MINUTES
Marathon Days 25-28 November 2024

Monday, 25 November 2024
Medicinal chemistry and structural biology
Moderators: Cecilia Pozzi and Theodora Calogeropoulou
Link
https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135211346?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

Attendance list
Uploaded in the platform

Program of the day

Moderators: Cecilia Pozzi and Theodora Calogeropoulou

14.30: Welcome (Action Chair: Maria Paola Costi)

14.40: Andrea Ilari, National Research Council of Italy (CNR), Rome, Italy
Exploring the Ubiquitin-Proteasome System for Structure-Based Design of Effective PROTACs targeting Trypanothione Reductase

15.00: Thomas J. Schmidt, University of Münster, Germany
Natural Products against Neglected Disesases

15.20: John Igoli, Joseph Sarwuan Tarka University, Nigeria
Novel scaffolds from African medicinal plants for antiparasitic drug discovery

15.40: Ineta Meldaikytė, Kaunas University of Technology, Lithuania.
Investigation of bipyrazole derivatives

16.00: Break

16.10: Ieva Bartkeviciute, Kaunas University of Technology, Lithuania
Synthesis of substituted oxadiazoles and evaluation of anthelmintic properties

16.30: David C. Magri, University of Malta, Malta
Molecular Logic Gates Derived from Fluorescent Natural Products

16.50: Maria Paola Costi, University of Modena and Reggio Emilia, Italy
High Throughput screening deselection of Leishmania dihydrofolate reductase for highly selective pteridine reductase inhibitors

17.10: Maria Laura Bolognesi, University of Bologna, Italy
Opportunities and challenges in the discovery of PROTACS for Vector-borne Parasitic Diseases.

17.30: Elisa Uliassi, University of Bologna, Italy
Exploring the chemical space of small molecules targeting trypanothione reductase for parasitic diseases

17.50: Closing remarks

Abstract
Medicinal chemistry and structural biology.
Moderators: Cecilia Pozzi and Theodora Calogeropoulou
The 9 presentations were focused on medicinal chemistry topics were the PROTAC approach was described under different aspects such as the structural biology aspect, the medicinal chemistry. Andrea Ilari presented the PROTACs targeting of Trypanothione Reductase. Then both Maria Laura Bolognesi, (Opportunities and challenges in the discovery of PROTACS for Vector-borne Parasitic Diseases) and Elisa Uliassi showed interesting medicinal chemistry aspects and potentialities of the approaches. A few talks by **Thomas J. Schmidt, University of Münster, Germany** (Natural Products against Neglected Disesases) and **John Igoli**, (Tarka University, Nigeria) presenting Novel scaffolds from African medicinal plants for antiparasitic drug discovery, showing the activities of plants extracts against Leishmaniaisis. Very important progress were highlighted regarding the anti-cutaneous Leishmaniasis activity of the sesquiterpenes from *Arnica montana*. **Ineta Meldaikytė**, Kaunas University of Technology, Lithuania, presented the Investigation of bipyrazole derivatives as anticancer agents with potential anti-trypanosomatidic activities. While **Ieva Bartkeviciute**, (Kaunas University of Technology, Lithuania) Synthesis of substituted oxadiazoles and evaluation of anthelmintic properties.

Meeting Summary

This summary is related to session of the COST Action meeting Marathon DAYS held on **November 25, 2024**, focusing on cutting-edge strategies in drug discovery, specifically targeting parasitic diseases like Leishmaniasis. The session included presentations on chemical probes for protein degradation, natural product screening, novel fluorescent tools, and high-throughput screening efforts against key parasitic enzymes.

Research Presentations

1. Fluorescent Ubiquitin Proteasome System for Structure-Based Design (Andrea Ilari, CNR)
Focus: Developing novel compounds that induce the **degradation** of the essential parasitic enzyme **Trypanothione Reductase (TR)** in *Leishmania* using the **Proteolysis-Targeting Chimeras (PROTACs)** technology.
Mechanism: The goal is to design a chimera molecule (a "Protac") that simultaneously binds the TR (protein of interest) and a parasite-specific E3 ligase, effectively tagging TR for destruction by the proteasome.
Key Findings:
Identified a **putative E3 ligase** in *Leishmania* with a thalidomide-like binding domain.
Synthesized a **Protac-like compound (C30/AP41)** that acts as a potent TR inhibitor and, crucially, was shown to **decrease the concentration of TR protein** in parasite cells (amastigotes) after treatment, providing a proof-of-concept for target degradation.
The compound C30 also showed good selectivity and efficacy in killing intracellular amastigotes.

2. Natural Products as Potential Hits Against Neglected Diseases (Thomas J. Schmidt)
Focus: A retrospective and current review of his 20+ years of research on natural products as anti-protozoal agents,emphasizing the use of **ethnobotany** and **structure-activity relationships (SAR)**. **Examples of Active Compounds/Classes:** Sesquiterpene lactones (e.g., **Helenalin** from *Arnica*, potent anti-*T. brucei* activity), Naphthoquinones (from walnut, strong anti-*Trypanosoma* and anti-*Leishmania* activity), Chromene compounds (potent anti-*P. falciparum* hits), Triterpenoids (from frankincense and myrrh), and Steroidal Alkaloids (from *Holarrhena*, strong anti-parasitic activity).

Clinical Success Story (Arnica): Presented results from a clinical study in Colombia using **Arnica tincture**topically against **cutaneous Leishmaniasis (CL)**. *Arnica* contains active sesquiterpene lactones with both **anti-leishmanial** and **anti-inflammatory** properties, leading to a near **100% cure rate** in uncomplicated CL cases in the observational study, without the need for painful injections. A larger, comparative clinical trial is planned.

3. Novel Scaffolds from African Medicinal Plants (John Igoli, Joseph Sarwuan Tarka University, Nigeria)
Focus: Exploring novel chemical scaffolds from unique African plants and organisms (including marine sources and propolis) for anti-parasitic drug discovery.
Key Findings: Highlighted newly isolated and structurally unique compounds, such as a **Triterpene** from *Dracaena* and a **Heptanoid** from *Gardneria*, that show high activity and excellent **selectivity** against various **Trypanosomatids** (e.g., *T. brucei*, *T. cruzi*, *L. major*) but are inactive against *P. falciparum* or human cell lines.
Call for Collaboration: Requested medicinal chemists in the network to help perform **SAR studies** on these novel,promising scaffolds (like **C-Phononylone**), as the isolation percentage is low and synthetic modification is needed for further development.

4. Investigation of Bipyrazole Derivatives (Ineta Meldaikytė, Kaunas University of Technology, Lithuania)
Focus: Synthesis and biological evaluation of new functionalized **bipyrazole derivatives**.
Methodology: Synthesized a range of bipyrazole compounds with diverse aromatic substituents, primarily aiming for S-alkylation reactions.
Key Findings: While most compounds showed only average antimicrobial and antioxidant activity, some analogues (e.g., those with a TNL or methoxyphenyl group) showed promising results in **anti-cancer activity**assays (lung and breast cancer cell lines). The potential for **anti-helminthic** activity (targeting Succinate Dehydrogenase) was explored using the free-living nematode **C. elegans** as a model organism, with some pyridine-based compounds showing a positive trend.

5. Synthesis of Substituted Oxadiazoles and Evaluation of Anti-helminthic Properties (Ieva Bartkevičiūtė, Kaunas University of Technology, Lithuania)
Focus: Synthesis and evaluation of 1,3,4-oxadiazole derivatives, a stable and medicinally promising scaffold, for anti-helminthic properties.
Methodology: Synthesized various S-alkylated oxadiazole derivatives, including those with quinoline and pyridine bases, achieving good to very good yields. Used **molecular docking** to predict active compounds *before* synthesis.
Key Findings: Tested compounds against *C. elegans* using a **chitinase enzyme assay** (a marker of nematode viability). Pyridine-based compounds, particularly those with no additional substituent or a fluorine atom, showed the most significant positive effect.

6. Molecular Logic Gates Derived from Fluorescent Natural Products (David Magri, University of Malta)
Focus: Developing **fluorescent logic gates** by chemically modifying or polymerizing natural products,demonstrating their potential utility beyond traditional medicinal use.
Innovation: Took the **Cinchona alkaloids** (like quinine) and created **co-polymers** with acrylamide.
Key Findings: The Cinchona co-polymers could be demonstrated to act as:
Inhibit Logic Gates (fluorescence output) in water, where acid turns the fluorescence ON, but the addition of chloride (or other halides) turns it OFF.
AND Logic Gates (colorimetric output) in THF/Water, where the presence of both acid and iodide causes a visible color change.
Perspective: Suggested using these fluorescent co-polymers as **alternative anti-parasitic agents** (to circumvent resistance) or as **diagnostic/environmental monitoring tools** (fitting the ecotox theme).

7. High Throughput Screening and Target Validation of *Leishmania* Dihydrofolate Reductase (Maria Paola Costi)
Focus: A large-scale effort to identify highly **selective inhibitors** of **Pteridine Reductase 1 (PTR1)**, an enzyme that contributes to **drug resistance** in *Leishmania* parasites by taking over the function of inhibited Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS).

Objective: Find compounds with **Ki in the sub-micromolar/nanomolar range** and **≥1000-fold selectivity** over DHFR.
Methodology: Collaborated with the **European Lead Factory (ELF)** for a high-throughput screening (HTS) of ≈600,000 compounds using a validated kinetic assay.
Key Findings: The HTS and subsequent selection yielded **15 high-quality hit compounds** with the desired selectivity profile (active against PTR1, inactive against DHFR). These hits were confirmed by dose-response curves and thermal shift assays (TSA).
Next Steps: Proceeding with **hit confirmation** (orthogonal assays), **SAR expansion** (synthesis of analogues),**biological testing** (active hits confirmed by partners in Porto), and X-ray **crystallography** for structural validation.

📝 **Next Steps and Collaboration**
Natural Products Group: There was an urgent discussion about formalizing the growing number of natural product researchers into a structured **Horizontal Group** to facilitate SAR studies, funding applications, and collaboration with synthetic chemists (like those requested by John Igoli).
ELISA/Target Degradation: Further mechanistic studies are needed to definitively prove that Protac-like compounds induce **proteasomal degradation** of the target enzyme (TR), not just a decrease due to other cellular effects.

OHD Marathon days WG2
Tuesday, 26 November 2024
Integration of Ecotoxicology in drug discovery
Moderators: Maria Paola Costi and Crtomir Podlipnik

Link to Teams
https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135292941?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

Moderators: Maria Paola Costi and Crtomir Podlipnik

10.00: **Welcome (Action Chair: Maria Paola Costi and Anabela Cordeiro)**

10.10: **Dominga Evangelista**, University of Bologna, Italy
Characterisation of persistence, bioaccumulation and toxicity of biologically active compounds with deep learning-based methods

10.30: **Daniele Aiello (STSM)**, University of Modena and Reggio Emilia, Italy
Integrating Ecotoxicological Profiling and Computational Approaches for Sustainable Drug Discovery Against Neglected Parasitic Diseases

10.50: **Lorenzo Raffellini**, Department of Pharmacy, University of Pisa, Italy
Waste of Punica Granatum: a source of bioactive components to develop new antiinfective treatments

11.10: **Črtomir Podlipnik**, University of Ljubljana, Slovenia
Machine learning models for assessing toxicity of aquatic model organisms

11.30: **Break**

11.40: **Bianca Martinengo**, University of Bologna, Italy
Green chemistry strategies for vector-borne parasitic disease drug discovery: design, synthesis and biological evaluation of cashew nut shell liquid derivatives

12.00: **Tahira Iftakhar**, University of Agriculture Faisalabad, Punjab, Pakistan, KBCMA College of Veterinary and Animal Sciences (CVAS) Narrowal, Punjab, Pakistan
Participatory And Molecular Surveillance Of Tick-Borne Theileriosis, And Anaplasmosis In District Bahawalpur, Punjab, Pakistan

12.20: **Daniel Sojka**, Institute of Parasitology, Biology Centre of The Czech Academy of Sciences (IoP BC CAS), Czech Republic
ASP3a/b - the promising druggable plasmepsin IX/X analogues from Babesia

12.40: **Closing remarks**

Meeting Summary

This summary is related to the session of a COST Action Marathon DAYS meeting held on **November 26, 2024**, focusing on integrating **ecotoxicology** and **computational methods** into the early stages of drug discovery, primarily targeting neglected parasitic diseases. The session featured presentations by young researchers and discussions on new collaborative opportunities.

Presentations and Key Research Highlights

1. Characterization of Persistence, Bioaccumulation, and Toxicity (PBT) of Compounds with Deep Learning (Dominga Evangelista, University of Bologna)

Objective: To develop a robust **Deep Learning (DL)** model using a **Message Passing Neural Network (MPNN)**to classify compounds as **PBT** or non-PBT early in the drug discovery process.

Methodology:

Collected and pre-processed a dataset of over 5,000 chemicals, classifying them as binary (PBT or non-PBT).

Employed three data splitting strategies (random, cluster splitting, and **cluster centroid splitting**) to ensure high structural dissimilarity between the training and test sets, simulating a real-world scenario.

Used the **cluster centroid splitting** model as the proposed model due to its robustness.

Key Findings: The DL model outperformed traditional Quantitative Structure-Property Relationship (QSPR) models, especially in minimizing **false negatives** (potential safety issues). The model identified common substructures (e.g., polycyclic aromatic rings, halogenated rings) associated with PBT behavior, aligning with QSPR findings. Applying the model to pharmaceuticals identified known environmental contaminants (antidepressants, opioids, steroids) as potential PBT risks.

2. Integrating Ecotoxicological Profiling and Computational Approaches for Sustainable Drug Discovery (Daniele Aiello, University of Modena)

Objective: To show how integrating **ecotoxicity parameters** can change the priority and selection of compounds in the early drug discovery pipeline.

Methodology:

Developed the **Compound Database Project**, a virtual library integrating *in vitro* activity, selectivity, *in vivo*data, and computational ecotoxicity parameters (obtained via ADMETlab) for compounds active against various neglected disease parasites (*Schistosoma*, *Babesia*, *Trypanosoma*, *Leishmania*).

Conducted two parallel case studies (one on anti-parasitic natural compounds, one on Calpain inhibitors) to test the effect of introducing ecotoxicity filters.

Key Findings: The inclusion of ecotoxicity filters alongside traditional toxicity (Tox) filters drastically **changed the final selection of hit compounds**, demonstrating the significant impact of prioritizing ecotoxicity early on. The goal is to build a robust workflow to select the safest and most promising scaffolds.

3. Waste of *Punica Granatum* as a Source of Bioactive Components (Lorenzo Raffellini, University of Pisa)

Objective: To develop anti-infective agents from pomegranate (*Punica Granatum*) peels, an agri-food waste product, using an eco-friendly approach.

Methodology:

Produced an **ellagic acid-rich extract** from pomegranate peels using a greener, enzyme/microorganism-based extraction method (avoiding strong acids).

The biological extract was found to contain significantly more ellagic acid and two glycosidic precursors (punicalagin isomers) than commercial juice.

Designed and synthesized novel small molecules (*urolithins* analogues) inspired by ellagic acid metabolites.

Future Plans: Assess the anti-infective potential of the biological extract (already showing antibacterial activity) and the novel urolithin analogues against neglected diseases like *Leishmania* and *Zika virus*. The speaker is applying for an **STSM with Sheraz Gul** to perform ADMETox and ecotox profiling.

4. Green Chemistry Strategies for Vector-Borne Parasitic Diseases and Drug Discovery (Bianca Martinengo, University of Bologna)
Objective: To develop new anti-parasitic drugs from Cashew Nut Shell Liquid (CNSL), an inedible, inexpensive,and readily available agri-industrial waste product, using green chemistry methods.
Methodology: Developed a small library of **phosphonium salts** derived from CNSL phenolic lipids.
Key Findings:
Compounds (especially **Compound 1 and 3**) showed excellent activity against the bloodstream form of *T. brucei* (**sub-nanomolar** EC50 values) and high selectivity over human cells (human foreskin fibroblasts).
Activity was also observed against *T. cruzi* and *Leishmania*.
Initial mechanism of action studies suggested the compounds may act by inhibiting the **Trypanosome Alternative Oxidase (TAO)** enzyme and/or causing **membrane perturbation** (supported by EPR studies).
Future Plans: Continue to investigate the mechanism of action and conduct *in vivo* and ecotoxicological studies (collaborating with Baylor University and others).

5. Experimental Platforms for Tick-Borne Pathogens and Drug Discovery (Dan Sojka, Institute of Parasitology, Czech Republic)
Objective: To present the lab's advanced experimental platforms for tick-borne diseases, highlighting opportunities for chemical and pharmacological collaboration. (The speaker changed the title from a specific drug-related topic to emphasize collaboration.)
Research Areas:
Tick Biology: Studying protein digestion and turnover in *Ixodes ricinus* (major European tick vector),including the development of functional genomic tools (RNAi) and *ex vivo* feeding models for testing compounds and pathogens.
Pathogen Research (*Babesia*): Studying *Babesia divergens* and *Babesia microti*. They are targeting specific parasite enzymes like **Plasmapepsins (Plm)**, **Calcium-Dependent Protein Kinases (CDPKs)**, and the **Proteasome** system.
Key Platforms/Findings:
Ectoparasite Drug Testing: Demonstrated high efficacy for FDA-approved proteasome inhibitors (e.g.,**Bortezomib**) against ticks.
Babesia Drug Testing: Showed that Plm inhibitors block invasion, while CDPK inhibitors block egress (exit from the red blood cell), illustrating clear anti-parasitic phenotypes.
Proteasome Inhibitors: Research aims to develop highly specific proteasome inhibitors (selectivity index ≥2000) by visualizing binding using Cryo-EM.
Advanced Microscopy: Developed highly effective **Expansion Microscopy** to visualize the parasite's ultrastructure.
Functional Genomics: Developing conditional knockouts/knockdowns in *Babesia* to validate targets.
Collaboration Offer: Explicitly sought collaboration for designing and synthesizing compounds (e.g., peptide mimetic inhibitors) tailored to their validated targets.

🤝 **Collaborative Opportunities and Closing Remarks**
STSM & Collaboration: Lorenzo Raffellini's application for an STSM with Sheraz Gul was highlighted. Annabela Cordeiro offered her lab's expertise for *in vivo* anti-parasitic testing (Leishmania, Trypanosoma, Malaria) once promising, non-toxic compounds are identified.
Chemoinformatics Summer School: Črtomir Podlipnik (Chair) announced the upcoming **Erasmus Mundus Master program Chemoinformatics Plus Summer School** in Ljubljana (early July) and offered **slots for invited lectures and young researchers** from the COST Action network.
Overall Sentiment: Maria Paola Costi was highly satisfied with the session, noting the excellent integration of different fields, the high quality of research, and the potential for new, more selective, and safer drugs by including ecotoxicological requirements from the earliest stages of the discovery process.

OHD Marathon days WG3
Wednesday, 27 November 2024
Parasitology and pharmacology

Moderators: Guy Caljon and José Maria Alunda

Link to Teams
https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135378158?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

1. LIST OF ATTENDANTS
The list of attendant is uploaded in the eCOST platform
2. PROOF OF ATTENDANCE
A proof of attendance is provided as screenshots made during the meeting (annex 2).
Program
Moderators: Guy Caljon and José Maria Alunda

14.30: Welcome (Action Chair: Maria Paola Costi and Guy Caljon)

14.40: André Lopes, Centre of Chemistry, University of Minho, Portugal
Recent Structural Optimizations of Pyrimido[5,4-d]pyrimidines Exhibiting Antileishmanial Activity

15.00: Sener Cintesun, Yildiz Technical University, Turkiye
Investigation of the bifunctional enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS) from Leishmania major

15.20: Cassandra Present, University of Antwerp, Belgium
A drug discovery journey: exploring N6-Methyltubercidin as an alternative antileishmanial drug treatment in a cutaneous Leishmania amazonensis mouse model

15.40: Lori Doko, eCampus University, Albania
Robotic Workflow for Antiparasitic Screening: Streamlined Cell Culture and Data Analysis in Pharmacology

16.00: Break

16.10: Estefania Calvo Alvarez, University of Milan, Italy
The role of NOD2 in macrophage activation and its therapeutic targeting in Leishmania infections

16.30: Tomilola Akingbade, Computer-Aided Therapeutic Discovery and Design Platform, Federal University of Technology, Nigeria
Synergistic inhibition of Plasmodium's vital proteins: A multi-target strategy using Buxus sempervirens

16.50: *Rokaya Ahmad**, University of Antwerp, Belgium***
Towards knowing your target in Leishmania: establishment of validated in situ assays for antileishmanial drug discovery

17.10: Gaia Mazza, University of Milan, Italy
Immunomodulatory effects of Helicobacter pylori Neutrophil Activating Protein (HP-NAP) on human and canine macrophages infected with Leishmania infantum

17.30: Stephanie A. Blandin, Inserm U1257, CNRS UPR9022, University of Strasbourg, IBMC, Strasbourg, France
Plasmodione antimalarial activity is partially mediated by the NADH dehydrogenase PfNDH2

17.50: Gomes-Alves AG, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal and CEB – Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal
Π-Π stacking stabilized polymeric micelles for hydrophobic drug delivery in
the treatment of leishmaniasis

18.10: *Closing remarks*

Abstract
The workshop (see programme in annex 3) included 9 presentations covering research on the major pathogenic protozoa, *Plasmodium* and *Leishmania*. Multiple research lines presented at the workshop are the result of collaborative work within OHD. Some presentations were approaching antiparasitic drug discovery from the chemical series or from bioactives from nature (plants and bacteria). **André Lopes** (University of Minho, Portugal) presented recent structural optimizations of pyrimido[5,4-d]pyrimidines with antileishmanial activity. **Cassandra Present** (University of Antwerp,

Belgium) presented N6-methyltubercidin as an alternative antileishmanial drug treatment for cutaneous leishmaniasis, whereas **Tomilola Akingbade** (Federal University of Technology, Nigeria) described the medicinal activities of alkaloids of *Buxus sempervirens*. **Gaia Mazza** (University of Milan, Italy) explored the use of a natural Neutrophil Activating Protein of *Helicobacter pylori* for the immunomodulation of human and canine macrophages infected with *Leishmania*. Other presentations were more target-oriented, including parasite and host targets. **Sener Cintesun** (Yildiz Technical University, Turkiye) explored the bifunctional enzyme dihydrofolate reductase-thymidylate synthase as antileishmanial target. From a host target perspective, **Estefania Calvo Alvarez** (University of Milan, Italy) explained the role and the therapeutic targeting of the host pattern recognition receptor NOD2 during *Leishmania* infections in macrophages. **Lori Doko** (eCampus University, Albania) reflected on the workflow for antiparasitic screening. Two presentations reported on mode of action studies with the development of novel assays. **Stephanie A. Blandin** (Inserm, University of Strasbourg, France) revealed that the antimalarial activity of Plasmodione is partially mediated by an NADH dehydrogenase *Pf*NDH2. **Rokaya Ahmad** (University of Antwerp, Belgium) identified a drug target of antileishmanial aminopyrazoles and developed a new assay to identify inhibitors of nuclear pore transport. Due to technical reasons, the presentation by **Ana G. Gomes-Alves** (Utrecht University, The Netherlands; Universidade do Porto and Universidade do Minho, Portugal) could not be given.

Summary

This meeting summary covers a session focused on **integrating medicinal chemistry and parasitic biology** with an eye toward **sustainable drug discovery** and **environmental safety (Ecotox)**, featuring presentations on new chemical scaffolds, target degradation strategies, and computational methods.

Research Highlights and Key Findings

1. Pyrimidal Pyridines: Discovery and Optimization (André Lopes, University of Minho)

Focus: A case study detailing the discovery and structure-activity relationship (SAR) optimization of a new class of **pyrimidal pyrimidine** compounds with potent **anti-leishmanial activity**.

Key SAR Findings:

Activity Requirements: High activity against both promastigotes and intracellular amastigotes requires **hydrogen bond accepting properties** in the blue (R) group, particularly a para-methoxy and a para-fluorine substitution.

Chemical Freedom: While the blue group is restrictive, the red (R1) group allows for chemical changes, provided the substituents remain **hydrophilic**; hydrophobic substitutions lead to a loss of amastigote activity.

Pharmacology: Demonstrated good **ADMETox profiles** (low cardiotoxicity, low cytochrome P450 inhibition) in collaboration with the Fraunhofer Institute.

Next Steps: Focusing on improving **solubility** (salts and encapsulation studies are ongoing), determining **pharmacokinetics**, and elucidating the unknown **molecular target** through computational studies (3D-QSAR).

2. DHFR-TS Production and Mutagenesis (Şener Çintesun, Yıldız Technical University)

Focus: Successful production and purification of the **bifunctional enzyme DHFR-TS** (Dihydrofolate Reductase-Thymidylate Synthase) and its isolated DHFR domain from *Leishmania major*.

Methodology: Used a bacterial expression system (Arctic Express) and affinity/size-exclusion chromatography.

Key Findings: Successfully generated and confirmed **three specific point mutations** in the DHFR active site. This work establishes a platform for future functional studies to understand how specific amino acids influence the enzyme's activity and inhibitor binding. (Kinetic characterization is planned.)

3. Nucleoside Analogues and Combination Therapy (Cassandra Present, University of Antwerp)

Focus: Investigating the efficacy of the nucleoside analogue **CL5564** (A6-methylaminotubercidin) alone and in combination with **miltefosine** against *Leishmania amazonensis*.

Key Findings:

In Vitro: CL5564 was **6.5-fold more potent** than miltefosine against intracellular amastigotes and showed a higher selectivity index.

In Vivo (Mouse Model): The compound was safe at a 10 mg/kg **intralesional** dose (unlike previous toxic oral doses of 50 mg/kg) and showed similar efficacy to miltefosine.

Combination: The CL5564/miltefosine combination showed an **additive effect** *in vitro* and was 128-foldmore effective *in vivo*, achieving a **sterile cure in two-thirds of the mice**.
Significance: Nucleoside analogues remain promising anti-leishmanial alternatives, especially in combination treatments for CL.

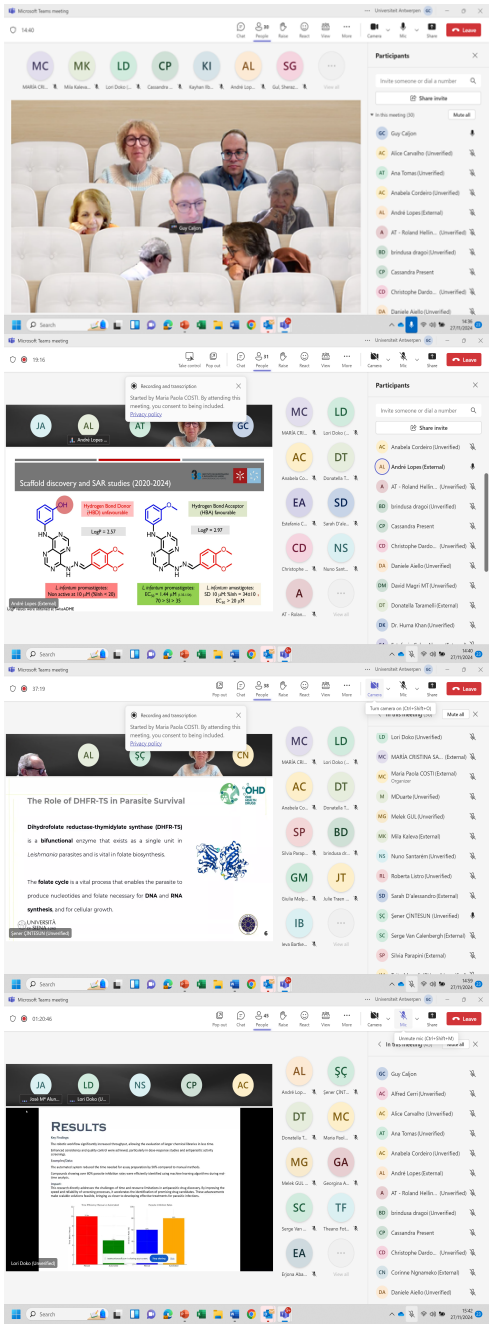
4. Target and Assay Project Development (Rokaya Ahmad, University of Antwerp)
Focus: Validating two new druggable pathways in *Leishmania* and developing robust **high-content assays** for screening.
Part 1: Endosomal Assembly/Trafficking:
Target Validation: Used chemical mutagenesis (ENU/EMS) followed by CRISPR/Cas9 gene editing to confirm that a mutation in a **Focal Adhesion Kinase (FAK)-like domain protein** (a PI(4,5)P2 binding protein) is the molecular target for the anti-leishmanial compound **aminopronazole**.
Mode of Action: The protein localizes to **endocytic vesicles** (endosomes), suggesting the drug interferes with endosomal assembly and recycling.
Part 2: Nuclear Protein Import:
Assay Development: Engineered *Leishmania* with an **M-Cherry fluorescent protein** fused to a **Nuclear Localization Signal (NLS)**.
Validation: Treatment with the universal import inhibitor **Importazole** caused the M-Cherry protein to accumulate outside the nucleus (forming distinct rings around it), confirming a dose-dependent inhibition of nuclear transport. This provides a clear, high-content visual assay for screening nuclear transport inhibitors.

5. Host Immunomodulation and Parasite Killing (Estefania Calvo Alvarez & Gaia Mazza, University of Milan)
NOD2 Signaling (Estefania):
Focus: Investigating the role of the host cytoplasmic pattern recognition receptor **NOD2** (recognizes bacterial **MDP** peptidoglycan) in the innate immune response to *Leishmania infantum* infection in macrophages.
Key Findings: *L. infantum* infection **upregulates NOD2/NOS2 expression** and induces the release of nitric oxide (NO) and TNFα. Using knockout cells and inhibitors (GSK 717), the **NO production and killing ability were shown to be NOD2-dependent**. Treatment with the NOD2 ligand MDP boosted NO and TNFαrelease, demonstrating its potential as an **immunomodulator** for immunotherapy.
HP-NAP Protein (Gaia):
Focus: Evaluating the immunomodulatory effects of the *Helicobacter pylori* protein **HP-NAP** (a known TH 1enhancer) against *L. infantum* in both human and canine macrophages (One Health approach).
Key Findings: HP-NAP showed a **direct toxic effect** against *L. infantum* promastigotes (approx. 45%growth inhibition). In infected macrophages, it **reduced the intracellular parasite burden** and **increased the production of the TH 1 cytokine IL−12** (20-fold increase) while IL−10 levels remained low, supporting its role as an immunomodulatory agent that enhances TH 1 responses.

6. High-Throughput Screening (HTS) and Computational Methods
PTR1 HTS (Maria Paola Costi): Successfully completed HTS of ≈600,000 compounds via the **European Lead Factory (ELF)** to find highly **selective inhibitors of PTR1** (Pteridine Reductase 1), which is implicated in drug resistance. The screening identified 15 high-quality hits with Ki in the nanomolar range and excellent selectivity over the DHFR partner, providing novel, non-folate-like scaffolds for development.
Robotic Workflow Concept (Lori Doko): Presented a conceptual framework for a fully automated, **sustainable robotic workflow** for high-throughput anti-parasitic screening, data analysis (machine learning), and IC50 prediction.
Molecular Docking (Tomilola): Presented an ongoing computational project docking compounds from the medicinal plant **Buxus sempervirens** (boxwood) against three *P. falciparum* targets (FP−2, PfHT, FPTase). Identified several lead compounds, with **Bucharamine** showing the best profile against FPTase.

🤝 **Collaboration & Strategic Themes**
Natural Products Coordination: Discussion highlighted the strong need for a formal **Horizontal Group** within the COST Action to coordinate and focus the research on natural products, especially those originating from Africa, and link them to synthetic chemistry and safety profiling.
Integrating Ecotoxicity: Multiple presentations emphasized the intent to profile new hits using **Ecotox models**(e.g., ADMETox, LC50) to ensure the next generation of anti-parasitic drugs is **environmentally safe**.

Target Validation: The trend toward **target-based drug discovery** was reinforced by the development of sophisticated genetic tools and high-content assays (e.g., nuclear transport, endosome assembly) that enable rapid and reliable target validation and mechanism-of-action studies.



OHD Marathon DAY 4
Thursday, 28 November 2024
YRI presentations + STSM presentations & @13:00 Awards

Link to Teams

https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135479241?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

Moderators: Elisa Uliassi and Gulsah Bayraktar

9.30: **Welcome (Action Chair: Maria Paola Costi and Elisa Uliassi)**

9.40: **Rodrigue Keumoe (STSM)**, Inserm U1257, CNRS UPR9022, University of Strasbourg, IBMC, Strasbourg, France
Imaging of Fe2+ gradients as a ferroptosis marker in malaria parasites using a fluorogenic labile heme reporters

10.00: **Lorenzo Tagliazucchi (STSM)**, Unimore, Italy
Revealing the Mechanism of Action of the innovative antileishmanial agent H80 through fractionated MS Proteomics, untargeted metabolomics, and fluorescence imaging

10.20: **Aleksandar Cvetkovski (STSM)**, Goce Delcev University, Faculty of medical science, Stip, N. Macedonia
Molecular docking study on antiparasitic nucleoside drugs

10.40: **Theano Fotopoulou (STSM)**, National Hellenic Research Foundation, Institute of Chemical Biology, Greece
Synthesis and biological evaluation of new antiparasitic 4-thiazolidinone bioisosters of alkylphosphocholines

11.00: Break

11:10: **Sheraz Gul** Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP),
Developing a Target Product Profile in drug discovery

11.10: **Giulia Saporito**, Unimore ,Italy
Addressing the drug resistance issue by targeting the folate cycle proteins in Trypanosoma brucei and Leishmania major parasites

11.30: **Graikioti Dafni (STSM)**, University of Patras, Greece
Libraries of analogues of Eucalyptus G-endoperoxides, antiparasitic activities, mechanisms of action

11.50: ***Roberta Listro**, University of Pavia, Italy*
Towards the discovery of novel Sortase A inhibitors as potential antibiofilm agents

12.10: **Cristina Sanz Cuesta**, University of Salamanca, Spain
Sulfonamides as antitrypanosomatid agents: A preliminary study

12.30: ***Clara Lima***, University of Porto, Portugal
Preparedness of veterinarians to face emergency of animal leishmaniosis in urban settings of Morocco

12.50: **YRI awards and closing**

Abstract
The **OneHealthdrugs Marathon days** took place online on **November 25-28, 2024** and has been a great opportunity for all young investigators to share and discuss their research and to network with all attendees. The dedicated YRI session held on Nov 28 has been chaired by Elisa Uliassi (HG4 YRI leader) and Gulsah Bayraktar (WG5 leader). This day has provided the ideal international platform for YRI to present their research work in the field of vector-borne parasitic diseases (VBPDs), covering medicinal chemistry/green chemistry, pharmacology/parasitology and ecotoxicology topics. The marathon was well attended by YRI (>50% of presenters and participants). The YRI session included 5 presentations on STSM conducted during the 2nd year, 5 YRI oral communications and 1 inspiring lecture given by Dr. Sheraz Gul. The session and the discussion after each presentation were highly interactive and interesting. We underscored the urgent

need to broaden the drugs available to fight VBPDs and the need to substantially change existing approaches to drug discovery and development. At the end of the presentations, a committee consisting of Prof. Anabela Cordeiro-da-Silva, Dr. Elisa Uliassi and Dr. Gulsah Bayraktar awarded 2 YRIs that presented in the Onehealthdrugs marathon: 1 for STSM and 1 for YRI. The 2 winners are:

- **Lorenzo Tagliazucchi** (STSM), Unimore, Italy
  Revealing the Mechanism of Action of the innovative antileishmanial agent H80 through fractionated MS Proteomics, untargeted metabolomics, and fluorescence imaging
- **Rokaya Ahmad**, University of Antwerp, Belgium

Towards knowing your target in Leishmania: establishment of validated in situ assays for antileishmanial drug discovery

We hope that we have helped to strengthen YRI's foundation in OHD topics and to increase their involvement in our OHD Action.

I would like to thank all the organizers, speakers and participants for their invaluable contributions and active participation in this marathon. A special thanks goes to Prof. Anabela Cordeiro-da-Silva. We thank Prof. Paola Costi for her guidance and dedication to the OneHealthdrugs COST Action.

Once again, we thank everyone who made this event possible.

Summary of the COST Action Young Researcher and Innovators Day

The summary is related the Marathon DAY 4 held on **November 28, 2024**, dedicated to the COST Action's Young Researcher and Innovators Day. The session featured presentations of short-term scientific missions (STSMs) and research projects, concluding with the announcement of two awards for the best STSM presentation and the best overall young researcher presentation.

📣 Opening Remarks and Logistics

Welcome and Awards: Elisa Uliassi and Maria Paola Costi (COST Action Coordinator) opened the session, welcoming young researchers and innovators. They highlighted that the day was dedicated to their work, with prizes to be awarded for the **best STSM presentation** and the **best overall young researcher presentation**.

Committee: Anabela Cordeiro and Gulsah Bayraktar joined Elisa and Maria Paola Costi on the committee that would select the award winners.

Future Meeting: A young researcher meeting was scheduled for **December 19th at 5:00 PM** to discuss future activities and provide updates.

Anabela Cordeiro's Offer: Anabela Cordeiro extended an invitation for young researchers to collaborate with her group, offering facilities to test compounds *in vitro* and *in vivo* in the preclinical phase.

🔬 Short-Term Scientific Mission (STSM) Presentations

The core of the meeting consisted of presentations on research conducted during STSMs and related projects:

1. Imaging Viron Gradients and Ferroptosis in Malaria Parasite (Rodrigue)

Host/Context: STSM at the Hamble Institute (Tobias Spielmann).

Focus: Investigating **ferroptosis**—a form of regulated necrotic cell death driven by iron-dependent lipid peroxidation—as a potential mechanism of action for the anti-malarial compound **Plasmodium** (from the benzene family).

Key Findings: Rodrigue showed that the compound induces **iron accumulation** (likely the heme complex, as clarified in discussion) within the infected red blood cells but not in non-infected cells, using fluorogenic probes like Silox and Asif Phenox. This suggests a **selective mode of action** that leads to parasite cell death, supporting the ferroptosis hypothesis.

Discussion: The discussion centered on whether the probe detects free iron or the heme-iron complex and the selectivity of the mechanism, given that *Plasmodium* parasites degrade hemoglobin to form toxic heme, which they polymerize into harmless hemozoin.

2. Revealing the Mechanism of Action of H80 (Lorenzo Tagliazucchi)

Host/Context: STSM at the National Hellenic Research Foundation (Prof. Katsila) and work at the University of Modena.

Focus: Characterizing the novel anti-leishmanial agent **H80** to identify its target or pathway using a **multi-omics integration** approach (ADMETox, Mass Proteomics, and Mass Metabolomics).

Key Findings:

Fluorescence Imaging: Showed H80 selectively accumulates **inside the parasite** (within the infected macrophages), suggesting a selective internalization pathway.
Proteomics: Revealed the upregulation of the **ribosomal complex**, **energetic metabolism** (TCA cycle, oxidative phosphorylation), and **trans-membrane transport**.
Metabolomics: Identified perturbations in **phenylalanine, amino sugar, and pyrimidine metabolism**. This aligns with an earlier hypothesis that H80 may target proteins involved in pyrimidine/purine biosynthesis (e.g., PTR1).
Discussion: Questions were raised about the initial short resistance-induction studies and the compound's internalization pathway. Lorenzo suggested it might be **endocytosis** based on kinetics, but conceded that transporter-mediated uptake is also possible and will be investigated further.

3. Molecular Docking Study on Anti-parasitic Nucleoside Drugs (Aleksandar Cvetkovski)
Host/Context: STSM at the University of Tartu (Professor Alfonso Garcia Sosa).
Focus: Using **molecular docking** to predict the binding poses and efficiencies of 24 nucleoside analogue drugs against **adenosine kinase (AK)** from *Trypanosoma cruzi* and *Leishmania mexicana*.
Key Findings: The study found **structural differences** in the active site of the AK between the two parasites, despite high overall protein similarity. The results, consistent with experimental data, showed that **Tubercidin** (a known drug) had the highest docking score and efficiency against *T. cruzi* AK but was less efficient against *L. mexicana* AK. Surprisingly, the study found **minimal interactions** with the purine ring of the ligand.
Discussion: The discussion focused on the unexpected lack of interaction with the purine moiety and the resulting low selectivity, which is counterintuitive for an adenosine kinase inhibitor. The goal is to identify and optimize new substrates for both parasites.

4. Synthesis and Biological Evaluation of New Fiazolidinone Analogues (Theano Fotopoulou)
Host/Context: Work at the National Hellenic Research Foundation.
Focus: Synthesizing a library of **fiazolidinone derivatives** as **bioisosteric replacements** for the phosphate group in active ether phospholipid anti-leishmanial agents, aiming for improved selectivity and reduced toxicity.
Key Findings: A library of 32 novel compounds was synthesized using multi-component reactions. The most potent analogues showed high activity against *L. infantum* promastigotes and amastigotes, with **low toxicity** against THP-1 macrophages. Compounds with a **longer chain** between the ring and the core showed better activity against *T. brucei*. Early ADMETox showed favorable metabolic stability.
Discussion: The presentation was lauded for the extensive synthetic and SAR (Structure-Activity Relationship) exploration. Questions were raised about the synthetic challenges and the required selectivity index (at least 10x, ideally 20x to 50x) over human cells.

5. Targeting the Folate Cycle Proteins (Giulia Saporito)
Host/Context: Work at the University of Modena, in collaboration with the University of Geneva.
Focus: Evaluating a new series of **1,3,5-triazine derivatives** against key enzymes in the parasite folate cycle: **Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS)** from *T. brucei* and *L. major*, and **Trypanothione Reductase 1 (TB PTR1)**. **Key Findings:** The compounds showed good activity in the low micromolar range against both parasitic DHFR-TS enzymes. One compound, **Is 10**, showed potential as a **dual inhibitor** targeting both DHFR-TS and TB PTR1.
Discussion: The main point of discussion was the lack of selectivity over **human DHFR**. While acknowledging that new anti-parasitic drugs should ideally be selective, the point was made that non-selective inhibition can sometimes be managed with folate supplementation, as is done in cancer therapy.

6. Libraries of Analogues of Endoperoxides (Dafni Graikioti)
Host/Context: STSM at the ICCF in Toulouse, France (Dr. Michel Baltas).
Focus: Synthesizing novel **endoperoxide analogues** as potential anti-malarial agents, specifically aiming to create **hybrid molecules** by combining the endoperoxide scaffold (like G-Map) with other anti-malarial pharmacophores (like chloroquine or artemisinin) via **click chemistry**.
Key Findings: Dafni successfully synthesized the endoperoxide core and several intermediates, including key alkene and azide-bearing precursors. Challenges were faced in the final functionalization steps (e.g., with bromides), requiring further optimization.

Discussion: The synthetic efforts were highly praised. Questions were asked about the stability of the crucial **endoperoxide bond** (which is sensitive to acids and heat) and the importance of checking for off-target effects like red blood cell hemolysis for the resulting hybrid molecules.

7. Towards the Discovery of Novel Antibiotics (Roberta Listro)
Host/Context: First time presenting at the COST Action, from the University of Pavia.
Focus: Addressing **Antimicrobial Resistance (AMR)** by targeting the bacterial enzyme **Sortase A (SrtA)**, which is essential for anchoring virulence factors to the cell wall and is key to **biofilm formation**.
Key Findings:
Virtual Screening: Used computational docking and molecular dynamics on *Staphylococcus aureus* SrtA against a natural product database (ZINC 20) to identify 24 hits, which were filtered into 7 unique scaffolds.
Inhibition Assay: Two compounds showed promising ≥50% inhibition in an *in vitro* SrtA assay.
Adhesion Assay: The best compounds showed a preliminary 15−20% inhibition of bacterial adhesion to a fibrinogen substrate at a low concentration (100µM).
Future Work: Will focus on identifying other synergistic metabolites in the natural matrix or modifying the best-performing natural scaffolds into small-molecule SrtA inhibitors