Abdel-Mogib, M., *et. al.*, Molecules. 2002.
4. Codex Alimentarius Commission. *Rev. 1(1987). Rev. 2(2001). Codex Stan 12-1981.*
5. Amr E. Edris. Phytother Res. 2007 Apr;21(4):308-23.

Antimicrobial resistant *Staphylococcus* colonization in pets and their owners

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Dogs and cats are increasingly regarded as family members. Therefore, the question about mutual transmission of pathogens is of particular interest. In households, staphylococci can be transmitted either through direct contact during owner-pet interactions or through secondary contact with contaminated surfaces [1]. In the human community, *Staphylococcus aureus* colonizes the anterior nares about 25–35% of healthy persons. Dogs often carry methicillin-resistant staphylococci asymptomatically [2]. The aim of this study is to identify S*taphylococcus* species isolated from nasal swabs of healthy dogs, cats and their owners, and to investigate the antimicrobial resistance. The study involved dogs (n=61), cats (n=45) and their owners (n=107). In total, 71 households were investigated. Amies transport medium was used for taking swabs from both nostrils of owners, dogs and cats. Coagulase positive staphylococci isolates have been identified using multiplex polymerase chain reaction (M-PCR). Polymerase chain reaction was used to detect genes associated with resistance to beta-lactams (*blaZ*) and methicillin (*mecA*). The results were considered statistically significant if P<0.01 or P<0.001. In 42 (59.2%) of the 71 sampled households there was at least one individual (either owner or pet) that carried coagulase-positive staphylococci. In this study the frequency of *S. aureus* carriage in owners was 30.8%, meanwhile *S. pseudintermedius* was identified only in 2.8%. Colonization of *S. aureus* in humans was significantly higher than in cats (P = 0.001) or dogs (P < 0.001). The high prevalence of *S. pseudintermedius* in dogs (45.9%) was detected in our research. In comparison, S. *aureus* colonization in dogs was rare - 6.6%. *S. pseudintermedius* was more common in dogs compared to humans (P < 0.001) and cats (P < 0.001). *S. aureus* was isolated from 3 cats (6.7%) and S. *pseudintermedius* in 1 cat (2.2%). Methicillin-resistant *S.aureus* and *S. pseudintermedius* strains were not determined in this study. 71% of *S. pseudintermedius* strains isolated from pets showed resistance to at least one antimicrobial agent. 22.6% of all *S. pseudintermedius* isolates from dogs were identified as multidrug resistant. Among the 33 strains of *S. aureus* isolated from humans, 22 (66.7%) isolates were resistant to at least one antimicrobial. 48.5% of beta-lactamase producing *S. aureus* strains were isolated from healthy humans. The prevalence of beta-lactamase-producing *S. pseudintermedius* strains isolated from dogs were 36.7 %. Genetic analysis showed that two pairs of *S. pseudintermedius* strains isolated from dogs and humans living in the same household were 100% identical, the third pair showed high 99.7% similarity. These results suggest that dog contact does increment the possibility to carry *S. pseudintermedius* in humans. After analyzing the sequences *of S. aureus* strains *nuc* gene, we found 100% similarity between strains isolated from the dog and its host. These data show a real flow of these bacterial species within household settings, and subsequently, the risk of bacterial transference.

References:

1.Sakr A, et all., *Staphylococcus aureus* nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Front. Microbiol. 2018 9, 2419.

2. Cuny C.,et al., Colonization of dogs and their owners with *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in households, veterinary practices, and healthcare facilities. *Microorganisms.* 2022, 10, 677.

N-Phenylpyrrolamide inhibitors of DNA gyrase with improved antibacterial activity

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The growing threat of antibacterial resistance increases the need for antibiotics with new mechanisms of action. The ATP binding site on DNA gyrase and topoisomerase IV is an attractive target for the development of new antibacterial agents. DNA gyrase and topoisomerase IV share 40% sequence identity and have similar active sites, providing a good opportunity for dual targeting.

In this work, we focused on advancing the development of *N*-phenylpyrrolamides as DNA gyrase B inhibitors with in vitro activity against "ESKAPE" pathogens. Based on the recently determined crystal structure of our *N*-phenyl-4,5-dibromopyrrolamide inhibitor-DNA gyrase B complex (Figure 1),[1] we prepared a series of improved *N*-phenylpyrrolamides and tested them against DNA gyrase and topoisomerase IV. The IC_{50} values for the most effective compounds were in the low nanomolar range. The minimum inhibitory concentrations (MICs) against selected Gram-positive and Gram-negative bacteria were in the low micromolar range. Resistance development, post-antibiotic effect (PAE), time-kill assays and toxicity were determined for the most promising compounds.[2-5]

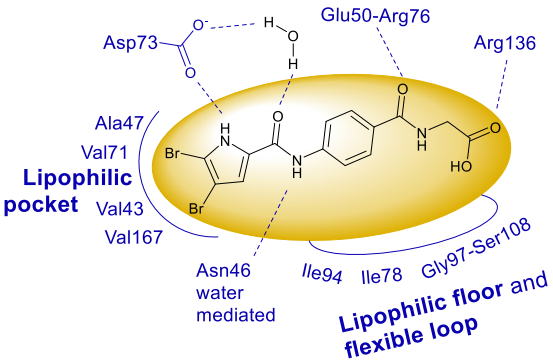

Figure 1. Representative *N*-phenylpyrrolamide DNA gyrase inhibitor and substrate-enzyme interactions.

References

1. Zidar, N.; Macut, H.; Tomašič, T.; Kikelj, D. et al. *J Med Chem*, **2015**, *58*, 6179-6194.
2. Zidar, N.; Tomašič, T.; Macut, H.; Kikelj, D. et al. *Eur J Med Chem*, **2016**, *117*, 197-211.
3. Durcik, M.; Lovison, D.; Skok, Ž.; Zidar, N. et. al. *Eur J Med Chem*, **2018**, *154*, 117-132.
4. Zidar, N.; Macut, H.; Tomašič, T.; Kikelj, D. et al. *MedChemComm*, **2019**, *10*, 1007-1017.
5. Benedetto Tiz, D., Skok, Ž.; Durcik, M.; Zidar, N. et al. *Eur J Med Chem*, **2019**, *169*, 269-290.