Deliverable reports

Deliverables 10: Report on imaging and target engagement studies

(Action Deliverables are distinct, expected, and tangible outputs of an Action which are meaningful in terms of the Action's overall Objectives, such as reports, documents, technical diagrams, software, etc.)

Challenge of reference:

Challenge 2. The impact of pharmaceutics and their R&D process on the environment is high and it is responsible of huge loss due to contaminated water that affects human and animal health, generate drug resistance problems. *Integrated multidisciplinary efforts (design, synthesis/extraction, in vitro and in vivo biological/animal studies, delivery) should be developed to reduce the drugs impact on the environment at every step of the drug research and development process. This requires the coordinated action of researchers and stakeholders (governmental bodies, patients' organizations, industries and SME***.**

Objective of reference (Research Coordination Objective)

Objective 3. **Coordination of the translation from *in vitro*-to-*in vivo* activities to obtain high quality leads and candidates**. Actions: Introduction of omics technologies (genomic, proteomics and transcriptomics) and imaging for a limited number of validated leads. Drug delivery of biodegradable nanotechnology and drug targeting tools applications for both H&A R&D, pharmacology (pharmacokinetics and pharmacodynamics) on animal models by changing drug regimen and study of the effects of the different drug bioavailability tools. This can be acquired through European RTD organization and allow the achievement of high-quality leads with associated biological properties tailored for the H&A VB parasitic infections drug research program. **KPI**: 3 metrics adopted to measure objective 3 to be reviewed once a year. 2.1 Number of successful examples of biodegradable nanotechnology formulation on dose regimen for each infection. 2.2 Number of projects developed in collaboration with the RTD platform and other stakeholders. 2.3 Number of animal studies including the degradable formulation and targeting vectorization.

Working group of reference:

WG3. Coordination of *in vitro*-to-*in vivo* translation *of One Health* leads and candidates. (Challenge 3**) Objectives.** Promoting and strengthening of innovative technologies required in the translation of leads and candidates from animal to humans and vice versa to ensure the progression of qualified leads and candidates to the end of the pre-clinical phase and de-risk studies in clinical phase 1. This is restricted to advanced leads and candidate.

T3.2 *Coordination of imaging and target engagement studies.* The EU RTD platforms and centres (EMBL, ESFR, OPENSCREEN) will be available to collaborate with the Action and accept PhD students for projects and experiments. (D3.2).

Deliverable description

Vector-borne diseases pose global health challenges, requiring innovative drug discovery approaches. Target identification and mechanism of action (MoA) studies are essential to understand pathogen-host interactions and identify molecular targets crucial for pathogen survival, replication, or transmission. Target engagement studies complement these by confirming drug-target interactions, with imaging technologies like fluorescence microscopy providing real-time visualization of drug effects. In combination with omics technologies (genomics, proteomics, and transcriptomics) and imaging, applied to a select number of validated leads. Imaging enhances preclinical studies by tracking pathogens, drug localization, and host-pathogen dynamics, bridging the gap between molecular insights and therapeutic efficacy. However, knowledge gaps, such as limited access to tools and lack of standardized protocols, hinder integration into workflows. Addressing these through collaboration and training can accelerate drug discovery, improve understanding of drug mechanisms and targets, and advance treatments for vector-borne diseases.

Description of what we have done (the meetings, training schools, STSM workshop performed, the *reports, documents, technical diagrams, software etc. with reference – link to the website or other external document of interest)*

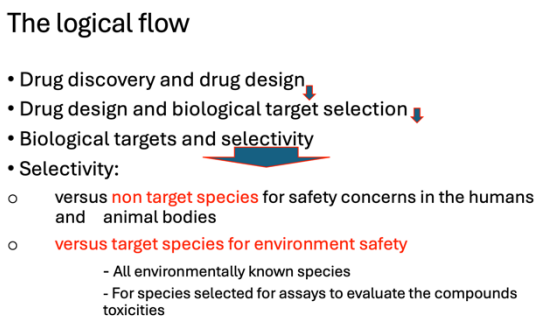
Workshops

Novel leads and drugs for vector borne diseases: Targets and off targets (toxicity and ecotoxicity) and mechanism of action. Presential event: September 19-20 2024, National Hellenic Research Foundation, Athens, Greece.

The workshop aims to provide up-to-date information and strategies for sustainable drug discovery targeting vector-borne diseases (VBDs). Special emphasis will be placed on identifying new targets for VBDs, methods to study target engagement, and leveraging imaging techniques in preclinical drug discovery. This collaborative event seeks to address critical gaps in knowledge, enhance understanding of methodologies, and promote their integration into preclinical studies. Additionally, the workshop will focus on biodegradability and toxicity considerations, highlighting their impact on the environment. By bringing together scientists, academics, and industry professionals, the platform will foster the sharing of insights and advancements toward environmentally conscious antiparasitic drug discovery. https://www.onehealthdrugs.com/events/scientific-meeting/novel-leads-and-drugs-for-vector-borne-diseases-targets-and-off-targets-toxicity-and-ecotoxicity-and-mechanism-of-action/

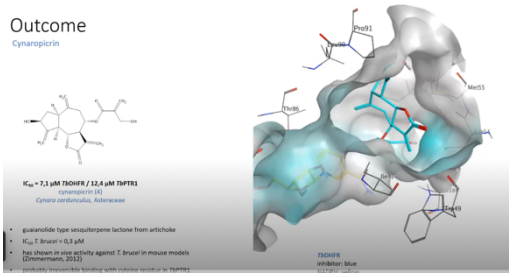
WG1 workshop - 17th April 2024 - 14:00-18:00 CEST online. Structural and functional aspects of targets involved in vector borne diseases

Investigating the structural and functional aspects of macromolecular targets provides essential insights for the rational development of drugs against vector-borne diseases. Structural biology, which integrates techniques such as NMR, X-ray crystallography, bioSAXS, and cryo-EM, is a powerful field for obtaining atomic-level information on these targets. Functional aspects are explored using diverse approaches, including in silico modeling, bioinformatics, artificial intelligence, and biochemical and biophysical methods. To ensure comprehensive safety, ecotoxicological considerations will be incorporated into target



characterization, promoting drug development that safeguards not only human and animal health but also environmental organisms—aligning with the principles of the One Health approach. We try to find an answer to the question "*Why structural biology and x-ray crystallography are important for the design of drugs safe for humans and animals, and the environment?*" This workshop seeks to advance knowledge on the structural and functional aspects of key targets in vector-borne diseases, fostering innovation and sustainability in drug discovery. It can be argued that we can connect structural biology and drug safety. https://www.onehealthdrugs.com/events/scientific-meeting/structural-and-functional-aspects-of-targets-involved-in-vector-borne-diseases/

15 May 2023, 14:00 - 17:00 CEST. Novel leads and drugs and their mechanism of action in the field of vector borne parasitic disease (web address https://www.onehealthdrugs.com/events/scientific-meeting/novel-leads-and-drugs-and-their-mechanism-of-action-in-the-field-of-vector-borne-parasitic-disease/)



The workshop aims to bring together experts to discuss and establish common definitions of novel leads, drugs, and their mechanisms of action, with a particular focus on their relevance to One Health needs. It will provide an overview of the state of the art in the field and serve as a platform to develop a strategic roadmap for achieving the core objectives of the COST Action.

STSM

STSM - Dafni Graikioti - 10/06/2024 to 24/07/2024. Libraries of analogues of Eucalyptus G-endoperoxides, antiparasite activities, mechanisms of action.

The team has developed a family of naturally occurring endoperoxides known as G-factors (G1–G3). These compounds are six-membered unsaturated cyclic endoperoxides fused to another six-carbon ring derived from syncarpic acid. They are naturally found in the leaves of Eucalyptus grandis and other myrtaceous plants. Two synthetic methodologies have been successfully developed and optimized, including one that enables the production of carbon-labeled derivatives.

Our studies on the antimalarial activity of these synthesized compounds have revealed the following key findings:

a) The G3 compound exhibited very weak activity against Plasmodium falciparum (IC50 = 36 µM), whereas its methylated derivative, G3Me, showed significantly improved antimalarial activity (IC50 = 280 nM).

b) Alkylation at the R1 (or R2) and R positions resulted in compounds with highly potent antiplasmodial activity.

c) Hybrid molecules were synthesized with exceptional potency, achieving IC50 values of 13 nM and 31 nM against the chloroquine-resistant W2 strain and the chloroquine-susceptible 3D7 strain, respectively.

Interested stakeholders

Stakeholders interested in imaging and target engagement studies for pharmaceutical development against parasite vector-borne diseases include Pharmaceutical and Biotechnology Companies related to identification and validation drug candidates and drug targets through advanced imaging techniques to accelerate development and approval of treatments for diseases like malaria, leishmaniasis, and Chagas disease; Academic and Research Institutions in advancing scientific understanding of parasite biology, drug mechanisms, and host-pathogen interactions using cutting-edge imaging technologies; Imaging Technology Developers by partnering with researchers to design and implement imaging tools tailored for target drug studies in vector-borne diseases.

By collaborating, these stakeholders play a critical role in leveraging imaging and target engagement studies to address vector-borne parasitic diseases more effectively.

Scientific impact (from the MoU)

- a shared experience for researchers, industry stakeholders and national/international organizations opening the way to novel fruitful collaborations for transfer of knowledge/ new knowledge creation about targets, drug research strategies, hits and leads elaboration, assays for HTS approaches, nanotechnology for drug delivery and animal studies; ecotoxicology and environmental tools applied to the research process.
- the engagement of novel and specific targets based on H&A biology comparative studies in particular in the field of degradome and soluble protein transporters (membrane proteins) for drug design, including specific targets against vectors;
- the prevention or overcoming of drug resistance such as host-parasites targets engagement using chimeric compounds as an innovative approach, more effective in drug resistance;
- omics and imaging studies, with synthetic conjugation technologies and structural biology in combination with advanced molecular biology for the mechanism of action studies, new targets identification.

The long-term benefits (from the MoU

- substantial improvements of the biological profile in treating parasitic diseases caused by VB parasitic diseases affecting H&A;
- engagement of RTD platforms active in the field;
- a permanent on-line network of stakeholders in antiparasitic drug discovery and development to maintain a transfer of knowledge, new One Health knowledge creation and strengthen collaboration.

Innovation

Cross-sectorial and interdisciplinary networking approach to advance the drug discovery and development field in VB parasitic diseases in H&A (because an effective cure of the human infections can be achieved if animals' infections are cured or eliminated). Integration of the innovative approaches with the environmental impact concepts, will involve also pharmaceuticals manufacturing and use.

Hits and lead compounds database tailored on H&A VB parasitic diseases and obtained during the Action lifetime will foster new research activities.

The Action will facilitate such innovation through active promotion of the database (IP regulated) and search for collaborators in academia and industry. IP on new advanced candidates with promising or relevant pharmacological activity will be promoted.