Deliverable 7

Title: Report on HTS assays and structural biology

Month of release: M42

Working group and HG involved: WG1, WG2, WG3

Challenge of reference:

Challenge 1. Existing drugs for human and animal neglected infections against VBD are scarce, with limited efficacy and toxicity. Therefore, a collective effort for innovative compounds and prediction tools should be identified to improve the therapeutic properties of the antiparasitic drugs and identify new drugs.

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.

Challenge 3. Not enough control and informed procedures are available to link the environmental impact to drug production and use. Specific guidelines do not exist to address the problem during the whole process from conception to manufacturing and use. Concepts and strategic support in a guideline/white paper format should be given to the stakeholder in the field for a standard approach to develop safer drugs for humans and animals.

Objective of reference (Research Coordination Objective)

Objective 1. Coordination and integration of the medicinal chemistry programs by collecting and re-using large compounds databases available from different sources. Associate the libraries with in silico prediction of compounds behaviors (e.g. molecular and chemioinformatic properties, metabolic and chemical degradation, ADME-tox properties of metabolites) to improve the properties of the antiparasitic drugs. Actions: Increase the number of compounds to screen in a coordinated approach (from virtual or phenotypic approaches). Coordination of the compounds' libraries available and usable for screening and repurposing studies by the participants. The compounds libraries that are IP free (with no intellectual protection), discovered previously or/and during the 4 years of the Action, will be made available to all participants. Compounds degradation prediction. Collection of the information in an open access database repository dedicated to OneHealth*drug*, such as FAIRDOM database (FAIRDOM is open source database). **KPI**: the Action will adopt 3 metrics to measure objective 1.1 activities to be updated, at least every 6 months 1.2 Number of compounds identified belonging to different libraries >10000. 1.3 Number of in silico studies including metabolic (enzyme assisted) and chemical degradation.

Working group of reference:

WG1. Compound libraries coordination and integration of compound design. (Challenge 1) (Dx from MoU)

Objective: Increase the number of compounds available for the drug research projects that should be screened adopting a coordinated approach (virtually or in phenotypic approaches) to ensure innovation in anti-parasitic drug discovery. Collection and coordination of data and information about compound design.

T1.3 *Targets selection, structure-based drug design by advanced modelling/compound* docking on available and novel 3D protein structure (X-ray crystallography and cryo EM-electron microscopy); chemoinformatic methods and artificial intelligence (AI) data analysis from HTS technologies (Bayesan models, and others). (D1.3)

T1.4 *Compounds biodegradability pattern prediction*. This can be performed using in silico tool that can provide insight into the chemical degradation of organic compounds under various environmental conditions. (D1.4)

WG2. Integration of early phase studies and low environmental impact actions. (Challenge 2)

Objectives: Obtain new compounds from different sources and study those compound *in vitro* for profiling and selection for advances in the process.

T2.3 *HTS screening of library from WG1 and T2.2 to evaluate ADME-Tox properties, biological data from assays panels*. Compounds metabolic degradation analysis. All data are included in FAIRDOM database. Connection with the international database CDD Vault50 will be established (D2.3).

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.

T3.3 *Coordination and integration of omics studies (PROTEOMICS, Genomics, TRANSCRITTOMICS) and validation technologies*, to better qualify the mechanism of action and drug resistance. All data will be deposited in the FARIDOM database. Protein targets and biological pathways from the omics studies will be validated through the evaluation of differential expression of proteins and their functional studies in cells models. (D3.3)

Deliverable description.

The deliverable reports the activities carried out to support the identification and prioritization of new antiparasitic drug candidates for vector-borne parasitic diseases, addressing the limitations of current therapies and the need to reduce environmental toxicity from the earliest stages of drug discovery. Within this framework, biological targets and compound collections made available by CA21111 members were collected, organized and integrated into two complementary resources: the BioTarget Database and the Virtual Chemotheca. These resources provided the basis for a coordinated structure-based screening workflow in which target and compound information were cross-referenced through a docking campaign conducted within the OHD:LUB project. The project addresses the urgent need for novel treatments by integrating large-scale, structure-based

screening campaigns with the development of a specialized digital infrastructure: **FAIRMol DockDB**. This interactive database platform (available at https://fairmoldockdb.molekule.net) serves as a central hub for securely storing, intuitively visualizing, and comprehensively analyzing the vast amounts of data generated from molecular docking simulations. These virtual studies were performed using, among others, the compounds from the OHD compounds database (https://www.cclab.unicz.it/ohd/index.php) against all the target included in the BioT-DB containing the antiparasitic protein targets collected from the OHD network participants.

The **OHD1 Target Database** project established the **BioTarget Database (BioT-DB)**, a structured resource for collecting and sharing information on biological targets investigated by CA21111 members. The database is organized into ten coordinated sections. Each target record combines general identifiers and sequence information, including links to UniProtKB and BRENDA, with functional annotation, production protocols, biochemical and biophysical characterisation, omics evidence, medium- and high-throughput screening data, and selectivity or specificity profiles. A dedicated structural section records available and unpublished information derived from X-ray crystallography, cryo-EM, BioSAXS and NMR, with links to PDB, EMDB, SASBDB and BMRB. Ecotoxicological relevance is documented through SeqAPASS or related approaches.

The resource was designed to facilitate internal collaboration by associating experimental information with contributor contact details and by enabling future integration with the CA21111 compound databases. As summarised in Table 1, the current dataset comprises 15 target entries from Leishmania and Trypanosoma species, covering redox enzymes, polyamine metabolism, sirtuins, folate and pteridine pathways, phosphohydrolase activity and a chaperone protein. Following scientific curation and validation, the information associated with these targets will be published in the BioTarget Database FAIRDOM-HUB, supporting FAIR access, traceability, reuse and continued community updating.

Then, the OHD:LUB Project connected compound collections (Virtual Chemotheca), target structures and environmental prioritisation. Compound libraries were docked against the official panel of 20 antiparasitic targets, and the resulting poses were processed through a decision layer combining docking information, predicted ADME/Tox properties and ecotoxicological scores. The computational workflow generated 32,143 docked compound states. This value represents protonation, tautomeric or docking states and must not be interpreted as the number of unique compounds in the Chemotheca.

Table 1. Targets available in the BioTarget Database (BioT-DB).

	TARGET	ORGANISM
1	Trypanothione Reductase (TR or TryR)	*Leishmania infantum*
2	Trypanothione Reductase (TR or TryR)	*Trypanosoma brucei brucei*
3	Ornithine decarboxylase (ODC)	*Leishmania infantum*
4	Silent Information Regulator 2 related protein 1 (LiSIR2rp1, or sirtuins)	*Leishmania infantum*
5	Bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS)	*Trypanosoma brucei brucei*
6	Dihydrofolate reductase (DHFR), isolated domain	*Trypanosoma brucei brucei*
7	Bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS)	*Trypanosoma cruzi*
8	Bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS)	*Leishmania major*
9	Dihydrofolate reductase (DHFR), isolated domain	*Leishmania major*
10	Pteridine reductase (PTR1)	*Trypanosoma brucei brucei*

11	Pteridine reductase (PTR1)	*Trypanosoma cruzi*
12	Putative pteridine reductase 2 (PTR2)	*Trypanosoma cruzi*
13	Pteridine reductase (PTR1)	*Leishmania major*
14	5'-deoxynucleotidase (or phosphohydrolase, PH)	*Leishmania donovani*
15	Tir chaperone protein (CesT)	*Leishmania donovani*

HTS assays

The OHD project operates as a collaborative initiative within the framework of the OneHealthdrugs COST Action, dedicated to accelerating the discovery of new antiparasitic compounds through the strategic application of advanced computational methods. This approach accelerates the hits identification. The project addresses the urgent need for novel treatments by integrating large-scale, structure-based virtual screening campaigns with the development of a specialized digital infrastructure: FAIRMol DockDB. This interactive database platform (available at https://fairmoldockdb.molekule.net) serves as a central hub for securely storing, intuitively visualizing, and comprehensively analyzing the vast amounts of data generated from molecular docking simulations.
The innovative aspect is that the HIT compounds identified are selected not only for the ADME-TOX but also for ecotoxicity parameters using the GreenDrugScore (Leveraging Ecotoxicity Parameters and Machine Learning to Rethink the Drug Discovery Pipeline, Journal of Pharmaceutical Analysis, 2026, 101734, https://doi.org/10.1016/j.jpha.2026.101734.)
The primary scientific objective driving this effort is to bridge the gap between computational prediction and experimental validation. By screening a diverse collected chemical library comprising more than 30,000 compounds against 22 distinct protein target configurations relevant to various parasitic diseases, the project aims to rapidly identify promising ligand–target interactions. To manage this scale, specific objectives included the rigorous preparation of biological targets and chemical structures, alongside the development of a robust prioritization protocol designed to select the most biologically relevant docking results for further investigation. Ultimately, the OHD project seeks to establish a sustainable framework that connects computational researchers with compound owners and laboratories capable of performing biological testing, thereby facilitating the seamless transition of promising candidates into experimental evaluation.

Description of the activities performed

Training schools (https://www.onehealthdrugs.com/events/training-schools/)

"Expression, purification and basic characterization of target protein samples for drug binding studies" 8-10/9/2025, Riga, Latvia. The focus of this training school was to provide basic knowledge and hands-on experience on protein expression protocols using bacterial cells, protein purification methods, and biophysical techniques for the rapid assessment of protein stability and purity. The school featured e a combination of lectures and practical laboratory activities allowing the students to familiarize with the most common procedures for large scale protein expression in bacteria, standard protein purification protocols using liquid chromatography techniques as well as some basic biophysical techniques, including Circular Dichroism (CD), Differential Scanning Fluorimetry (DSF), and Mass Spectrometry (MS).

“Natural products in antiparasitic drug discovery: experimental and computational approaches” 18-20/6/2024, Naples, Italy. The focus of the training school through lectures and hands-on experiments was to explore the principles underlying i) methods for isolation and structural characterization of antiparasitic metabolites from plants and marine organisms, ii) in silico methods for hit identification and optimization, and iii) green synthetic approaches for structural optimization.

“MedChem and structural biology: tools and strategies for hit and lead optimization in the One Health perspective” 27-29/09/2023, **Siena, Italy** During this training school the importance of computational methods and tools for chemical safety and sustainability assessment, regulatory compliance, Read Across, and the applicability of in silico tools for predicting toxicity were discussed. Practical sessions using VEGA software provided hands-on experience, enabling attendees to assess chemical compound characteristics and toxicities. The event fostered networking and collaboration opportunities among participants. Overall, the significance of sustainable and innovative strategies in advancing drug development and addressing infectious diseases has been critically highlighted.

“Drug discovery against parasitic vector borne diseases in-vitro assays development and screening in drug discovery”. 8-10/5/2023, Hamburg, Germany. The initial aim of drug discovery is to develop compounds that exhibit appropriate safety and efficacy profiles which can be progressed to clinical evaluation. The focus of this Training School was to explore the principles underlying target identification, biochemical and cell-based assay development, small molecule screening and in-vitro ADME-Tox profiling to produce compounds ready for clinical studies, whilst maintaining low environmental impact.

Workshops and meetings

Workshop online - Virtual screening of OHD Compound libraries against parasitic targets
13/05/2026 14:00-16:00 CET Online
The OHD:LUB Project is the OneHealthdrugs COST Action's premier success story, showcasing how interdisciplinary synergy can fast- track drug discovery on a budget. Its workflow bridges the gap from supercomputing to sustainable therapy through three key phases:
Phase I: High-Velocity Virtual Screening – Leveraging the Vega HPC system, the team (led by Podlipnik and Jukić in collaboration with D. Aiello) has screened 36,000+ compounds against 22 targets. With 21 targets already docked, they are now using 3D interaction fingerprints to refine the most promising hits.
Phase II: The "Green" Triage – In a pioneering move, the project uses ML-driven ecotoxicity models (in collaboration with Daniele Aiello) to filter hits. This creates a "Green Drug Score," ensuring candidates are both potent and environmentally benign from the outset.
Phase III: Experimental Validation – With a deadline of October 2026, the project has launched a "call to action." While labs prepare protein purification, Sheraz Gul (Fraunhofer) provides HTS and ADMET testing infrastructure. STSM funding is prioritized for young researchers to travel and conduct the final physical assays.
https://www.onehealthdrugs.com/events/scientific-meeting/meeting-2026/virtual-screening-of-ohd-compound-libraries-against-parasitic-targets/

Workshop online - The OHD:LUB project results. 1st round Target Screening and OHD-compound prioritization” Monday, 22nd June 2026 14:30-16:30 (CET)
The workshop was organized with the aim to develop and realize the OHD:LUB project. This is open to medicinal chemists and interested scientists of the OHD COST Action. In particular, those who participated to the OHD:LUB project by contributing with targets suggestions, compounds in house or from the OHD database, assay

performance (antiparasitic, ecotox evaluation) and ecotox prediction, protein availability, structural biology (X-ray). The aim is to show the first-round results, start the organization of the next step experimental process, promote medicinal chemistry networks and program. Specific appointments will be planned in dates to be defined. Agreement among the interested parties will be necessary to ensure the most fruitful and smart exploitation of the computational work. This workshop will be culminating event of this collaborative project providing the opportunity for exploitation also after the end of the OHD COST Action. The agenda is the following:
Project Overview: Presentation of the OHD:LUB initiative.
Technical Framework: Detailed description of current tools and methodologies.
Team & Collaboration: Updates on participating scientists and guidelines for collaborative activity management.
Experimental Roadmap: Planning upcoming experiments and reviewing available exploitation platforms for testing, including a breakdown of available expertise. Strategic Planning: Coordination of compound-protein target-assay workflows.
https://www.onehealthdrugs.com/events/scientific-meeting/meeting-2026/workshop-online-the-ohd-lub-project-
results-1st-round-target-screening-and-ohd-compound-prioritization/

"Parasitology and Biological Evaluation for Drug Screening in One Health Perspective" Online event. 19/2/2026. This Young Researchers and Innovators meeting focused on parasitological assays and the biological evaluation of compounds for antiparasitic drug screening. Case studies connected screening workflows with the One Health framework.

Workshops and meeting 2025 (https://www.onehealthdrugs.com/events/scientific-meeting/next-meeting/)

"Imaging and Biosensor Strategies in Drug Discovery for Vector-Borne Diseases" Online event. 26/9/2025. This workshop explored imaging, fluorescent probes and biosensor cell lines for target identification, target engagement and assay development. It included high-throughput biochemical, high-content microscopy and flow-cytometry approaches for monitoring drug-induced effects.

"Advanced Technologies Integration into Medicinal Chemistry Programs" Online event. 30/6/2025. This workshop presented the strategic integration of high-throughput screening, AI-driven drug design and omics technologies into medicinal chemistry pipelines. The approaches were discussed as tools to improve target specificity and accelerate antiparasitic drug discovery.

"OHD 2025 Annual Conference: Strategies for Breaking the Chain Between the Parasite Reservoir in Animals and Infections in Humans" In presence event. 4-6/6/2025, Antwerp, Belgium. The conference addressed assays for target and off-target effects and the identification and structural characterization of therapeutic targets for vector-borne diseases. These topics were integrated with medicinal chemistry, parasitology and the One Health assessment of environmental impact.

Workshops and meeting 2024 (https://www.onehealthdrugs.com/events/scientific-meeting/past-meetings-in-2024/)

"Novel Leads and Drugs for Vector-Borne Diseases: Targets and Off-Targets (Toxicity and Ecotoxicity) and Mechanism of Action" In presence event. 19-20/9/2024, National Hellenic Research Foundation, Athens, Greece. This WG1/WG2/HG7 workshop examined new targets, target engagement and mechanisms of action in

sustainable antiparasitic drug discovery. The programme included structure-guided inhibitor design and phenotypic and target-based screening approaches.

"Advancements in Structural and Functional Drug Discovery of Vector-Borne Diseases" In presence event. 3-5/7/2024, Saïd Business School, Oxford, and Diamond Light Source, United Kingdom. This meeting brought together structural biology, biophysics and rational drug design to advance antiparasitic

drug discovery. It promoted the integration of chemical research with structural sciences for the investigation of disease-relevant targets.

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drug discovery. It promoted the integration of chemical research with structural sciences for the investigation of disease-relevant targets.

"Structural and Functional Aspects of Targets Involved in Vector-Borne Diseases" Online event. 17/4/2024. This WG1 workshop focused on the structural and functional characterization of macromolecular targets relevant to vector-borne diseases. Approaches included NMR, X-ray crystallography, bioSAXS, cryo-EM, bioinformatics and biochemical and biophysical methods.

Workshops and meeting 2023 (https://www.onehealthdrugs.com/events/scientific-meeting/past-meetings-in-2023/)

"Novel Leads and Drugs and Their Mechanism of Action in the Field of Vector-Borne Parasitic Disease" Online event. 15/5/2023. This joint WG1/WG2 workshop reviewed novel leads, drugs and mechanisms of action in vector-borne parasitic diseases. It provided a conceptual basis for target-oriented studies and for integrating compound information into the OneHealthdrugs discovery roadmap.

STSM

"Determination of in vitro ADME-Tox properties of newly optimized pyrimido[5,4-d]pyrimidines with antileishmanial activity"

Period: 29/04/2026-28/07/2026
Student / grantee: André Manuel Gomes Lopes
Host institution: Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Drug Screening & Compound Repurposing, Germany
The mission screened 566 compounds in five cell-based and ten enzymatic assays using high-throughput equipment, including an Echo liquid handler and an EnVision plate reader. The panel covered protein targets and enzymes such as HDAC6/8, PDE4C1/D2, ROCK1, G6PDH and CYP isoforms.

“High throughput cloning, production, and crystallization of Leishmania infantum calpain, a new promising target against Leishmaniasis”

Period: 16/06/2025-29/08/2025
Student / grantee: Cecilia Pozzi
Host institution: Diamond Light Source Ltd, Harwell Science & Innovation Campus, Didcot, United Kingdom
High-throughput cloning and expression screening generated a library of LiClp constructs and fusion-tag combinations, followed by Ni-NTA, affinity and size-exclusion purification workflows that improved the yield of the selected construct to approximately 2 mg/L of culture. The purified protein supported an automated crystallization campaign covering more than 500 conditions, establishing a route toward structural, biochemical and biophysical characterization of this new Leishmania infantum calpain target.

“In-vitro and in-silico analysis of neglected infectious disease compounds from an ADMET and ecotoxicology perspective”

Period: 13/01/2025-12/07/2025
Student / grantee: Lorenzo Raffellini
Host institution: Fraunhofer ITMP - Discovery Research ScreeningPort, Germany
This STSM combined compound screening with the development of an ecotoxicology assay in a 384-well HTS format. The miniaturized workflow enabled parallel testing with reduced reagent consumption and rapid data acquisition.

“Modelling of binding affinity of a potential new class of anti-parasitic drugs”

Period: 19/08/2024-31/08/2024
Student / grantee: Aleksandar Cvetkovski
Host institution: Institute of Chemistry, University of Tartu, Estonia
Adenosine-kinase structures were generated with AlphaFold, refined and compared in PyMOL, and used to dock 24 nucleoside compounds with GNINA. The work addresses structural interpretation of protein-ligand interactions, but reports computational rather than experimental biophysical measurements.

“Investigation of the bifunctional enzyme dihydrofolate reductase–thymidylate synthase (DHFR-TS) from Leishmania major”

Period: 17/06/2024 – 26/08/2024
Student: Şener Çintesun
Host institution: Not specified in the linked award list
The listed mission focuses directly on the bifunctional DHFR-TS enzyme from Leishmania major, a defined antiparasitic protein target. The public award list does not provide the experimental or structural methods used.

“Redox reactions of plasmodione with oxyhemoglobin or heme (Fe2+)”

Period: 24/04/2024-02/05/2024
Student / grantee: Narimantas Čėnas
Host institution: Laboratoire d’Innovation Moléculaire et Applications (LIMA), UMR 7042 CNRS-Unistra-UHA, Strasbourg, France
Biochemical experiments tested plasmodione reactions with oxyhemoglobin and heme, with LC-MS tracking of metabolite formation and PfFNR-related redox cycling. The stated objective included determination of kinetic parameters for the proposed activation pathway.

“Revealing the MoA of the innovative antileishmanial agent H80, leveraging MS omics tools combined with ADME/Tox/Ecotox chemoinformatics”

Period: 02/04/2024-14/06/2024

Student / grantee: Lorenzo Tagliazucchi

Host institution: National Hellenic Research Foundation (NHRF), Athens, Greece

MS proteomics and bioinformatics were used to compare differentially expressed proteins and identify pathways and putative targets affected by H80. The report also links ADME predictions with CYP450 enzymatic assays, making the mission adjacent to protein-assay workflows rather than protein production.

Publications

1. Synthesis and Biophysical and Biological Studies of N-Phenylbenzamide Derivatives Targeting Kinetoplastid Parasites. J. Jonathan Nué-Martinez; David Cisneros; María del Valle Moreno-Blázquez; Cristina Fonseca-Berzal; José Ignacio Manzano; Damien Kraeutler; Marzuq A. Ungogo; Maha A. Aloraini; Hamza A. A. Elati; Alexandra Ibáñez-Escribano; Laura Lagartera; Tomás Herraiz; Francisco Gamarro; Harry P. de Koning; Alicia Gómez-Barrio; Christophe Dardonville. *Journal of Medicinal Chemistry* (2023), 66, 13452-13480. DOI: 10.1021/acs.jmedchem.3c00697

2. High-Throughput Phenotypic Screening and Machine Learning Methods Enabled the Selection of Broad-Spectrum Low-Toxicity Antitrypanosomatidic Agents. Pasquale Linciano; Antonio Quotadamo; Rosaria Luciani; Matteo Santucci; Kimberley M. Zorn; Daniel H. Foil; Thomas R. Lane; Anabela Cordeiro da Silva; Nuno Santarem; Carolina B Moraes; Lucio Freitas-Junior; Ulrike Wittig; Wolfgang Mueller; Michele Tonelli; Stefania Ferrari; Alberto Venturelli; Sheraz Gul; Maria Kuzikov; Bernhard Ellinger; Jeanette Reinshagen; Sean Ekins; Maria Paola Costi, *Journal of Medicinal Chemistry* (2023), 66, 15230-15255. DOI: 10.1021/acs.jmedchem.3c01322.

3. The discovery of aryl-2-nitroethyl triamino pyrimidines as anti-Trypanosoma brucei agents. Pasquale Linciano; Cecilia Pozzi; Giusy Tassone; Giacomo Landi; Stefano Mangani; Matteo Santucci; Rosaria Luciani; Stefania Ferrari; Nuno Santarem; Lorenzo Tagliazucchi; Anabela Cordeiro-da-Silva; Michele Tonelli; Donatella Tondi; Laura Bertarini; Sheraz Gul; Gesa Witt; Carolina B. Moraes; Luca Costantino; Maria Paola Costi. *European Journal of Medicinal Chemistry* (2024), 264, 115946, DOI: 10.1016/j.ejmech.2023.115946. PDB codes: 8OF2

4. Synthesis and Anti-Chagas Activity Profile of a Redox-Active Lead 3-Benzylmenadione Revealed by High-Content Imaging. Nathan Trometer; Jérémy Pecourneau; Liwen Feng; José A. Navarro-Huerta; Danielle Lazarin-Bidóia; Sueli de Oliveira Silva Lautenschlager; Louis Maes; Amanda Fortes Francisco; John M. Kelly; Brigitte Meunier; Monica Cal; Pascal Mäser; Marcel Kaiser; Elisabeth Davioud-Charvet. *ACS Infectious Diseases* (2024), 10, 1808-1838. DOI: 10.1021/acsinfecdis.4c00137.

5. Discovery of 1,3,4-Oxadiazole Derivatives as Broad-Spectrum Antiparasitic Agents. Alexandra Ioana Corfu; Nuno Santarem; Sara Luelmo; Gaia Mazza; Alessandro Greco; Alessandra Altomare; Giulio Ferrario; Giulia Nasta; Oliver Keminer; Giancarlo Aldini; Lucia Tamborini; Nicoletta Basilico; Silvia Parapini; Sheraz Gul; Anabela Cordeiro-da-Silva; Paola Conti; Chiara Borsari. *ACS Infectious Diseases* (2024), 10, 2222-2238. DOI: 10.1021/acsinfecdis.4c00181.

6. Proteomic Profiling of Antimalarial Plasmodione Using 3-Benz(o)ylmenadione Affinity-Based Probes. Ilaria Iacobucci; Vittoria Monaco; Agnès Hovasse; Baptiste Dupouy; Rodrigue Keumoe; Bogdan Cichocki; Mourad Elhabiri; Brigitte Meunier; Jean-Marc Strub; Maria Monti; Sarah Cianférani; Stéphanie A. Blandin; Christine Schaeffer-Reiss; Elisabeth Davioud-Charvet. *ChemBioChem* (2024), 25, e202400187, DOI: 10.1002/cbic.202400187.

7. Identification of Innovative Folate Inhibitors Leveraging the Amino Dihydrotriazine Motif from Cycloguanil for Their Potential as Anti-Trypanosoma brucei Agents. Valeria Francesconi; Marco Rizzo; Cecilia Pozzi; Lorenzo Tagliazucchi; Claude U. Konchie Simo; Giulia Saporito; Giacomo Landi; Stefano Mangani; Anna Carbone; Silvia Schenone; Nuno Santarém; Joana Tavares; Anabela Cordeiro-da-Silva; Maria Paola Costi; Michele Tonelli. *ACS Infectious Diseases* (2024), 10, 2755-2774. DOI: 10.1021/acsinfecdis.4c00113. PDB codes: 8RHT, 8RHU, 8RHV, 8RHW, 8RHX, 8RHY

8. Sustainable Antiparasitic Agents from an Agro-Industrial Waste: Mitochondria-Targeting Cashew Nutshell Liquid-Derived Phosphonium and Ammonium Salts. Bianca Martinengo; Cecilia Baldassarri; Kayhan Ilbeigi; Hamed E. Alkhalaf; Aditya Sarode; Ehab Kotb Elmahallawy; Ba Reum Kwon; Amos Sarpong Agyei; Aigerim Abdimanova; Ludmila Ferreira de Almeida Fiuza; Raquel Azevedo; Ketlym da Conceição; Marcos Meuser Batista; Ellyêssa Nascimento Borges; Kleber Santiago Freitas e Silva; Éder Jéferson Souza Cardoso; Natália Cipriano Monteiro; Lais Flavia Nunes Lemes; Luiz Antonio Soares Romeiro; Antonio Alonso; Maria de Nazaré Correia Soeiro; Guy Caljon; Bryan W. Brooks; Harry P. De Koning; Maria Laura Bolognesi. *Journal of Medicinal Chemistry* (2025), 68, 19438-19462. DOI: 10.1021/acs.jmedchem.5c01617.

9. Design of 2-Aminobenzothiazole Derivatives Targeting Trypanosomatid PTR1 by a Multidisciplinary Fragment Hybridization Approach. Joanna Panecka-Hofman; Pasquale Linciano; Ina Pöhner; Edyta Dyguda-Kazimierowicz; Wiktoria Jedwabny; Giacomo Landi; Nuno Santarem; Gesa Witt; Bernhard Ellinger; Maria Kuzikov; Rosaria Luciani; Stefania Ferrari; Daniele Aiello; Stefano Mangani; Cecilia Pozzi; Anabela Cordeiro-da-Silva; Sheraz Gul; Maria Paola Costi; Rebecca C. Wade. *Journal of Medicinal Chemistry* (2025), 68, 20595-20618. DOI: 10.1021/acs.jmedchem.5c01799. PDB codes: 9HUP, 9HUT, 9HUU, 9HUV, 9HUW

10. AgamOBP1-Directed Discovery of Repellents to Control the Spread of Mosquito-Borne Diseases. Evanthia Chazapi; Eftichia Kritsi; Constantinos Potamitis; Panagiota G. V. Liggri; Katerina E. Tsitsanou; Christina E. Drakou; Antonios Michaelakis; Dimitrios P. Papachristos; Spyros E. Zographos; Maria Zervou; Theodora Calogeropoulou. *ChemMedChem* (2025), 20, e202500555. DOI: 10.1002/cmdc.202500555. PDB codes: 3N7H, 5EL2

11. From pan-active to parasite-selective antiparasitic agents: A scaffold hopping approach. Chiara Borsari; Nuno Santarem; Dina Coertzen; Asia Mazzolari; Alexandra Ioana Corfu; Catarina Coelho; Francisca Barbosa; Lucia Tamborini; Lyn-Marié Birkholtz; Lorenzo Raffellini; Oliver Keminer; Nicoletta Basilico; Silvia Parapini; Sheraz Gul; Anabela Cordeiro-da-Silva; Maria Paola Costi. *European Journal of Medicinal Chemistry* (2025), 300, 118095. DOI: 10.1016/j.ejmech.2025.118095.

12. The heme A synthase Cox15, as a target of redox-active 3-benzylmenadiones with antiparasitic activity. Marcelo L. Merli; Claudia Serot; Cindy Vallières; Julia A. Cricco; Bogdan I. Iorga; Elisabeth Davioud-Charvet;

Brigitte Meunier. *Antimicrobial Agents and Chemotherapy* (2026), 70, e01161-25. DOI: 10.1128/aac.01161-25.

Interested stakeholders

Experts in medicinal chemistry, parasitology, and environmental toxicology who focus on drug discovery. Computational chemists and biologists involved in in silico prediction modeling. Organizations seeking innovative antiparasitic compounds for drug development pipelines. Stakeholders in pharmaceutical R&D emphasizing environmentally sustainable practices. Agencies interested in setting standards and policies for sustainable drug development with low environmental impact. Researchers participating in "OneHealthdrugs" initiatives or similar global programs aiming at interdisciplinary solutions for health challenges.

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.
- relevant scientific training for Young Researchers and Innovators in a multidisciplinary context favouring their career development.
- 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 discovery and optimization of high number of antiparasitic hits and lead compounds for H&A use with expected environmental low impact and higher quality profile than before;
- the development of multitarget compounds with different profile (finalized at PROTAC and other multitarget strategy) with chimeric structures; host-parasite chimeric compounds design
- the identification of novel hits and leads from organic material from the human activity (hemp, hives, frass and others) recycling the waste from industrial production;
- new biological assays for HTS and HCS screening and for lab scale studies of the mechanism of action together with the reduction of parasite growth;

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 long-lasting impact on antiparasitic drug development in Europe due to targeted training of Young Researchers and Innovators;

Innovation

The innovation of D12 lies in an integrated target-to-hit workflow connecting high-throughput protein production and screening with structural biology and computational prioritization. Miniaturized biochemical, cell-based and

ADME-Tox assays enable the rapid, parallel evaluation of compound libraries while reducing reagent consumption. Structural biology and complementary biophysical methods provide and share experimentally grounded information on target architecture, protein quality and ligand binding.

The BioTarget Database standardizes these data and links target information to production protocols, screening results and structural repositories. Its integration with the Virtual Chemotheca and the OHD docking workflow supports evidence-based compound prioritization and experimental validation. This coordinated pipeline is designed to shorten iterative cycles between target preparation, screening, structural interpretation and hit optimization. Overall, the approach accelerates the identification of validated antiparasitic hits and strengthens structure-guided drug discovery within a sustainable One Health framework.