Green and bioinformatic approaches including biodegradability prediction in antiparasitic drug discovery

Tuesday, 18th March 2025

Teams platform and Cloudpharm P.C

Cana Laboratories, Athens, Greece

One Health drugs against parasitic vector borne diseases in Europe and beyond

OneHealth*drugs*

This workshop will bring together experts and researchers to explore innovative molecular modeling and experimental tools in the field of biodegradability and antiparasitic drug discovery that align with the goals of the COST CA21111 Action

BOOK OF ABSTRACTS

Meeting Venue

This will be a mixed event on Teams platform and Cloudpharm P.C Cana Laboratories - 446 Irakliou Avenue, Iraklio 14122, Attica, Greece

Organizing committee

Sandra Gemma, University of Siena, Italy
Marina Roussaki, Cloudpharm P.C., Greece
Theodora Carogelopoulou, National Hellenic Research Foundation, Greece
Maria Paola Costi, University of Modena and Reggio Emilia, Italy
Anabela Cordeiro da Silva, IBMC, Portugal

Program

TUESDAY 18th March 2025	
9:30 - 10:30	Registration
10:30-10:40	Opening remarks: **Sandra Gemma**, **Theodora Carogeropoulou**, **Marina Roussaki**
10:40 - 10:50	**Maria Paola Costi** Action Chair – OneHealthdrugs updates
Session I	Opening and Environmental Impact of Pharmaceuticals **Chair**: Anabela Cordeiro Silva
10:50 - 11:10	Presentation of Cloudpharm P.C. and CANA Laboratories - **Panagiotis Zoumpoulakis** Cloudpharm P.C., Greece, **Dimitris Moraitis** Cosmos Health Inc., Greece
11:10 – 11:30	Presentation of the EU-Funded Project "ENVIROMED" - **Marina Roussaki** Cloudpharm P.C., Greece
11:30 – 11:50	Biodegradability and Pharmaceutical metabolites in Environmental Toxicology - **Eleni Chontzopoulou** Cloudpharm P.C., Greece
11:50– 12:10	Coffee Break
Session II	Computational Approaches in Ecotoxicology & Drug Discovery **Chair**: Theodora Calogeropoulou
12:10 – 12:30	Machine Learning based predictive models for ecotoxicity using LC50 and bioconcentration factors - **Vagelis Tsoukas** Cloudpharm P.C., Greece
12:30 – 13:00	Isalos Analytics Platform: A Zero-Code Machine Learning Solution for Cheminformatics and Beyond - **Antreas Afantitis** PhD, MB, NovaMechanics MIKE, Greece
13:00 – 14:00	Visit to the facilities of CANA Laboratories
14:00 - 15:00	Break and refreshment
Session III	Advanced Research in One Health and Drug Development **Chairs**: Sandra Gemma (University of Siena), Maria Paola Costi (University of Modena and Reggio Emilia)
15:00 - 15:40	Biodegradation of APIs – A Cross-Industry Perspective - **Tobias Harschneck** Boehringer Ingelheim Vetmedica GmbH, Germany (remote)
15:40 – 16:10	Emerging Contaminants in the Environment: A One Health Crisis - **Maria Clara Starling** - Universidade Federal de Minas Gerais, Brazil (remote)

16:10 – 16:40	Targeting Leishmaniasis: Integrating In Silico and Experimental Approaches for Scaffold Optimization within the One Health Framework - **Sandra Gemma** University of Siena, Italy
16:40 – 17:10	Break
Session IV	Young Researchers' Contributions **Chair**: Marina Roussaki (Cloudpharm)
17:10 – 17:30	One Health Approach to Antiparasitic Drug Discovery: Integrating Structural Analysis and Ecotoxicological Profiling for Eco-Friendly Compound Prioritization - **Daniele Aiello** University of Modena and Reggio Emilia, Italy
17:30 – 17.45	A Design of Experiment (DoE) approach for a sustainable synthesis of chalcones as promising antimicrobial scaffold - **Ludovica Marotta** University of Siena, Italy
17:45 – 18:00	Closing Remarks

Presentation of the EU-Funded Project "ENVIROMED"

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The EU-funded project ENVIROMED aims to reduce the environmental footprint of pharmaceuticals by enhancing our understanding of their lifecycle impacts. This initiative focuses on assessing the presence, persistence, and toxicity of pharmaceutical compounds in the environment, employing both in-vitro and in-vivo models. Additionally, ENVIROMED seeks to develop greener manufacturing processes and advanced monitoring technologies to detect pharmaceutical micropollutants in wastewater and natural environments. By integrating these approaches, the project aspires to promote sustainability within the pharmaceutical industry and mitigate ecological risks associated with pharmaceutical pollutants

Biodegradability and Pharmaceutical metabolites in Environmental Toxicology

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Pharmaceuticals enter ecosystems through various pathways, including wastewater effluents, agricultural runoff, and improper disposal. Due to their widespread use, their presence in the environment raises significant concerns for public health, ecosystems, and biodiversity (Kayode-Afolayan et al., 2022). Evaluating pharmaceutical metabolites is essential for a comprehensive risk assessment of their environmental impact, since metabolites can exhibit different toxicological profiles, persistence and ecological behaviors compared to the parent drug (Figure 1). Pharmaceuticals and their metabolites can contaminate water, soil and air, potentially leading to toxicity and long-term ecological effects. In general, these compounds undergo chemical transformation in the environment due to factors such as temperature and pH or enzymatic transformation (metabolization) in the human body by a variety of different enzymes (Baillie, 2008). Once these metabolites enter aquatic systems, they may pose unique environmental risks distinct from those of the original compound. Since not all drugs have been thoroughly studied for metabolite formation, various prediction tools are available to anticipate their chemical structures and enhance our understanding in the overall environmental impact of these chemicals. Likewise, microbial transformations of pharmaceuticals in wastewater treatment can generate different metabolic products, which can also be predicted using *in silico* tools. Since accurately predicting the potential chemical structures of drug metabolites is crucial for environmental risk assessment, we have compiled a dataset of drugs along with their experimentally reported metabolites. Finally, we evaluated the accuracy of various software tools to identify the most reliable publicly available option.

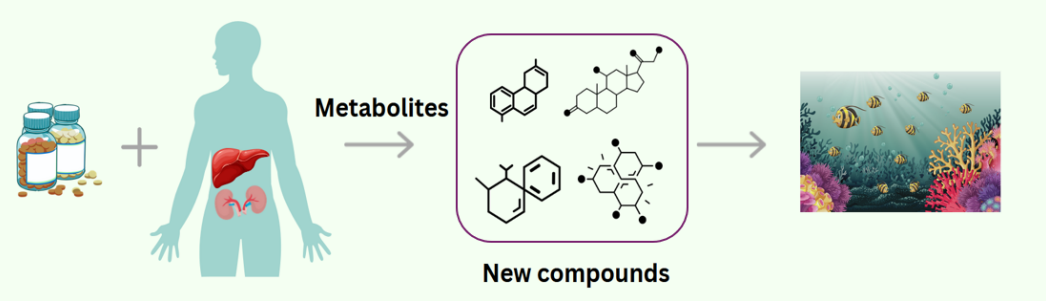

Figure 1. Human metabolites derived from pharmaceuticals as a threat for aquatic organisms.

References

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Kayode-Afolayan, S. D., Ahuekwe, E. F., & Nwinyi, O. C. (2022). Impacts of pharmaceutical effluents on aquatic ecosystems. *Scientific African*, *17*, e01288. https://doi.org/10.1016/J.SCIAF.2022.E01288

Machine Learning based predictive models for ecotoxicity using LC50 and bioconcentration factors

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Nowadays, significant amounts of chemical substances are detected in various environmental compartments. In particular, the aquatic environment has become a reservoir for drugs and fertilizers released through the discharge of effluent water [1,2]. The release of these emerging contaminants into the environment can negatively impact ecosystems[3]. It is thus critical to reduce the overall environmental impact of pharmaceuticals by developing novel computational tools aiming to contribute to green-by-design compounds.

For this purpose, Cloudpharm PC[4] has designed and developed a multimodal platform that offers state-of-the-art cheminformatics-based classification models to assess the potential effect of pharmaceuticals on the aquatic environment. Predicting the bioconcentration factor (BCF) and Lethal Concentration, 50% (LC50) of pharmaceuticals in aquatic organisms is critical for the relevant environmental risk assessment. Since the experimental determination of BCF and LC50 are of high experimental cost and requires a large number of vertebrate animals to perform the *in vivo* assays, in silico methods such as quantitative structure-activity relationships (QSARs) powered by machine learning (ML) models were employed to assess the environmental risk of pharmaceuticals[5,6] in aquatic organisms. In this study, we developed effective classification models which aid in eliminating candidates in the earlier processes of the drug discovery pipeline based on their potential environmental impact, and thus shape future compounds in terms of green-by-design ambitions. To fully evaluate the environmental risk of pharmaceuticals, regarding their bioaccumulative and ecotoxic effects on aquatic organisms, it is essential to assess these properties in their metabolic products, as they also influence the overall ecotoxicity profile of pharmaceutical compounds. Therefore, we have incorporated software into our platform to predict the most likely human metabolites for each drug, which will be further assessed using our newly developed BCF and LC50 prediction models to evaluate their alignment with the principles of green chemistry [7].

The platform aims to be accessible to scientists in the field of drug discovery and serve as a novel screening methodology within drug discovery pipelines, which also functioning as a regulatory checkpoint in the pharmaceutical industry. In conclusion, this platform demonstrates the impact of machine learning in the computational prediction of key biological properties of chemicals, which are crucial for determining their potential applications in health protocols and industry.

References

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2.Białk-Bielińska, A. et al. Ecotoxicity evaluation of selected sulfonamides. Chemosphere 85, 928–933 (2011).

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Isalos Analytics Platform: A Zero-Code Machine Learning Solution for Cheminformatics and Beyond

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NovaMechanics MIKE, Greece

The Isalos Analytics Platform (https://isalos.novamechanics.com/) is a zero-code machine learning solution designed to empower domain experts without any programming background. It employs a tab-based, node-like workflow where each tab acts as a node in a pipeline, allowing users to import data, apply transformations, and build predictive models through a sequence of intuitive operations. Using this visual interface, scientists and analysts can perform complex data preprocessing, model validation, and analytics tasks entirely through point-and-click interactions, eliminating the need to write code. Key features of Isalos include robust data transformation tools, comprehensive statistical evaluation modules, and a suite of state-of-the-art algorithms for classification and regression (e.g., XGBoost, k-Nearest Neighbors, multi-layer perceptron networks, and radial basis function networks). Validated models can be directly deployed as web services via the Enalos Cloud Platform (https://www.enaloscloud.novamechanics.com/), enabling broad accessibility and collaboration. Originally developed for cheminformatics applications, Isalos has been successfully applied to tasks such as experimental design, predictive modeling, and result validation in chemical research. Its no-code approach and flexible analytics pipeline extend beyond chemistry, empowering experts in business analytics, bioinformatics, and other domains to adopt machine learning solutions without coding barriers. By lowering the technical threshold, the Isalos Analytics Platform democratizes AI-driven analytics and accelerates research and data-driven decision-making across disciplines.

Biodegradation of APIs – A Cross-Industry Perspective

Tobias Harschneck

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Biodegradation is one important factor influencing the environmental fate and safety of molecules that are used in pharmaceutical, agricultural, and animal health applications. Current legislative frameworks like the EU Taxonomy or Urban Wastewater Treatment Directive further emphasize and incentivize reducing the environmental impact of active molecules through efforts such as designing for biodegradability. This presentation aims to show the relevance of API biodegradation for the pharmaceutical and related industries, highlights current approaches to address the topic along the R&D process, and gives an outlook on cross-industry efforts and collaborations to drive future developments in the field.

Emerging Contaminants in the Environment: A One Health Crisis

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Emerging contaminants (ECs), such as pharmaceuticals, personal care products, agrochemicals, and industrial compounds, have become significant environmental concerns due to their persistence and potential adverse effects on ecosystems and human health. The detection of these contaminants in various environmental matrices, often at concentrations ranging from nanograms to micrograms per liter, underscores the urgency of addressing this issue within the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health.

The issue of antimicrobial resistance (AMR) is intricately linked to the presence of ECs in the environment. Municipal wastewater treatment plant effluents and hospital effluents contain resistant bacterial strains carrying numerous resistance genes and their variants. Consequently, final outflows play a critical role in AMR dissemination, making the disinfection of these effluents essential. This is particularly concerning in low- and middle income countries where there is lack of sanitation infrasctructure. Conventional technologies used for water and wastewater treatment, such as chlorination and UV processes, have been proven ineffective. In contrast, advanced oxidative processes are viable alternatives, as oxidative radicals damage cell constituents and nucleic acid structures through free radical reactions. The photo-Fenton process, particularly when carried out under sunlight (solar photo-Fenton), has shown promise in effectively removing antimicrobial-resistant bacteria (ARB) and associated resistance genes (ARGs) from wastewater.

Despite these efficiencies, is is critical to follow the toxicity generated during the secondary wastewater treatment to avoid the discharge of potentially harmful by-products. This is when biological treatment using bacteria and alagae consortium and ecotoxicological assays come into play. A natural microalgae-bacteria consortium under low-intensity LED illumination achieved a 48.34% removal of sulfamethoxazole (SMX) and a 24.58% removal of trimethoprim (TMP). The symbiotic relationship between microalgae and bacteria facilitates the biodegradation of these antibiotics. Regarding the use of chemical treatment strategies, ecotoxicological assessments performed to evaluate chronic toxicity to *Raphidocelis subcapitata*, and phytotoxicity tests with *Lactuca sativa* after oxidative treatment of secondary wastewater containing ECs indicated 65-83% removal of the sum of ECs (ΣECs) rendering the samples either less or non-toxic across all bioassays.

In conclusion, the pervasive presence of emerging contaminants in the environment poses a multifaceted One Health crisis, impacting ecosystems and human health. Research offers valuable insights into the occurrence, risks, and treatment of these contaminants, emphasizing the critical need for continued investigation and the development of effective strategies to mitigate the impacts of ECs and AMR, ensuring the protection of environmental and public health, especially through ecotoxicological assays. These should be aligned with the development of alternative environmental friendly drugs as aimed by the One Health Drugs under the cost action.

Targeting Leishmaniasis: Integrating In Silico and Experimental Approaches for Scaffold Optimization within the One Health Framework

Sandra Gemma,[a] Sara Rossi,[a] Mirko Pineschi,[a] Stefania Butini,[a] Lorenzo Antonelli,[b] Cécile Exertier,[b] Giuseppe Campiani,[a] Andrea Ilari,[b] and Gianni Colotti[b]

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Leishmaniases are vector-borne diseases caused by protozoan parasites of the Leishmania genus, affecting both humans and animals. Leishmania infantum is a key pathogen responsible for visceral leishmaniasis (VL) in South America, the Mediterranean, and parts of Asia, as well as canine leishmaniasis (CanL) in Europe, highlighting the interconnected nature of human and animal health.

Leishmaniasis remains a major global health challenge due to the limited availability of effective and safe treatments. Current therapies are often associated with severe side effects, high costs, and the emergence of drug resistance, underscoring the urgent need for novel anti-leishmanial agents. Despite ongoing drug discovery efforts, most research focuses primarily on efficacy and pharmacokinetics, often neglecting the environmental impact of these compounds. Given the One Health perspective, which recognizes the interconnectedness of human, animal, and environmental health, it is crucial to integrate ecotoxicological considerations into the drug development process. The environmental fate of pharmaceuticals, including their persistence, bioaccumulation, and potential toxicity to non-target organisms, must be assessed to minimize ecological risks. Therefore, a holistic approach that balances potency, safety, and environmental sustainability is essential for the next generation of anti-leishmanial drugs.

Here, we present our ongoing efforts to discover novel antileishmanial hit compounds through an integrated approach that combines structure-based drug design, molecular modeling, and bioinformatics. Our strategy focuses on predicting the drug-like properties and environmental fate of new scaffolds, ensuring both therapeutic potential and ecological safety before experimental validation.

One Health Approach to Antiparasitic Drug Discovery: Integrating Structural Analysis and Ecotoxicological Profiling for Eco-Friendly Compound Prioritization

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The prioritization of compounds from diverse sources is a critical step in drug discovery, shaping the success of medicinal chemistry programs. Beyond potency and selectivity, incorporating ecotoxicological parameters enables the early identification of safer and more sustainable molecules. At OneHealthdrugs, we are developing a database of antiparasitic compounds active against *Trypanosoma*, *Leishmania*, *Schistosoma*, and *Babesia*, integrating structural information, molecular targets, IC50/Ki values, and selectivity against human cell lines. Two case studies were conducted to evaluate the impact of ecotoxicological parameters on compound selection. The first analyzed a virtual screening dataset targeting *Leishmania infantum* calpain[1-3], while the second focused on natural compounds active against *Trypanosoma brucei*. These datasets were assessed using ADMET[4] and ecotoxicological profiling, molecular docking, MM-GBSA ΔG Bind calculations[5], and structural clustering via Tanimoto similarity and scaffold decomposition. Currently, our work is focussed on compounds active against *Trypanosoma brucei*, including both synthetic and natural products. To refine compound selection, we are developing algorithms for scaffold analysis and studying molecular similarities using two complementary fingerprinting approaches. The first, based on the classic Tanimoto similarity of molecular fingerprints, emphasizes structural commonalities. The second, a "pharma fingerprint," integrates key pharmacophoric features related to biological activity, offering a functional perspective on molecular similarity. This dual approach aims to uncover scaffold patterns that balance potency with improved ecotoxicological properties. A key aspect of our study is the identification of eco-friendly scaffolds, guiding the design of new compounds with lower environmental impact while maintaining high antiparasitic activity. Future steps include refining our workflow and visualizing correlations between IC50 values and ecotoxicological parameters to enhance compound prioritization strategies and underscores the importance of integrating ecotoxicological profiling and structural analysis in early-stage drug discovery, promoting the selection of effective and sustainable antiparasitic agents.

References

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A Design of Experiment (doe) approach for a sustainable synthesis of chalcones as promising antimicrobial scaffold

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Background: Our investigation of chalcones as potential scaffolds for combating *Leishmania* will be here presented. Their demonstrated antileishmanial activity, particularly against the amastigote form of the parasite, and their structural simplicity and ease of synthesis make them attractive candidates for drug development. Several studies indicated that chalcones can exert their effects by targeting specific metabolic pathways within the parasite, such as inhibiting mitochondrial functions. Additionally, structure-activity relationship (SAR) studies have highlighted the importance of substituents on the chalcone structure, which can significantly influence their biological activity. This combination of favorable properties, positions chalcones as promising leads in the search for effective treatments against leishmaniasis. So, in the frame of the One Health concept, it is important to develop appropriate low-impact synthetic strategies for exploring the SARs. Chalcones can be synthesized through environmentally friendly methods, specifically utilizing the Claisen-Schmidt condensation reaction. However, the yield of this synthetic methodology is highly dependent on the substitution pattern of the starting materials. Here we present our method to optimize this reaction using green solvents and the Design of Experiment (DoE) approach (**Figure 1**), thereby promoting sustainable practices in drug discovery.

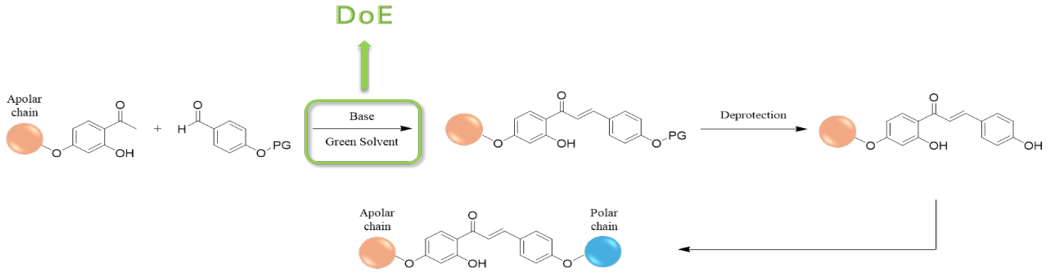

Figure 1. Scheme of synthetic process to obtain the final compounds; the computational optimization step is highlighted in green.

In conclusion, through this research a small library of novel chalcones was prepared and preliminary evaluation of their antileishmanial properties will be also presented.