CA21111 - One Health drugs against parasitic vector borne diseases in Europe and beyond (OneHealth.drugs)

STSM REPORT

STSM Application number: COST-STSM- CA21111

STSM Grantee Name: Lorenzo Raffellini

Young Researcher and Innovator

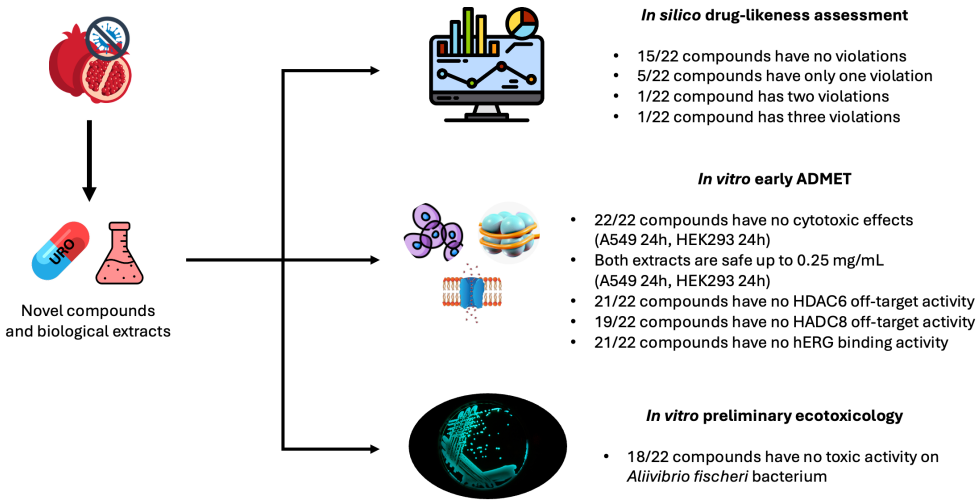
STSM title: *In-vitro* and *in-silico* analysis of neglected infectious disease compounds from an ADMET and ecotoxicology perspective

Home Institution: Department of Pharmacy, University of Pisa

Host Institution: Fraunhofer ITMP – Discovery Research ScreeningPort

STSM start and end date: 13/01/2025 to 12/07/2025

Working Group: WG1



Purposes of the STSM

Neglected infectious diseases (NIDs) are responsible of >500,000 deaths yearly and >billion people remain chronically injured or infected[1]. Leishmaniasis, a vector-borne NID caused by parasites such as *Leishmania donovani*, is increasingly resistant to standard treatments, with climate change contributing to its spread[2]. Another NID of concern is Zika Virus (ZV) infection, a vector-borne disease transmitted by mosquitos for which there are no specific or effective drugs[3].
Plant sourced substances are gaining attention in drug discovery for many pathologies and *Punica granatum* has beneficial effects against *Leishmania* and ZV models without toxicity[4,5]. Ellagic Acid (EA), a major constituent of pomegranate extracts, demonstrated interesting biological effects, such as anti-inflammatory, antioxidant, and most importantly antileishmanial activity[6]. Molecular docking and dynamics simulations also suggest EA can inhibit ZV NS3-helicase, supporting its antiviral potential[3]. This STSM builds on an ongoing collaboration between the applicant's institution, where various compound series have been designed, and Fraunhofer ITMP, where I will conduct *in vitro*

and *in silico* profiling of novel compounds and extracts. The goal is to identify safe, effective candidates with low off-target activity. The most promising will be tested against Leishmania and ZV to explore their potential as therapeutic agents for NIDs, aligned with OHDs principles.

Description of the work carried out during the STSM (max 500 words):

According with the aim of identifying NIDs of the COST Action, we assessed both newly synthesized compounds and organic waste-based extracts to analyze their safety and off-target activity. Thus, we run an early ADME-Tox profiling of these potential anti-infective substances. Such profiling dealt with assessment of cytotoxicity, hERG blockage, and HDACs inhibition. Another goal of OHD is to reduce the drugs environmental impact. For this reason, we also developed a protocol to assess preliminary Ecotoxicity with a bacterium model, namely *Aliivibrio fischeri.* Drug-likeness profile has been assessed using five suitable filters. In light of collected results, we selected the most suitable substances, and within the OHD network we tested them for their potential activity against a *Leishmania* model thanks to collaborators of the organization. During this experience, I took advantage of the possibility to participate to the "In silico prediction method for ecotoxicity and bioaccumulation [WG2+HG6+HG7]" training school that took place in (Athens), Greece, in order to enhance my knowledge upon machine-learning techniques to predict ecotoxic effects of old and novel substances.

Relevance to the "green" objectives of the CA21111: Ecotoxicity/environmental impact of the STSM project (max 500 words):

Environmental impact of chemicals is increasingly attracting attention, since it is linked to several health-linked phenomena, such as antibiotic resistance and spill-over events. In light of this and in line with the objectives of this COST Action, during this STSM we focused our efforts also on developing a HTS-format assay to assess *in vitro* ecotoxicology effect of novel compounds. *Aliivibrio fischeri* is a gram-negative bacterium that lives in symbiosis with many marine species, and so it is tightly linked to the aquatic ecosystem. This strain has been already used to assess ecotoxicological effect of samples of contaminated water samples, but the protocol required the usage of cuvettes. Thus, we bought the ecotox kit and then we scaled-down the assay itself using a 384-well plate instead of a cuvette. In conclusion, now it is possible to simultaneously test many compounds with less reagent consumption. Moreover, thanks to the characteristic of the assay, results are collected in less than an hour, further increasing efficiency in data production.

Description of the main results obtained:

In silico prediction about drug-likeness of synthetic derivatives tested through five different filters shows fifteen compounds with no violation, five compounds with a single violation, and two compounds with two and three violation, respectively. Tested compounds and extracts did not show any cytotoxicity on A549 and HEK293 cell lines. Specifically, extracts demonstrated to be safe up to 0.25 mg/mL. Three urolithin derivatives upon twenty-one slightly inhibit HDAC8 and just one of them showed off-target activity on HDAC6. Among them all, only one compound binds weakly hERG channel, therefore they seem to have no cardiotoxic potential. Then, four of them demonstrate slight preliminary ecotoxic effect on *Aliivibrio fischeri* model. All compounds have been tested at 10 μM concentration.

Mutual benefits for the Home and Host institutions:

The collaboration between the parties led to the acquirement of several useful data, according to what reported in the previous sections. Such data will be potentially translated into future publications and dissemination materials, which will highlight the work of both institutions, the importance of the addressed topics, and the great opportunities that the STSM of this COST Action can afford.
Lastly, both Home and Host institutions managed to increase the grantee's scientific cultural baggage with innovative procedures and techniques employed during this mobility period.

Future collaboration with the Host institution (if applicable):

Professor Rapposelli's group and Dr Gul collaboration was already well established, and this STSM has further strengthened it. Both Home and Host institutions are going to keep involved in a biunivocal cooperation useful to better address the complex drug discovery process.

Foreseen publications or conference presentations expected to result from the STSM (if applicable):

To date, data collection is still ongoing. However, the work conducted during this STSM will be surely published in case of positive results, in order to adhere to the dissemination goal of the COST Action itself. During this STSM I had the possibility to test compounds from other research groups which belong to OHD. This not only contributes to strengthen the collaborations within the COST Action network, but has also led to drafting two foreseen publications. Together with us, Dr. Chiara Borsari and her group submitted a paper that will be hopefully published soon. On the other hand, we are drafting a paper in collaboration with Prof. Sandra Gemma.