Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: 21111
Grantee name: André Manuel Gomes Lopes

Details of the STSM

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

Description of the work carried out during the STSM

From the 29th of April to the 28th of July of 2026, the grantee (André Manuel Gomes Lopes) screened 352 compounds from his research group in an expanded and detailed ADME-Tox assay panel which consisted of the following:

- Cytotoxicity in three cell-lines (HEK-293, U2OS and A549),
- Two Histone Deacetylase enzymes (HDAC6 and HDAC8),
- Two Phosphodiesterase enzymes (PDE4C1 and PDE4D2),
- Four Cytochrome P450 Isoforms (CYP3A4, CYP2C9, CYP2C19 and CYP1A2),
- One Kinase enzymes (ROCK1),
- Glucose-6-Phosphate Dehydrogenase enzyme (G6PDH),
- *h*ERG liability (cardiotoxicity),
- Ecotoxicity (*Aliivibrio fischeri* bioluminescence bacteria to assess WaterTOX) and
- Mitochondrial toxicity assay (Mito-Tracker) on the previously identified 16 hit compounds for the treatment of Leishmaniasis.

All compounds were initially tested at 10µM and their % Inhibition determined. For some compounds which yielded >50% Inhibition at 10µM, they were progressed to dose response assays and their IC_{50} were determined (e.g. G6PDH, HDAC and CYP3A4 assays). In the cytotoxicity assays, compounds were initially tested at 10µM with 24h and 48h incubation and most of the compounds were not associated with cytotoxicity. In order to expand the data, additional cytotoxicity assays were performed with 48h incubation with 50µM and 100µM compound, allowing for a more detailed ranking the compounds and identification of safer derivatives for progression.

Additionally, the grantee screened compounds from other libraries in collaboration with different research groups, namely (1) Portugal - 162 compounds, (2) Italy/Bologna - 24 compounds (Ritonavir and its derivatives were screened in the CYP3A4 assay at 100nM and as expected all of them showed inhibition over 50% and thus their IC_{50} were determined), (3) Italy/Pisa - 97 compounds (10 compounds were extensively screened in the HDAC6 and HDAC8 assays to determine their IC_{50}), (4) Greece - 12 compounds and (5) OneHealthDrugs COST Action 21111 - 21 veterinary drugs (screened in a simple ADME-Tox Panel (HEK-293, HDAC6, CYP3A4, *h*ERG and WaterTOX).

Overall, the grantee completed and exceeded the proposed workplan.

Description of the STSM main achievements and planned follow-up activities

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the Grant Holder Manager to verify the grant documents compliance and proceed with the payment.

The main goal of the STSM was to profile the 352 compound library from the grantee's research group to identify and confirm high-quality candidates for the next step in his PhD which is the *in vivo* assays. This work complemented and aligned perfectly with his PhD and the OneHealthDrugs principals as it reduced animal testing and only allow the best and safer compounds to advance to the next stage of pre-clinical drug development.

During the STSM several scientific and personal objectives were achieved by the grantee who undertook an extensive amount of highly technical research work by performing five cell-based assays and ten different enzymatic assays. A total of 566 compounds were screened, while also learning how High-Throughput Screening equipment (e.g. Echo Liquid Handler, EnVision plate reader, etc) work. This allowed the grantee to extend his experience in cell culture, biochemical assays and improve his laboratory skills in the future. From the proposed workplan, every assay and learning step was performed and achieved flawlessly with highly scientific accuracy. The CYP2D6 and AMPK assays could not be performed as the respective kits and reagents were not available.

Additionally, the results obtained from this STSM also allowed early identification of unfavourable chemotypes in the different compound series. This information is essential in the upcoming design and synthesis of new derivatives, as it allows a deeper understanding of what makes a molecule safer. In this STSM, the grantee identified several HDAC6 and HDAC8 inhibitors, which on first glance may seem sub-optimal, however, these could be progressed as anti-cancer compounds as they are highly potent. From the grantee's research group, 45 compounds were designed *in silico* specifically to inhibit the G6PDH and 28 of these showed >50% inhibition at 10μM. The screening identified 6 compounds with IC_{50} <10μM and validated the computational model, and these results published and the research will continue.

Regarding the future follow-up activities, the grantee will present this work in the final OHD-STSM meeting. The results for the compounds from the collaborators will be published and in the case of results relating to the grantee's PhD, it will be included in his thesis to be published and help him achieve his scientific and personal goals.