

COST ACTION CA21145

European Network for diagnosis and treatment of antibiotic-resistant bacterial infections

(EURESTOP)

Lisbon, 26-27 January 2023



COST action CA21145, 1st Meeting

Research Center for Biosciences & Health Technologies (CBIOS)

Universidade Lusófona de Humanidades e Tecnologias

Lisbon, Portugal

Location and dates: Lisbon, Portugal 26-27 January 2023

Venue: CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024

Remote access: <https://videoconf-colibri.zoom.us/j/91185124432>

Scientific Committee:

Mattia Mori (Italy) - Action Chair

Patricia Rijo (Portugal) - Vice Chair

Cristina Nativi (Italy) - Science Communication coordinator

Priyanka Sahariah (Iceland) - Grant Awarding coordinator

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Younes Smani (Spain) - WG2 Leader

Carole Devaux (Luxembourg) - WG3 Leader

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Patrícia Rijo (Local Organizer)

Vera Isca (Portugal)

Márcia Filipe (Portugal)

Gabrielle Bangay (Portugal)

Daniel Santos (Portugal)

Ana Mourato (Portugal)

Andreia Rosatella (Portugal)

Management: Carolina Altília - COST Action CA21145 - info@eurestop.eu

CA21145 SUMMARY

The emergence and spread of drug-resistant bacteria is an important health and socioeconomic threat with global dimensions, which is developing towards an emergency/pandemic crisis. No drugs are available to address the disease, and diagnostic tools are poorly effective. This negatively impacts the treatment and survival of critically ill patients. As such, drug-resistant bacteria may spread outside hospital settings, representing a critical risk for the global population. Current research in this field is highly fragmented and mostly monodisciplinary, thus limiting the development of innovative diagnostic and therapeutic solutions.

This COST Action will bring together industrial and academic European scientists with different skills and expertise in a multidisciplinary and concerted initiative. The Action will combine disciplines such as chemistry, physics, bioinformatics, genetics, biology, immunology, and medicine in understanding the genetic and molecular bases of bacterial drug resistance, developing innovative diagnostic tools, and delivering lead/pre-clinical candidates, antibody-based therapies, and clinical-ready repurposed drugs towards the personalized treatment of drug-resistant bacterial infections. The further challenge of the Action is to enhance networking among European scientists and to increase the competitiveness of European research by promoting the exploitation of translational research outcomes, e.g., by the creation of novel SMEs. Finally, by knowledge creation and sharing, the Action will train a new generation of young scientists skilled in the multiple aspects related to bacterial drug resistance. Career development of Young Researchers and Innovators and research impulses in Inclusiveness Target Countries (ITC) will be considered as a priority in the Action.

www.eurestop.eu

<https://www.cost.eu/actions/CA21145/>

PROGRAM

26th January 2023

<https://videoconf-colibri.zoom.us/j/91185124432>

8:15 – 9:00 Registration of participants

9:00 – 9:15 **Opening:** Mattia Mori – CA21145 Chair
L. Monteiro Rodrigues - CBIOS Director
Patrícia Rijo – Local organizer

Session 1 | Chairman: CRISTINA NATIVI (Italy)

9:15 -10:00 **Invited Expert:** Marco Maria D'Andrea Università di Roma "Tor Vergata", Italy
Antibiotics and antibiotic resistance: lessons from the past to guide future actions

10:00 -10:15 Magne Sydnnes University of Stavanger, Norway
Post-use photodecomposition – quicker removal of antimicrobial agents from the environment

10:15 -10:30 Abidelfatah Nasser Beitberl College of Education, Israel
Environmental Dissemination of antibiotic resistant bacteria (ARB) and genes (ARG) by the discharge of Wastewater Effluents

10:30 – 10:45 Vladimír Křen Czech Academy of Sciences, Czech Republic
Flavonolignans from silymarin as MDR inhibitors

10:45 – 11:15 *Coffee break*

Session 2 | Chairman: DANA REICHMANN (Israel)

11:15 – 11:30 Simone Carradori University of Chieti-Pescara, Italy
Pseudomonas aeruginosa carbonic anhydrase inhibitors as innovative antibacterial agents to combat antibiotic resistance: ciprofloxacin derivatives

11:30 – 11:45 A. Basak Kayitmazer Bogazici University, Turkey
Soft Matter for Understanding and Treating Antibiotic-Resistant Bacterial Infections

11:45 – 12:00 Ivanka Tsakovska Bulgarian Academy of Sciences, Bulgaria
Development and application of in silico approaches to predict pharmacological/toxic effects of bioactive compounds

12:00 – 12:15 Nuno R. Candeias University of Aveiro, Portugal
Phenolic Mannich bases as antibacterials agents for Staphylococcus aureus

12:15 – 12:30 Yves Mèly University of Strasbourg, France
Developing new strategies to image, target and kill gram negative bacteria

12:30 – 12:45 Lul Raka National Institute of Public Health of Kosova, Kosovo
Addressing antimicrobial resistance in Kosova

12:45 – 14:20 *Lunch break (& group picture at 14:20 in front of the Auditorium entrance)*

Session 3 | Chairman: CAROLE DEVAUX (Luxembourg)

14:30 – 14:45 **Vera M. S. Isca** Universidade Lusófona, Portugal

*Antimicrobial screening of *Plectranthus* extracts for applications against antibiotic resistant bacterial infections*

14:45 – 15:00 **Ana Martins** Biological Research Centre, Hungary

Laboratory evolution as a key tool in antimicrobial development

15:00 – 15:15 **Ismail Ocsoy** Erciyes University, Turkey

Development of natural and synthetic anthocyanin incorporated phenotypic tests for colorimetric, fast and sensitive detection of antibiotic resistance bacteria in clinic

15:15 – 15:30 **Constantinos Athanassopoulos** University of Patras, Greece

Natural product antibacterial agents and rediscovery of old antibiotics

15:30 – 15:45 **Michail Christodoulou** University of Milan, Italy

4-(1,2,2-triphenylvinyl)aniline as self-assembling inducer

15:45 – 16:15 *Coffee break*

16:15 – 16:30 **Matthew Borg** COST Association

Q&A – reimbursement rules and eligible expenses

16:15 – 17:15 **Working Groups Meeting**

WG1 – Room D.1.11

Remote connection: <https://videoconf-colibri.zoom.us/j/99354119338> Meeting ID: 99354119338

WG2 – Room D.1.13

Remote connection: <https://videoconf-colibri.zoom.us/j/96883839475> Meeting ID: 968 8383 9475

WG3 –conference auditorium

Remote connection: <https://videoconf-colibri.zoom.us/j/91185124432> (same as the main conference)

17:15 – 18:45 **MC Meeting** – ONLY FOR MC MEMBERS

Conference auditorium

Social Dinner – Chimarrão Campo Grande - 19:30-22:00

(<https://chimarrao.pt/>)

27th January 2023

<https://videoconf-colibri.zoom.us/j/91185124432>

Session 4 | Chairman: PATRICIA RIJO (Portugal)

9:15 – 10:00 **Invited Expert: Artur Silva** University of Aveiro, Portugal

Biologically active oxygen and nitrogen heterocyclic compounds from cycloaddition and conjugate addition reactions on chromones

10:00 – 10:15 **Tomislav Mestrovic** University North, University centre Varaždin, Croatia

Estimating the burden of bacterial antimicrobial resistance in Europe and beyond: a systematic data-driven approach for research and implementation priority setting

10:15 – 10:30 **Stephen J. Fey** CelVivo ApS, Denmark

Culture Conditions Matter

10:30 – 10:45 **Fabienne Dumoulin** Acibadem Mehmet Ali Aydınlar University, Turkey

Photodynamic therapy - Are light-triggered oxidative damages the magic solution to AMR?

10:45 – 11:15 Coffee break

Session 5 | Chairman: PRIYANKA SAHARIAH (Iceland)

11:15 – 11:30 **Younes Smani** University of Pablo de Olavide, Spain

Innovative antimicrobial strategy for the treatment of MDR bacterial infection

11:30 – 11:45 **Bengt Erik Haug** University of Bergen, Norway

Novel ligands for the flavin mononucleotide riboswitch

11:45 – 12:00 **Bruno L. Victor** University of Lisbon, Portugal

How can the Structure-based Molecular Modeling group from FCUL boost the EURESTOP

12:00 – 12:15 **Kaja Kasemets** National Institute of Chemical Physics and Biophysics, Estonia

Antimicrobial chitosan-nanocomposites for biomedical applications

12:15 – 12:30 **Maria M. M. Santos** University of Lisbon, Portugal

Spirooxindoles for targeting cancer and malaria. Perspectives of antibacterial activity

12:30 – 12:45 **Giorgia Giovannini** Swiss Federal Laboratories for Materials Science & Technology, Switzerland

Diagnosis and treatment of bacterial infections

12:45 – 14:30 Lunch break

Session 6 | Chairman: YOUNES SMANI (Spain)

14:30 – 14:45 **Rossella Grande** University of Chieti-Pescara, Italy

Antimicrobial and Antibiofilm Activities of Carvacrol and Thymol: Effective Carbonic Anhydrase Inhibitors versus Helicobacter pylori

14:45 – 15:00 **Gabrielle Bangay** Universidade Lusófona, Portugal

Chemical design of halimane diterpene for enhanced antimicrobial activity

15:00 – 15:15 **Ryszard Ostaszewski** Institute of Organic Chemistry, Poland
Application of multicomponent reactions for the synthesis of a new potential antimicrobial drugs

15:15 – 15:30 **Ivana Kovačević** University of Novi Sad, Serbia
Natural products with γ -butyrolactone moiety as potential antibiotics

15:30 – 15:45 **Sanja Glisic** Institute of Nuclear Sciences VINCA, Serbia
Efficient in silico approach for selection therapeutics against DR bacteria and identification of new therapeutic targets

15:45 – 16:00 **Entela Haloci** University of Medicine, Albania
A comparative study of antibacterial and antifungal activities of some essential oils

16:00 – 16:30 *Coffee break*

Session 7 | Chairman: TOMISLAV MESTROVIC (Croatia)

16:30 – 16:45 **Nace Zidar** University of Ljubljana, Slovenia
Discovery of new inhibitors of DNA gyrase and topoisomerase IV to overcome antimicrobial resistance

16:45 – 17:00 **Paulo J. Costa** University of Lisbon, Portugal
Studying molecular recognition phenomena using computational tools: the case of halogenated drugs

17:00 – 17:15 **Jitka Viktorova** University of Chemistry and Technology Prague, Czech Republic
Antibiotic resistance platform for high-throughput screening of adjuvants for combination therapies

17:15 – 17:30 – **Carole Devaux** Luxembourg Institute of Health, Luxembourg
*Development of immunotherapeutic complexes eliciting complement activation towards multidrug-resistant *Pseudomonas aeruginosa**

17:30 – 17:40 – **CA21145 Chair and Vice-Chair**
Closing remarks, next meetings and activities

**Abstracts of invited experts
(in order of presentation)**

Antibiotics and antibiotic resistance: lessons from the past to guide future actions

Marco Maria D'Andrea (marco.dandrea@uniroma2.it)

Lab. of Applied Microbiology - Dep. Of Biology – University of Rome “Tor Vergata”, Rome, Italy

The therapeutic efficacy of antibiotics is seriously jeopardized by the rapid and massive dissemination of antibiotic resistant bacteria on a global scale. This phenomenon is particularly worrisome in hospital settings, where it represents a leading cause of mortality and morbidity, as well as of prolonged hospital stays and substantial increases of health-care associated costs. Indeed, recent estimates trying to quantify the burden of antimicrobial resistance highlight that 4.95 million deaths were associated to antimicrobial resistance in 2019, including 1.27 million deaths directly attributable to this issue. It is now widely recognized that a global, coordinated and multidisciplinary action plan is urgently needed to minimize the negative effects due to antibiotic resistant bacteria. Such a plan should include not only the discovery and development of new antibiotics, antibiotic classes and of alternative antimicrobial strategies, but also effective directions at international level to stop the abuse and misuse of the available antimicrobial armamentarium. This latter important goal will be reached only if interventions such as improvement and optimization of i) sanitation, ii) surveillance of drug resistance, iii) the antibiotic use in all contexts, iv) diagnosis of infectious diseases and v) vaccines will be implemented soon.

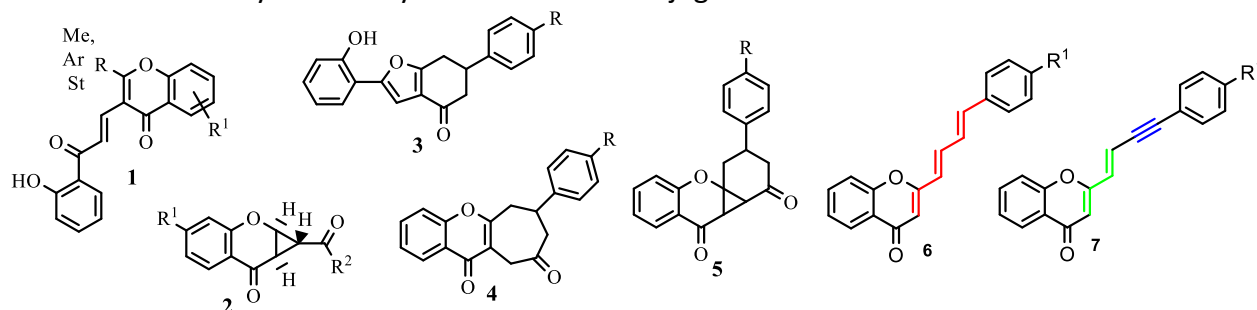
BIOLOGICALLY ACTIVE OXYGEN AND NITROGEN HETEROCYCLIC COMPOUNDS FROM CYCLOADDITION AND CONJUGATE ADDITION REACTIONS ON CHROMONES

Artur M. S. Silva, artur.silva@ua.pt

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Oxygen and nitrogen heterocyclic compounds constitute the largest and most varied families of organic compounds, comprising a great number of classes according to the size, number of heteroatoms and heterocyclic ring oxidation. The important industrial and biological applications of these types of compounds and also some problems associated with their application, such as multiple drug resistance to some nitrogen heterocycles and potential carcinogenesis of high doses of oxygen heterocyclic-based antioxidants, led us to develop new synthetic methods for novel biologically active derivatives of both referred families of heterocyclic compounds.

Herein we will be focused on the use of chromones as building blocks for the synthesis of several other nitrogen and oxygen heterocycles, polysubstituted chromones **1**, and polycyclic compounds **2-5**. We also discuss new synthetic routes for 2-[(1*E*,3*E*)-4-arylbuta-1,3-dien-1-yl]chromones **6** and (*E*)-2-(4-arylbut-1-en-3-yn-1-yl)chromones **7** and studied the reactivity of their unsaturated systems in cycloaddition and conjugate addition reactions.



Acknowledgements:

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020 and Portuguese NMR Network.

Abstracts of Lectures (in order of presentation)

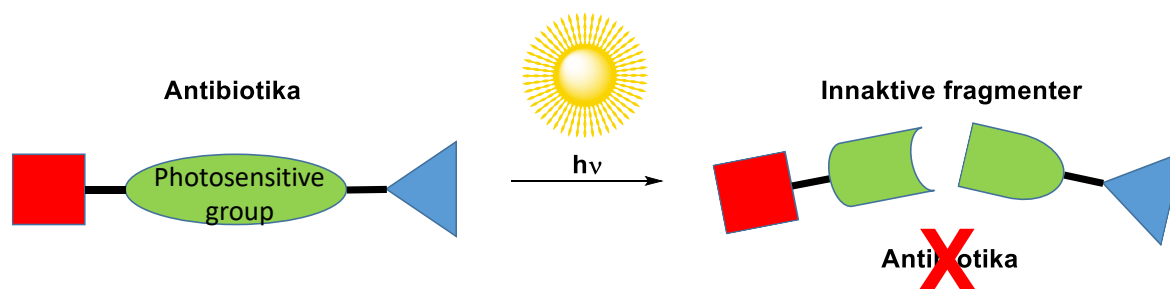
Post-use photodecomposition – quicker removal of antimicrobial agents from the environment

Magne Sydnes

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Faculty of Science and Technology, Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Norway

Accumulation of persistent antimicrobial agents in the environment after use is one of the drivers behind antimicrobial resistance. Quicker degradation of biological substrates would ease the pressure on resistance development. To facilitate quicker degradation of antimicrobial agents after excretion from the patient (humans or animals) we have developed chemistry that enables photodegradation. The talk will focus on the development of photodegradable ethanolamine moiety (Scheme 1), mechanism of decomposition, and antimicrobial activity of our compounds.



Scheme 1. Schematic outline of the photodegradable antimicrobial agents.

References

1. Eikemo, V.; Sydnes, L. K.; Sydnes, M. O. Photodegradable antimicrobial agents – synthesis, photodegradation, and biological evaluation. *RSC Adv.* **2021**, *11*, 32339-32345.
2. Eikemo, V.; Holmelid, B.; Sydnes, L. K.; Sydnes, M. O. Photodegradable Antimicrobial Agents: Synthesis and Mechanism of Degradation. *J. Org. Chem.* **2022**, *87*, 8034-8047.

Environmental Dissemination of antibiotic resistant bacteria (ARB) and genes (ARG) by the discharge of Wastewater Effluents

*Abidelfatah Nasser, abid@beitberl.ac.il and Heitham Fawaqa
Institute of Natural Sciences, Beitberl Collage of Education, Beitberl, Israel*

Antibiotics resulted in improving the quality of life worldwide; however, antibiotic resistance increases the morbidity and mortality caused by bacterial infections and the costs of treating infectious diseases. This study was conducted to evaluate the prevalence of multi drug resistant (MDR) fecal coliforms (FC) and genes, which confer resistance to β -lactams and tetracycline antibiotics (ampC, blaSHV, tetA and tetB) in secondary and tertiary wastewater effluents. The most commonly observed resistance profiles for FC isolated from secondary effluents and tertiary effluents treated by UV were for P, CT, F, while in isolates obtained from tertiary effluents treated by chlorine the highest resistance profile was recorded for P, CT, and AMP). The most frequently detected genes in all samples were ampC and tetB. In contrast, the tetA gene was found to be more prevalent in secondary effluents and tertiary effluents disinfected by UV compared to effluents disinfected by chlorine. The findings of this study that suggest wastewater treatment process may do not prevent the spread of ARB and ARGs in downstream receiving water bodies.

Flavonolignans from silymarin as MDR inhibitors

Vladimír Křen, kren@biomed.cas.cz

Jitka Viktorová, Martina Hurtová, Kateřina Valentová

Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, CZ142
00 Prague 4, Czech Republic

Silymarin is a complex of flavonoids and flavonolignans known for its broad spectrum of biological activities, including its ability to modulate drug resistance in cancer. We have tested 11, optically pure silymarin flavonolignans for their ability to reverse the multidrug resistance phenotype of *Staphylococcus aureus* and reduce its virulence (Fig. 1). Silybin A, 2,3-dehydrosilybin B, and 2,3-dehydrosilybin AB completely reversed antibiotic resistance at concentrations of 20 μM or less. Both 2,3-dehydrosilybin B and AB decreased the antibiotic-induced gene expression of representative efflux pumps belonging to the major facilitator (MFS), multidrug and toxic compound extrusion (MATE), and ATP-binding cassette (ABC) families. Most of the tested flavonolignans reduced cell-to-cell communication on a tetrahydrofuran-borate. Anhydrosilychristin was the only compound that reduced communication based on acyl-homoserine lactone (autoinducer 1) (IC_{50} 4.8 μM). Most flavonolignans inhibited *S. aureus* surface colonization, with 2,3-dehydrosilybin A being the most active (IC_{50} 10.6 μM). Particularly derivatives of 2,3-dehydrosilybin B, 2,3-dehydrosilybin AB, and silybin A are non-toxic modulators of *S. aureus* multidrug resistance and can decrease the virulence of the bacterium [1]. Laboratory of Biotransformation in Prague has a large library of natural and semisynthetic flavonolignans and other flavonoids (up to 100 compounds) and offer them to all COST participants on request.

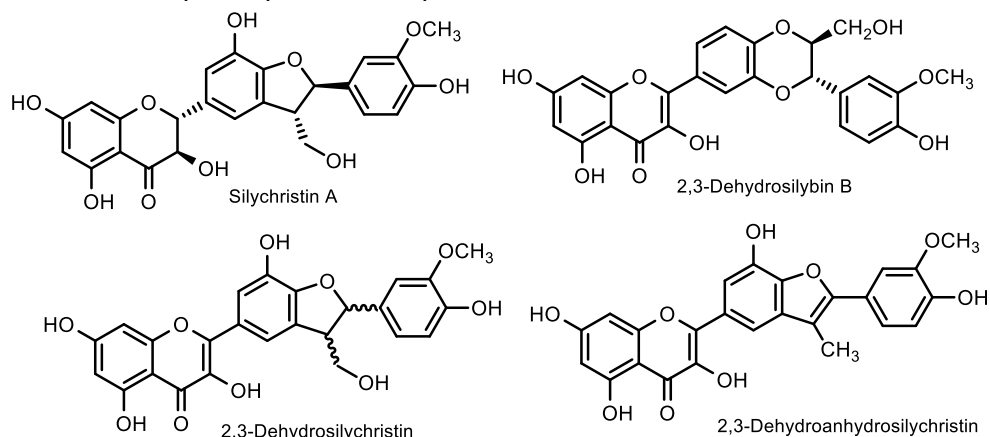


Figure 1. Natural and semisynthetic flavonolignans (selected) from silymarin.

Acknowledgment: COST Action CA21145 EURESTOP and grant 21-01799S from Czech Science Foundation are acknowledged.

[1] K. Holasová, B. Křížkovská, L. Hoang, S. Dobiasová, T. Macek, V. Křen, K. Valentová, T. Ruml, J. Viktorová: Flavonolignans from silymarin modulate antibiotic resistance and virulence in *Staphylococcus aureus*. *Biomedicine & Pharmacotherapy* 149, 112806 (2022).

***Pseudomonas aeruginosa* carbonic anhydrase inhibitors as innovative antibacterial agents to combat antibiotic resistance: ciprofloxacin derivatives**

Simone Carradori, simone.carradori@unich.it

Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Italy

The COVID-19 pandemic has exacerbated the anti-microbial resistance, increasing the inappropriate use of antibiotics. Therefore, there is an urgent need for new antimicrobials involving innovative mechanisms of action. Ciprofloxacin stands out as the main drug against the Gram-negative bacterium *Pseudomonas aeruginosa*, despite the frequent occurrence of resistance. Its mechanism of action is expressed with inhibition of DNA gyrase and blockage of bacterial DNA replication.

Within the BacCAD (Targeting bacterial Carbonic Anhydrases – towards a new generation of antibacterial drugs) project and using the structure of this quinolone as a lead compound, we have introduced chemical functionalities to interact with other targets as Carbonic Anhydrases (CAs), essential for the survival, growth, virulence and pathogenicity of bacteria. More than fifty compounds demonstrated the ability to selectively inhibit CAs of *P. aeruginosa* with respect to the human ones and the Minimum and Bactericidal Inhibitory Concentration against this pathogen were determined. The more active compounds also had the ability to eradicate the mature biofilm of this bacterium and demonstrated low toxicity against a human fibroblast cell line. These results highlight a promising new line of research focused on an innovative bacterial target for the design of new drugs to be used alone or in combination.

Soft Matter for Understanding and Treating Antibiotic-Resistant Bacterial Infections

A. Basak Kayitmazer (basak.kayitmazer@boun.edu.tr)
Department of Chemistry, Bogazici University, Türkiye

Ionic polysaccharides such as hyaluronic acid and alginate are present in the extracellular matrix of healthy tissues and bacterial biofilms, respectively. In our lab, we focus on understanding the interactions between these biopolyelectrolytes with macromolecular ligands such as proteins and surfactants. We also study model systems to understand membraneless organelles which have recently been suggested as an alternative mechanism for bacteria to manage the crowding effect in the cell. For this purpose, we rely on experimental soft matter techniques such as isothermal titration calorimetry, dynamic light scattering, small-angle neutron scattering, rheology, and zeta potential measurements.

Development and application of *in silico* approaches to predict pharmacological/toxic effects of bioactive compounds

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Petko Alov, Merilin Al Sharif, Antoniya Dyukendhieva-Todorova,
Iglika Lesigiarska, Tania Pencheva, Ilza Pajeva

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Bulgaria

The contemporary *in silico* approaches combine various ligand- and structure-based methods that aim at characterization of the relationship between the chemical structure of the compounds and their effect - therapeutic, toxic, etc. *In silico* approaches effectively help in understanding and elucidation of mechanisms by which compounds interact with target biomacromolecules, thereby explaining fundamental processes in the living organisms. This presentation provides an overview of the research that is being performed at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences to implement *in silico* modelling approaches in the field of drug design and computational toxicology. A number of case studies are discussed: (i) *in silico* analysis of selected polyphenols from the chemical class of hydroxyanthraquinones to interact with the bacterial enzymes DNA gyrase and DNA topoisomerase IV [1]; (ii) QSAR analysis of diaryl ethers and their analogues as potential antiviral agents [2]; (iii) *in silico* identification of multi-target ligands as promising hit compounds for neurodegenerative diseases [3].

References

1. Alov P et al. *Molecules*, 2022, 27, 3274.
2. Nikolova et al. *ChemistrySelect*, 2022, 7(34), e202203088.
3. Alov P et al. *Int.J.Mol.Sci.* , 2022, 23, 13650.

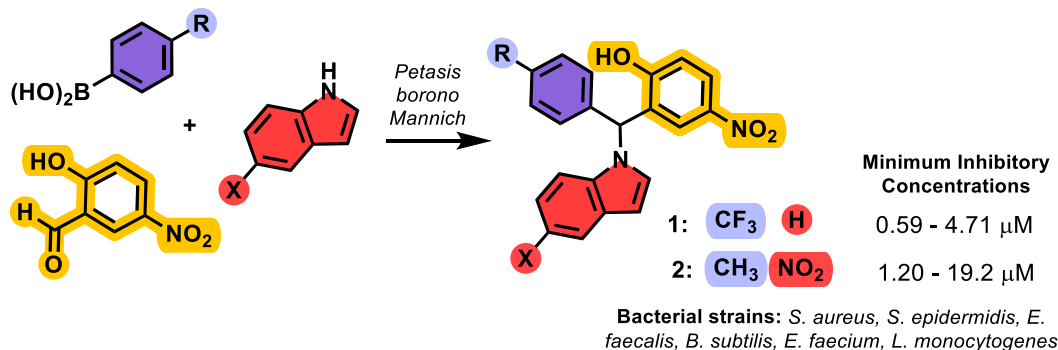
Phenolic Mannich bases as antibacterials agents for *Staphylococcus aureus*

Nuno R. Candeias, ncandeias@ua.pt

Alexandra Nunes, Patrícia Rijo

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro 3810-193, Portugal

Bone and implant-related infections caused by *Staphylococcus aureus*, including methicillin-resistant strains, remains one of the main concerns in modern orthopedic surgery. The rapid increase of *S. aureus* antimicrobial resistance together with their impact in healthcare, highlights the urgent need of alternative therapeutic compounds, eventually with new modes of action, lesser prone to yield resistance. Aminoalkylphenols, easily prepared through a Petasis borono-Mannich multicomponent reaction¹, have been evaluated as antibacterial agents resulting in the identification of 2-hydroxy-5-nitrophenyl and indolinyl as key moieties for conferring the intended antibacterial properties². Testing a library of aminoalkylphenols against a large panel of bacteria resulted in the identification of a particular lead with Minimum inhibitory concentrations (MICs) as low as 1.18 μM against a large panel of Gram-positive bacteria, especially multidrug-resistant *S. aureus* and *S. epidermidis* (MRSA and MRSE included), at concentrations that are not cytotoxic³. The same compound (**1**) displayed low toxicity against two *in vivo* models (*Artemia salina* brine shrimp and *Saccharomyces cerevisiae*). By using an *in vitro* selective approach, *S. aureus* isogenic clones propagated without and under **1** sub-lethal concentration showed a lack (or very low) potential to induce antimicrobial resistance after prolonged usage when compared to antibiotics conventionally used to treat these infections.



Acknowledgments

This work received support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020, UIDP/50006/2020, CEE-CINST/2018 and PTDC/QUI-QOR/1786/2021.

Funding

This work received financial support from PT national funds (FCT/MCTES) through the projects UIDB/50006/2020, UIDP/50006/2020 and PTDC/QUI-QOR/1786/2021, and Janne and Aatos Erkkö Foundation.

References

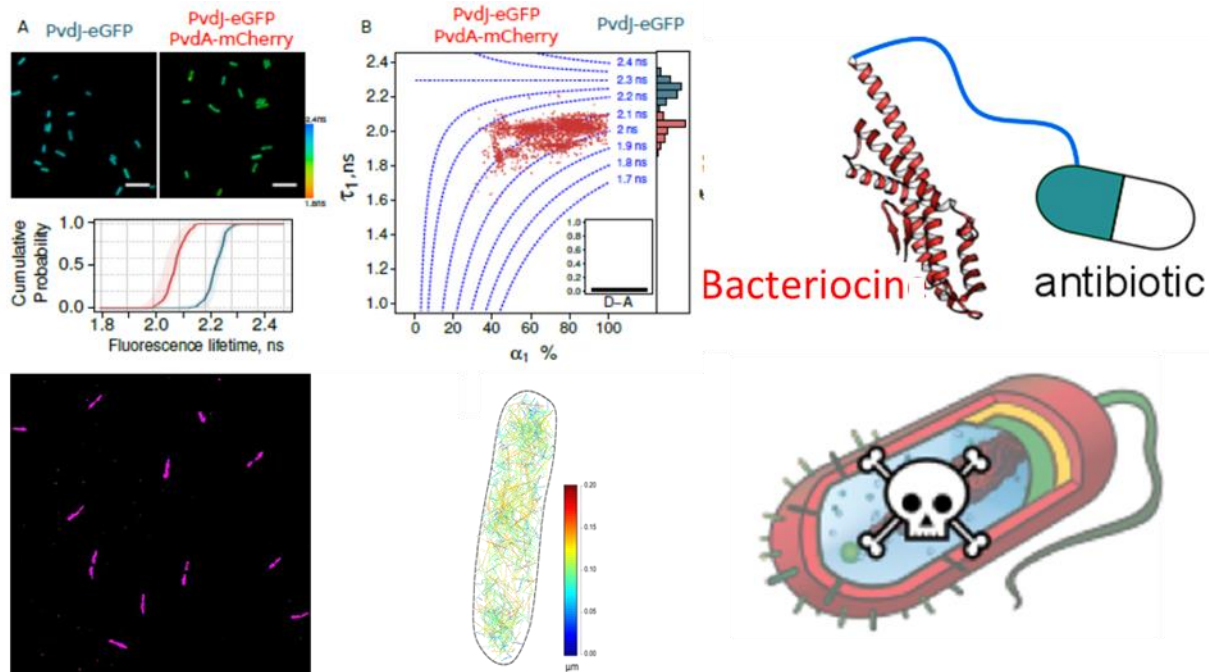
- a) N. R. Candeias, F. Montalbano, P. Cal, P. M. P. Gois, *Chem. Rev.* **2010**, *110*, 6169-6193; b) P. Wu, M. Givskov, T. E. Nielsen, *Chem. Rev.* **2019**, *119*, 11245-11290.
- a) I. Neto, J. Andrade, A. S. Fernandes, C. P. Reis, J. K. Salunke, A. Priimagi, N. R. Candeias, P. Rijo, *ChemMedChem* **2016**, *11*, 2015-2023.; b) T. Rimpilainen, J. Andrade, A. Nunes, E. Ntungwe, A. S. Fernandes, J. R. Vale, J. Rodrigues, J. P. Gomes, P. Rijo, N. R. Candeias, *ACS Omega* **2018**, *3*, 16191-16202.
- T. Rimpilainen, A. Nunes, R. Calado, A. S. Fernandes, J. Andrade, E. Ntungwe, G. Spengler, N. Szemerédi, J. Rodrigues, J. P. Gomes, P. Rijo, N. R. Candeias, *Eur. J. Med. Chem.* **2021**, *220*, 113459.

Developing new strategies to image, target and kill gram negative bacteria

Yves Mély^a, Shahriar Safari^a, Dmytro Dziuba, Béatrice Roche^a, Abdallah Basma^a, Mezouarhi Chaimae^a,
Fechter Pierre^b, Choulier Laurence^a, Patrice Rassam^a

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The activities of our lab in the bacterial field are focused on three main axes.

- 1) We develop advanced quantitative and super resolution microscopy techniques that are used to investigate fluorescently-labeled proteins, nucleic acids, lipids and drugs in bacteria. Being specialized in fluorescence techniques and methods, we have also developed fluorescence-based assays for screening antibacterial agents.
- 2) We design strategies combining bacteriocins and drugs, to efficiently and specifically target *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumoniae* bacteria, tackling some of the main resistance mechanisms. Our aim is to functionally validate their antimicrobial efficacy at the single-cell level both *in vitro* and *in vivo* and identify the molecular mechanisms and actors involved in their uptake. We also develop new prototypes to monitor and prevent biofilms, which strongly hinder bacteria eradication
- 3) Within a consortium of collaborative labs, our aim is to identify new anti-bacterial combinations of molecules from ancient medical manuscripts. We also develop innovative targeting strategies, based on aptamers. We have validated an aptamer that is able to detect *Pseudomonas aeruginosa* in complex mixtures of bacteria. Our final objective is to design multi-functional constructs that target infectious sites.

ADDRESSING ANTIMICROBIAL RESISTANCE IN KOSOVA

Lul Raka^{1,2,3,4}, lul.raka@uni-pr.edu

¹National Institute of Public Health of Kosova, Prishtina, Kosova

²Faculty of Medicine, University of Prishtina “Hasan Prishtina”, Prishtina, Kosova

³Ministry of Health, Chair, National Program for AMR & HAI

⁴President, Kosova Society for Microbiology

Introduction: Resistance rates of all microorganisms in Kosova are 2-5 fold higher than average in EU. Over the counter sale of antibiotics and misuse of antibiotics in primary care are the main challenges.

Methods: This review addresses challenges and current government initiatives to combat AMR in Kosovo.

Results: Wholesales data on antibacterial use in Kosovo was 26.3 DID in 2011 and decreased to 20.1 DID in 2018. In all country hospitals, 56.8% of inpatients used at least one antibiotic, with ceftriaxone as the most prescribed. Ceftriaxone use is the highest in Europe. Covid-19 significantly accelerated antibiotic misuse.

The Ministry of Health in Kosova completed two national action plans for AMR with investment of 1.2 millions in laboratory infrastructure. Budget alternatives through open call grants were a significant response to budget constraints. The WHO approach AWaRe (Access Watch and Reserve) was introduced within the New Essential Medicine List. The Faculty of Medicine has included the AMR as a new elective module at the undergraduate level, whereas translated educational package “E-bug” was launched for school children. Kosova participated in latest EURECA grant.

Conclusions: Key pillars of response to combat AMR will be “One Health” approach and antimicrobial stewardship.

Antimicrobial screening of *Plectranthus* extracts for applications against antibiotic resistant bacterial infections

Márcia Santos Filipe^{1,2} Vera M. S. Isca^{1,3}, Ana María Díaz-Lanza², Patrícia Rijo^{1,3}

1 CBIOS – Universidade Lusófona’s Research Center for Biosciences & Health Technologies, Lisbon, Portugal

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Antimicrobial resistance is a major cause of death worldwide. The number of microbial infections are increasing and becoming more difficult to treat. [1] The search for new antimicrobial compounds active against multidrug-resistant bacteria has great importance. Historically, natural products, have been an important source of new entities to overcome antimicrobial resistance. *Plectranthus* genus (Lamiaceae) is widely used in traditional medicine against a vast range of diseases. Furthermore, previous studies reported the occurrence of promising antimicrobial compounds in *Plectranthus* spp. such as abietane-type diterpenes. [2]

In this study, we investigate the antimicrobial activity of eight *Plectranthus* spp. (*P. ambigerus*, *P. barbatus*, *P. cylindraceus*, *P. ecklonii*, *P. fruticosus*, *P. grandidentatus*, *P. hadiensis*, *P. madagascariensis*). All species were subjected to ultrasound-assisted extractions using methanol as solvents (10%, w/v). The antimicrobial activity was assessed through the well diffusion method against a collection of Gram-negative, Gram-positive bacteria and yeast strains. Furthermore, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were also assessed.

All tested extracts displayed antimicrobial activity against the Gram-positive bacteria strains. The most promising results were obtained for the *P. grandidentatus*, followed by *P. ambigerus* and *P. hadiensis* extracts, against *Staphylococcus aureus*. Additionally, *P. barbatus*, *P. ambigerus* and *P. fruticosus* extracts showed significant potential against *Staphylococcus epidermidis*. Based on the reported results, additional studies are currently ongoing to evaluate the cytotoxic effect of the extracts for future applications against antibiotic resistant bacterial infections.

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Laboratory evolution as a key tool in antimicrobial development

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The study of the bacterial processes leading to evolution of antimicrobial resistance is a key tool to the discovery and understanding of its mechanisms. These can eventually contribute to the identification of new drug targets or to the chemical optimization of existing antimicrobials. An early identification of possible mechanisms of resistance of lead compounds can help to chemical improvement at an early stage in the development pipeline, saving time and money, and increasing efficiency.

In our lab we aim to identify and understand resistance mechanisms of old and new antibiotics against a panel of human pathogens with special attention given to the ESCAPE pathogens on the WHO high priority list for antimicrobial development. We use a wide variety of methodologies including automated laboratory evolution, frequency of resistance assay, genetic engineering, etc, in a high-throughput and systematic manner. We are also interested in translating the significance of our work to *in vivo* conditions.

Additionally, we also aim to set up an antibiotic-discovery pipeline where antibiotic producer bacteria will be forced to evolve in the presence of antibiotic-resistant human pathogens. Such experimental setup is expected to trigger the biosynthesis of new molecules that are active against the antibiotic-resistant bacteria. Such molecules will be identified, purified, and tested against a wide variety of human bacterial pathogens.

The work of the presenting author is supported by the grant: NKFI FK 137808

Development of Natural and Synthetic Anthocyanin Incorporated Phenotypic Tests For Colorimetric, Fast and Sensitive Detection of Antibiotic Resistance Bacteria in Clinic

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In this research, we developed anthocyanins, from red cabbage (*Brassica oleracea*) as a natural pH indicator, incorporated colorimetric tests for rapid and sensitive detection of model bacteria including antibiotic resistance and urease positive ones. By designing content of the tests, MRSA, MRSE, *K. Pneumoniae*, Carbapenem and Colistin resistant bacteria and *H. pylori*, which causes chronic gastritis and gastric cancer can be rapidly and specifically detected. In the diagnostic process, we have reduced the bacterial culture time from 24-48 hours to 1.5-2 hours with antibiotic susceptibility tests. The potential detection mechanism of these colorimetric tests relies on protonation or deprotonation of anthocyanins molecules by acidic or basic organic volatile compounds produced during growth of the bacteria. The pH dependent colorimetric response of the tests were detected by the naked eyes. For quantitatively supporting results detected by visually, we performed the Delta-E and G/B values with ImageJ software. We propose that that this anthocyanins-incorporated colorimetric test can be implemented in clinics for the detection of pathogens

Natural product antibacterial agents and rediscovery of old antibiotics

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Antibiotics changed the course of therapeutics and have saved countless lives worldwide since their first clinical introduction. However, shortly after their introduction, resistance began to emerge rapidly, and now multidrug-resistant bacteria and especially *Staphylococcus aureus* resistant to methicillin and gentamicin (MRSA), a pathogen responsible for 60-89% of hospital infections, have become a major concern for these infections' treatment.

Therefore, it has become evident that novel anti-infective agents with better efficiency and/or alternative modes of action are urgently required to battle the ever-evolving multidrug-resistant bacteria. Many research groups worldwide are now devoted to solving this crisis by either looking for new natural compounds since the chemo-diversity in nature offers a valuable source of new drugs, new drug leads, and new chemical entities (NCEs) or "rediscovering" known old antibiotics by chemical modifications.

In our group, during the last few years, we have been developing new antibacterial agents following both above strategies. In this short presentation, we will focus on some examples of natural and semisynthetic abietane diterpenoids with activity against MRSA and MSSA(1-3), as well as some examples of Chloramphenicol derivatives (4, 5).

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4-(1,2,2-triphenylvinyl)aniline as self-assembling inducer

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In recent years, nanomedicine became increasingly more important as a tool to improve the bioavailability of drugs used for the treatment of various diseases, as can help overcoming a range of different issues, including low bioavailability due to poor absorption and degradation by protective mechanisms after reaching the target site. Our research group is particularly interested in a research niche in nanotechnology that is represented by self-assembling drug conjugates able to spontaneously form nanoparticles (NPs) in aqueous media. These conjugates present the same general structure, where the drug is covalently linked to lipid moieties, able to induce the structural organization of building blocks as a consequence of specific local interactions. This kind of NPs are easy to obtain, and can reach high local drug concentrations in tissues weakening the systemic toxicity of drugs. The fundamental moiety for the synthesis of this kind of NPs is the self-assembly inducer, that could either be squalene, 4-(1,2-diphenylbut-1-en-1-yl)aniline or 20-hydroxyecdysone. The choice of the self-assembly inducer is important for the formation of nanoparticles, and here is where we set our interest. Thus, we envisage to use the 4-(1,2,2-triphenylvinyl)aniline scaffold (**1**, Figure 1) as the self-assemble inducer, in which the ethyl chain is replaced with a phenyl ring providing a self-assembled inducer with potentially more π -stacking interactions among the aromatic rings of the adjacent molecules and subsequently better formation and stabilization of the nanoparticles.

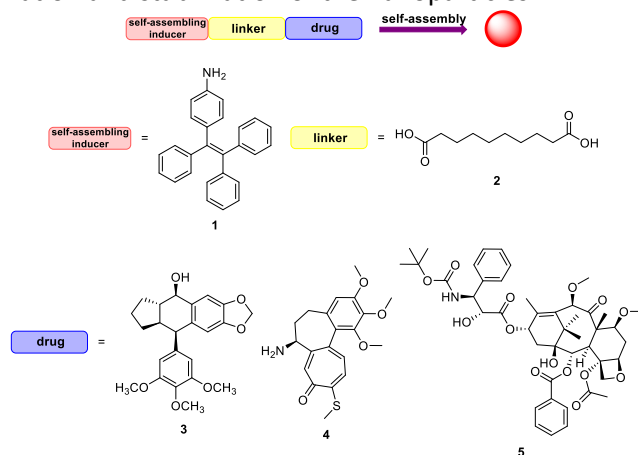


Figure 1. Building blocks of the conjugates.

Estimating the burden of bacterial antimicrobial resistance in Europe and beyond: a systematic data-driven approach for research and implementation priority setting

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Abstract text

The overarching goal of the Global Research on AntiMicrobial resistance (GRAM) Project – which represents a strategic partnership between the Institute for Health Metrics and Evaluation (IHME) / University of Washington (United States) and the University of Oxford (United Kingdom) – is to produce the most comprehensive set of estimates for establishing the true burden of antimicrobial resistance (AMR). A complex and multifaceted modelling approach and two salient counterfactual scenarios are utilized for that purpose, drawing upon the largest number of data sources to date from a myriad of international stakeholders. Globally, in 2019 there were 1.27 million (95% UI 0.911–1.71) deaths attributable to and 4.95 million (3.62–6.57) deaths associated with AMR, with the highest all-age death rate seen in western sub-Saharan Africa. In the WHO European Region, we have estimated 133 000 (90 100–188 000) deaths attributable to and 541 000 (370 000–763 000) deaths associated with bacterial AMR, with the highest age-standardized mortality rates observed in Tajikistan, Uzbekistan and Azerbaijan. These striking numbers show that AMR is a true public/global health hazard; moreover, specific differences among countries demonstrate how tailored, data-driven approach is essential for policy decisions regarding screening efforts, antimicrobial stewardship initiatives and antibiotic development.

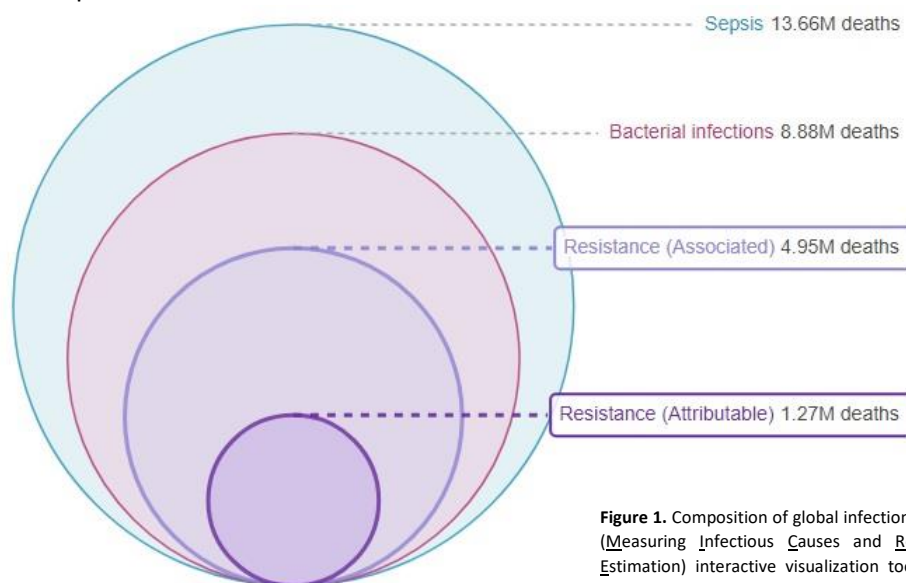


Figure 1. Composition of global infection-related deaths. Source: MICROBE (Measuring Infectious Causes and Resistance Outcomes for Burden Estimation) interactive visualization tool for exploring estimates of the burden of infections classified by the involved organ system (infectious syndrome), causative microorganism (pathogen), and resistance to treatment (antimicrobial resistance). Link: <https://vizhub.healthdata.org/microbe/>

Culture Conditions Matter

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The poor correlation between *in vitro* and *in vivo* antibiotic efficacy can often be attributed to the fact that culture conditions matter.

For example, bacterial biofilms confer 10 to 1,000 times more resistance to antibiotics compared with planktonic growth. This often leads to complications and treatment failure [1]. Hepatocyte-like cells grown as 3D cultures express 10- to 100-fold more drug-metabolising cytochrome P450 enzymes compared to the same cells cultivated in 2D culture [2].

HUVEC cells are sensitive to oncolytic viruses (OV) in 2D but when co-cultivated in 3D conditions become resistant to OV. [3]

The biofilms present in cystic fibrosis patients are often polymicrobial, commonly containing *Mycobacterium abscessus* and *Pseudomonas aeruginosa*. Clarithromycin is effective against *P. aeruginosa*, but only when grown on plastic. Both *M. abscessus* and *P. aeruginosa* can be induced by several antibiotics to form biofilms on plastic. However, on 3D cultures, antibiotic treatment can inhibit *P. aeruginosa* but not *M. abscessus* during biofilm formation. *In vivo* this can result in a competitive advantage for *M. abscessus*. Interestingly, the response of *M. abscessus* to antibiotics is influenced by the presence of *P. aeruginosa*, but not vice versa. These results illustrate that knowledge of both host cell-bacterial and inter-bacterial species interactions are important when selecting antimicrobials [4].

The conclusion to be drawn is that it is essential to use a relevant model system to determine whether a compound has antibiotic potential. I will present an easily decontaminated system that can be used for the effective, long-term, reproducible 3D culture of *in vivo* mimetic cells which is well suited for this task.

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Photodynamic therapy - Are light-triggered oxidative damages the magic solution to AMR?

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Photodynamic therapy is based on the generation of singlet oxygen (SO) and other reactive oxygen species (ROS) by a triplet photosensitizer that has been excited by light. These SO and ROS can then induce cell death due to their strong and immediate oxidative damages. A main application is anti-cancer PDT.

In the case of bacteria and other microbes, and because of the large scope of action, it is considered as very unlikely that resistance can be developed [1].

The general advantages of antimicrobial photodynamic therapy (aPDT), also called Photoantimicrobial chemotherapy (PACT) or Photodynamic Inactivation (PDI) [2] will be presented, followed by design considerations of photosensitizers. Finally, our works with phthalocyanines and their potential as aPDT photosensitizers will be presented [3].

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Innovative antimicrobial strategy for the treatment of MDR bacterial infection

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The development of new approaches that adjuvant the scarcity of clinically relevant antibiotics for treatment of infections by multidrug-resistant Gram negative bacteria (MDR-GNB) is an urgent need. Previously, we showed that tamoxifen, an anti-cancer drug, and metabolized to three major metabolites that exhibit high antibacterial activity against *Acinetobacter baumannii in vitro* [1]. However, the molecular target(s) of the tamoxifen metabolites (MET), remain to be determined.

Using a transposon library of *A. baumannii*, we select two mutants resistant to MET. To further determine whether MET affects their membrane structure, permeability and OMPs profile, we perform analysis by transmission electron microscopy, fluorescence assays, SDS-PAGE and qRT-PCR, respectively. We show that both mutant strains treated with MET presented lower membrane permeabilization and suffered morphological changes and reduction in OmpW expression. Molecular docking of MET against OmpW was performed. We find that MET binding to OmpW presented higher score. This data has been confirmed by the MET susceptibility profile of *A. baumannii* Δ OmpW which has presented MIC value 8-folds higher than the wt strain. Additionally, we perform checkerboard and time-kill curve analyses to determine whether MET can synergize with clinically used antibiotic such as colistin. We find that MET is more synergistic with colistin against colistin-resistant *A. baumannii* during.

Overall, our data showed that ompW might be involved in the mechanism of action of MET against *A. baumannii*, and advanced our knowledge on the antimicrobial activity of MET as new therapeutic indication.

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Novel ligands for the flavin mononucleotide riboswitch

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Riboswitches, which are mostly found in bacteria are considered as one promising class of targets for future antibiotics with a novel mechanism of action.^{1,2} Riboswitches are *cis*-acting gene regulatory elements which are located to the 5' untranslated region of mRNAs. They consist of an aptamer domain that binds to small ligands (typically an important metabolite) and an expression platform that controls the expression of the downstream gene(s) which are involved in the synthesis or uptake of the metabolite.

The flavin mononucleotide (FMN) riboswitch is found in 41 human pathogens and is the 3rd most widespread riboswitch known,² however, only a limited number of FMN riboswitch ligand classes are known. These are ribocil A-C (discovered by Merck), synthetic FMN analogues and the natural product roseoflavin.³ Based on results from virtual screening combined with information on the binding modes of FMN and ribocil, we have designed novel compound series and developed synthetic routes to these. These efforts have resulted in the identification of several compound series that show binding affinities toward the FMN riboswitch in the low micromolar range. The design and synthesis of these compound series will be discussed.

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How can the Structure-based Molecular Modeling group from FCUL boost the EUROSTOP

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Computational drug discovery is an effective strategy for accelerating and economizing drug discovery and development process. Because of the dramatic increase in the availability of biological macromolecule and small molecule information, the applicability of computational drug discovery has been extended and broadly applied to nearly every stage in the drug discovery and development workflow, including target identification and validation, lead discovery, and optimization and even preclinical tests. Since 2019, the Structure-Based Molecular Modeling laboratory @ FCUL [1] has been dedicated to the development and application of multiple computational methods to drug discovery and development projects focused on human and animal health. The successful application of methods based on Structure modelling, Molecular Docking, and Molecular Dynamics methodologies and focused on studying protein-protein, protein-ligand [2], protein-DNA [3], ligand-DNA [4], and ligand-membranes [5] interactions allowed us to contribute to the identification and optimization of several promising therapeutical approaches to diagnose and treat several animal and human diseases. In this communication we will present some of our most recent works, and also some of our current on-going scientific challenges. We will also highlight the methodological approaches we believe can be of utmost importance in fulfilling EUROSTOP (COST CA21145) goals.

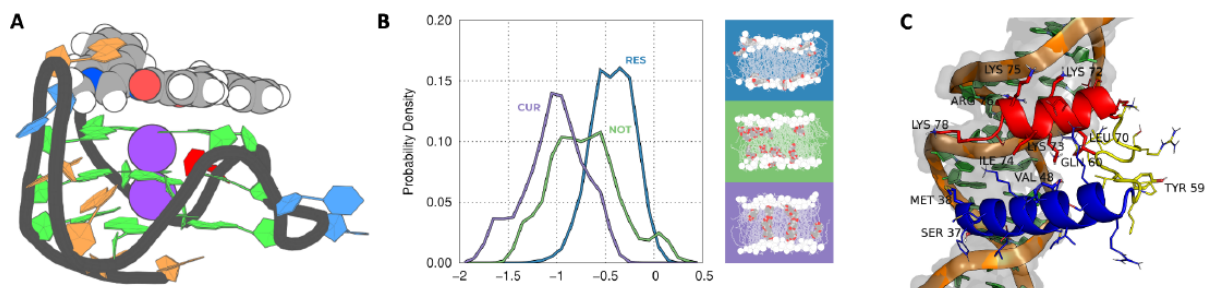


Figure 1 - Examples of past and current projects running on SBMM laboratory. A) Identification of active compounds to stabilize G-quadruplexes; B) Development of a computational protocol to identify membrane PAINS; C) Characterization of the interaction between DNA and P10 Protein used in the development of a diagnostic tool or vaccine to fight Swine Fever infections on pigs.

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Antimicrobial chitosan-nanocomposites for biomedical applications

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Health-care-associated infections and the development of antimicrobial resistance are serious public health problems worldwide. Bacterial and fungal infections are often difficult to treat because microbes can adapt rapidly to conventional antibiotics. Nowadays, nanotechnology holds promise for the development of novel antimicrobials for the treatment of infections. Nanoparticles (e.g. Ag, CuO and ZnO NPs) have multiple attack mechanisms against microbial cells, and therefore microbes rarely develop antimicrobial resistance.

The aim of the [Estonian Research Council project PRG749](#) “Antimicrobial chitosan-nanocomposites for biomedical application: efficiency and safety” (2020-2024) is to develop novel chitosan-silver and chitosan-copper nanocomposites with synergic beneficial properties - chitosan would provide an immune-stimulating and silver or copper nanoparticles antimicrobial effect.

We synthesize a library of nanocomposites and test their efficacy against the medically important pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and yeast *Candida albicans* and safety to human cells *in vitro* and a pro-inflammatory response (release of cytokines). As a result of the project, the physicochemical properties of the nanocomposites yielding the highest antimicrobial and immunomodulatory efficiency without harmful effects on human cells will be identified.

We showed that the studied Ag-chitosan-nanocomposites are effective antimicrobials and the potency of nanocomposites depends on the chitosan content.

Spirooxindoles for targeting cancer and malaria. Perspectives of antibacterial activity

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In this communication an overview of the results obtained in the Medicinal Organic Chemistry research group of the Research Institute for Medicines will be presented, focusing on the development of spirooxadiazoline oxindoles with dual-stage antimalarial activity [1], and on the discovery of dual inhibitors of the MDM2/4-p53 protein-protein interactions [2]. Finally, an overview of the compounds available in-house will be shown, including perspectives of antibacterial activity for these chemical scaffolds.

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Acknowledgments: Past and present members of the Santos Laboratory for their contributions to the research presented in this communication. This research was funded by National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) through grant numbers UIDB/04138/2020 (iMed.Ulisboa), and projects PTDC/QUI-QOR/29664/2017 and PTDC/QUI-QOR/1304/2020.

Diagnosis and treatment of bacterial infections

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Bacterial infections remain one of the most concerning health problems worldwide, causing millions of deaths and hospitalizations each year. In recent years, much effort has been invested in the development of new methods guaranteeing an early and specific diagnosis to treat infections promptly and selectively. This is of particular importance given the ease with which bacteria can spread and their capability to develop resistance against antibiotics. Different detection techniques have been developed to reduce the detection time, and avoid cultivation and sample enrichment, which are particularly time-consuming and could take between 24–48 hours.

In this presentation, I will present an overview of several projects aiming to design advanced diagnostic tools and smart delivery systems to efficiently detect and treat bacterial infections. I will focus on nanotechnology fluorescent-based approaches to sensitively and selectively detect bacteria, showing the results of a cost-efficient "off-on" fluorescent probe^{1,2} and I will introduce the progress achieved with alternative probes. Moreover, I will show the efficiency of our pH-responsive wearable drug delivery system in treating infected wounds³. Finally, I will present the rationale of the recently funded projects with which we aim to combine the diagnosis and treatment of infected wounds in a wearable self-care system.

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Antimicrobial and Antibiofilm Activities of Carvacrol and Thymol: Effective Carbonic Anhydrase Inhibitors versus *Helicobacter pylori*

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Helicobacter pylori is associated with the development of chronic gastritis, peptic ulcers, and gastric cancer in humans. It has been demonstrated that *H. pylori* eradication reduces the incidence of gastric cancer of about the 50% of patients, unfortunately, the current therapeutic plans suffer from an emerging bacterial resistance rate and poor patient compliance, for this reason, the World Health Organization listed *H. pylori*, clarithromycin-resistant, among the "priority pathogens" against which it is necessary to promote research and development of new antimicrobials. The study of the effects of compounds targeting key bacterial enzymes involved in essential pathways, such as Carbonic Anhydrases (CAs), versus clinically relevant pathogens or opportunistic pathogens could represent an alternative strategy of therapeutic treatments. Based on these concerns, it has been studied the anti-*H. pylori* activity of thymol and carvacrol in terms of biofilm production and release of outer membrane vesicles-eDNA associated. The microbiological data were correlated by the in vitro evaluation of *H. pylori* CA inhibition, isoform selectivity, and the assessment of their limited toxicity against three probiotic strains with respect to amoxicillin. Subsequently, we focused the attention on carvacrol and we evaluated the antibiofilm activity of (i) carvacrol (CAR), (ii) amoxicillin (AMX) and (iii) an urease inhibitor, such as salicylhydroxamic acid (SHA), alone and in combination each other, versus *H. pylori*. The data obtained showed a greater antimicrobial and antibiofilm efficacy of the combinations of CAR-AMX, SHA-AMX and CAR-SHA versus *H. pylori* in vitro compared to the same compounds used alone. The effective association of SHA-AMX and CAR-AMX might lead to decrease the concentration of amoxicillin necessary to eradicate the pathogen improving the patient compliance. Moreover, the association of CAR and SHA decreased the concentrations, necessary for *H. pylori* biofilm eradication, of the two compounds individually tested. The results obtained demonstrated that carvacrol and thymol could be considered as new lead compounds as alternative *H. pylori* CA inhibitors or to be used in association with currently used antimicrobials, in particular, we demonstrated that CAR, AMX and SHA in combination each other were capable of eradicating *H. pylori* mature biofilm, representing a potential innovative strategy that could contribute to reducing the spread of the antibiotic resistance phenomenon.

Chemical design of halimane diterpene for enhanced antimicrobial activity

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Plants of the *Plectranthus* genus are often cited for their medicinal properties. *Plectranthus ornatus* Codd. is traditionally used in central Africa for the treatment of gastric and liver diseases and their leaves are also used for their medicinal uses. The main constituent of *P. ornatus* is the halimane compound, 11*R**-acetoxyhalima-5,13*E*-dien-15-oic acid (**1**), described for a variety of biological properties; more specifically, this natural diterpene showed moderate anti-inflammatory activity and cytotoxicity against four cancer cell lines, MCF-7, FaDu, CCRF-CEM and lung A549 with IC₅₀ = 13.61, 15.12, 16.52 and 19.38 µg/mL, respectively [1,2]. Despite the natural diterpene **1** having reported moderate antimicrobial activity (MIC values between 15.63 and 62.50 µg/mL in nine out of ten strains of *Staphylococcus* and *Enterococcus*), semi-synthetic derivatives showed improved antibacterial activity across different strains [3]. Interestingly, in a previous study from our group, a derivate of **1** showed almost identical bacterial growth inhibition when compared to an antitubercular drug in clinical use [4]. In this study, we synthesised four derivatives, using different amines (morpholine, phenylalanine hydrochloride, piperidine and cyclohexylamine). The antimicrobial activity of the amine derivatives was not significant against *S. aureus* and *E. faecalis*, however current studies are on-going to assess the activity of these analogues in different antibiotic-resistant bacterial strains and their cytotoxicity.

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Acknowledgements

This work was financially supported in part by FCT – Fundação para a Ciência e Tecnologia grant UI/BD/151422/2021 and Fundação Calouste Gulbenkian grant N.º. 275123

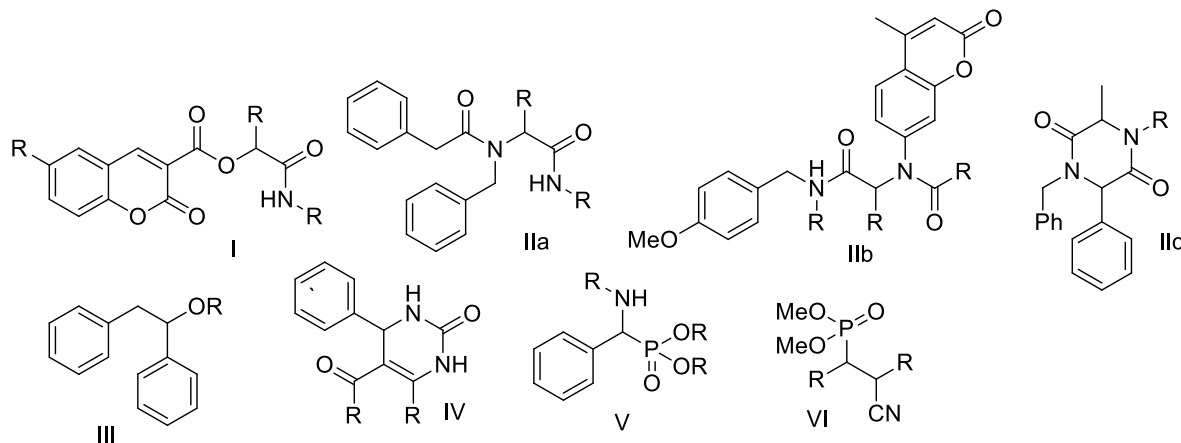
Application of multicomponent reactions for the synthesis of a new potential antimicrobial drugs

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Multicomponent reactions (MCR) are excellent tools for the synthesis of peptidomimetics which can be used for the synthesis of antimicrobial compounds. During our studies we have developed several synthetic methods based on MCR reactions for the synthesis of peptidomimetics of high biological activity.

Biological activity of synthesized compounds was important from the aspect of the increasingly common drug resistance of bacteria by lipopolysaccharide (LPS). For experiments *Escherichia coli* model strains K12 (without LPS in its structure) and R1–R4 (with different length LPS in its structure) were used and minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined for each synthesized compounds. The results of our studies on synthesis and antimicrobial properties of compounds using Passerini reaction of structure I [1], Ugi reaction of structure IIa, IIb, IIc [2], Pd acetate coupling of structure III [3], Biginelli reaction of structure IV [4], Kabachnik–Fields reaction catalysed by enzymes of structure V [5], Knoevenagel–Phospha–Michael reaction of structure VI [6] will be presented and discussed on selected examples.



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Acknowledgments: This work was supported by the National Science Center, Poland project OPUS 2019/33/B/ST4/01118.

Natural products with γ -butyrolactone moiety as potential antibiotics

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Modern medicine has improved and prolonged our lives. Despite all success, humanity is faced with the big challenge of antibiotic resistance, which threatens fundamental tools of modern medicine. COVID 19 pandemic showed us how fragile our medicinal systems are, and will have a complex long-term impact on antibiotic resistance. Basically, we are in an evolutionary arms race against microorganisms and viruses. Evolution might be the key to solving this problem, i.e. intrinsic bioactivity of natural products (NPs), could be an inspiration and a starting point in the quest for new and better antibiotic agents. One of the privileged structures of biologically active NPs is γ -butyrolactone. Our research in the medicinal chemistry of the NPs and their analogues with this structural motif will be presented. We believe that these compounds bear great potential as antibiotics.

Efficient *in silico* approach for selection therapeutics against DR bacteria and identification of new therapeutic targets

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New treatments are urgently needed for drug-resistant bacterial (DR) infections. *In silico* approaches have become integral to drug discovery, crucial in improving and accelerating this expensive and time-consuming process. Using molecular descriptors, electron-ion interaction potential (EIIP), and average quasi-valence number (AQVN), which describe long-range interactions between therapeutic molecules and their targets, we proposed a virtual screening criterion for choosing antibacterial agents and their combination for the treatment of multidrug-resistant bacterial infections. This criterion can be applied to the virtual screening of small molecular libraries (approved drugs, commercial, natural compounds, nutraceuticals) for candidate therapeutic molecules and their combinations. The AQVN and EIIP values of amino acids and nucleotides are input components in the Informational spectrum method (ISM), a virtual spectroscopy method employed to examine biological macromolecules. The ISM has been successfully applied for the structure-function analysis of different proteins, predicting new protein interactors, and identifying protein domains responsible for long-range interactions. ISM was applied earlier in investigating dangerous gram-positive bacteria for potential new targets useful for countermeasures against *B. anthracis*. New PA targets and interaction domains between PA and EMILINs were proposed and confirmed experimentally using recombinant proteins.

A comparative study of antibacterial and antifungal activities of some essential oils

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The evolution of antibiotic-resistant bacteria has been developed very rapidly lately over the world. In this point, there is an urgent need to develop alternative strategies to address this issue. Herbal drugs are a very promising group since they are used since ancient times for their antimicrobial, antifungal and anti-inflammatory properties. The essential oils obtained from medicinal and aromatic plants of Albanian origin, such as *Satureja montana*, *Thymus vulgaris*, *Origanum vulgare*, *Myrtus communis*, *Rosmarinus officinalis*, *Thymus capitatus* and *Salvia officinalis* are studied for antibacterial and antifungal properties in this study. On the other hand, current topical applications of these volatile compounds turn out to be complicated because of their problems of stability, evaporation and controlled release, which are major problems for their therapeutic uses; therefore, is studied their microencapsulation in polymers such as β -cyclodextrin which could be the solution to their chemical and physical problems. The essential oils were tested for antimicrobial and antifungal activity before and after microencapsulation. Positive controls of antibiotics and antifungals were used for comparison. Essential oils showed high inhibition zones against selected bacteria, and encapsulated ones were found to be more efficient. In conclusion these essential oils can be applied in many formulations due to their low risk of skin sensitizing and high antibacterial and antifungal activity they demonstrated after encapsulation.

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Discovery of new inhibitors of DNA gyrase and topoisomerase IV to overcome antimicrobial resistance

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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 an exceptional opportunity for dual targeting.

Based on the recently determined crystal structure of our *N*-phenyl-4,5-dibromopyrrolamide inhibitor-DNA gyrase B complex¹, we prepared a series of improved *N*-phenylpyrrolamides and tested them against DNA gyrase and topoisomerase IV (Figure 1). The IC₅₀ values for the most potent 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. One of the inhibitors had an IC₅₀ value of 2.8 nM against *E. coli* DNA gyrase and showed MIC values of 1-2 µg/mL against selected Gram-positive and 0.064-32 µg/mL against selected Gram-negative bacteria. In addition, some of our recently discovered *N*-phenylpyrrolamide inhibitors show promising on-target activity against *M. tuberculosis* DNA gyrase (IC₅₀; 27 nM) and promising MIC values against mycobacteria.²⁻⁶

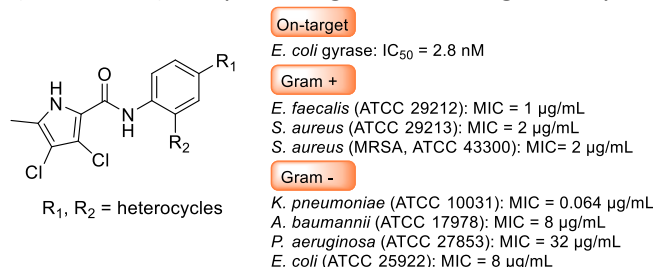


Figure 1. General structure of our *N*-phenylpyrrolamide DNA gyrase and topoisomerase IV inhibitors, and biological activity of the representative compound.

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Studying molecular recognition phenomena using computational tools: the case of halogenated drugs

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The usage of computational techniques is paramount not only for the drug discovery process but also to shed some light and rationalize the activity of such molecules based on their interaction with biomolecular targets. In this context, molecular docking and molecular dynamics simulations allow observing these interactions at the molecular level. The later computational technique is also powerful at estimating drug-likeness properties such as membrane permeability. In the past few years, the Computational Chemistry & Molecular Interactions Lab @ BioISI [1] has been investigating, using a wide range of computational techniques (QM calculations, docking, MD simulations), the molecular recognition phenomena mediated by unusual noncovalent interactions involving halogen atoms. In this communication, we will present our current research results on the theme. Specifically, we will show how we have been improving parameters for the description of halogenated drugs using force field methods [2-4], which could prove very useful in the context of virtual screening. We will also show our results on a novel and relevant interaction between halogenated drugs and phospholipid membranes (Fig. 1), previously overlooked, which eventually determines the pharmacological or toxicological activity of those molecules, therefore, with potential implications in drug discovery and development (e.g. improvement of molecular descriptors for QSAR models aiming at predicting permeability or toxicity) [5]. Finally, we will highlight how such modelling techniques could be useful in the context of screening and optimizing compounds able to interfere with the growth and replication of DR bacteria under the scope of EURESTOP CA2115 Action.

Acknowledgments: Fundação para a Ciência e a Tecnologia (FCT), Portugal, is acknowledged for grants UIDB/04046/2020 and UIDP/04046/2020 (toBioISI) and Individual Call to Scientific Employment Stimulus grant 2021.00381.CEECIND (P. J. Costa).

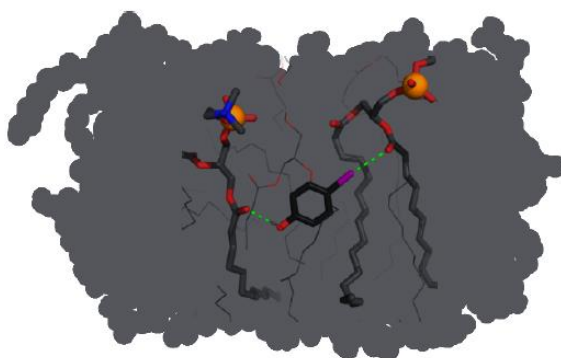


Fig. 1 – Schematic representation of a halogen bond and a hydrogen bond between a halobenzene derivative and two phospholipid molecules [5].

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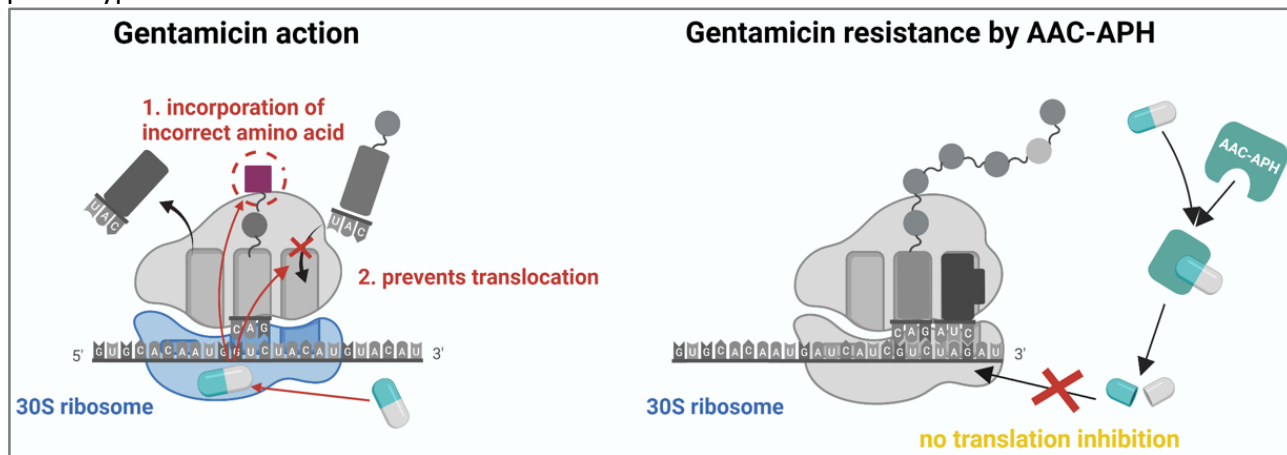
Antibiotic resistance platform for high-throughput screening of adjuvants for combination therapies

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We are currently facing the post-antibiotic era, when most antibiotics will no longer be able to effectively treat bacterial infections. The introduction of new antibiotics into clinical practice does not keep up with the development of resistance, and therefore adjuvant (combined) therapy appears to be a suitable alternative. It is based on the simultaneous administration of an antibiotic and an inhibitor of the resistance mechanism that the bacteria use to eliminate the antibiotic. This therapy was first approved in 1981, when Augmentin was launched on the market, a preparation combining clavulanic acid (β -lactamase inhibitor) and amoxicillin (β -lactam antibiotic).

The aim of this work is the identification of resistance mechanisms in clinical isolates of *Staphylococcus aureus* and their targeted inhibition using adjuvants. For this purpose, a library of genetically modified strains is being prepared, which contains strains carrying a single resistance determinant, usually the gene for destructase, an enzyme that modifies the structure of the antibiotic. Strains prepared in this way have increased resistance to the action of the antibiotic given. Subsequently, these strains are used for high-throughput screening of the library of substances and are searched for those capable of reverting the antibiotic-resistant phenotype back to the sensitive one.



Development of immunotherapeutic complexes eliciting complement activation towards multidrug-resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic bacterium causing a variety of life-threatening infections in immunocompromised patients. As a new approach to combat bacterial infections, we developed complement activating multimeric immunotherapeutic complexes (CoMiX) using the C4bp multimerising scaffold to activate complement on targeted bacteria. We combined on the C4bp scaffold a single-chain fragment variable (scFv) recognising the exopolysaccharide (Psl) matrix of *P. aeruginosa* to either an Fc-dimer inducing the complement classical pathway (CoMiX-Fc) or Factor H-related protein 1 (CoMiX-FHR1) competing with FH binding and eliciting the complement alternative pathway.

The binding of CoMiX-Fc was tested on reference strains and 29 antibiotic-resistant clinical isolates retrieved from sputa of cystic fibrosis patients or from tracheobronchial aspirates of subjects housed in medical Intensive Care Units (ICU) by whole cell ELISA. The anti-Psl CoMiX-Fc bound to ~80% of clinical isolates. CoMiX-Fc significantly increased C3b and C5b9 deposition ($p < 0.001$) on clinical isolates and reference strains as compared to controls. Supplementing human serum with 3 μ g of CoMiX-Fc or CoMiX-FHR1 resulted in a complete inhibition of the growth of luciferase-expressing PAO1 strain (PAO1-Luc) during 5 hours compared to serum alone.

In conclusion, we have developed new immunotherapeutic complexes having a direct killing effect on *Pseudomonas aeruginosa in vitro*.