Investigating the mechanism of action of the antiparasitic agent H80 through imaging techniques

Giulia Malpezzi [a], Lorenzo Tagliazucchi [a], Daniele Aiello [a], Alberto Venturelli [a], Anabela Cordeiro-da-Silva [b,c], Nuno Santarem [b,c], Glauco Ponterini [a], Maria Paola Costi [a]

(a) Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy. (b) Instituto de Investigação e Inovação em Saúde and IBMC-Institute for Molecular and Cell Biology, Universidade do Porto (c) Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto (FFUP), 4150-180 Porto, Portugal;

The primary objective of therapeutic research for Leishmaniasis is to replace the currently few existing drugs, that are fairly toxic and require prolonged parenteral administration, with an orally-available option that is less toxic and involves short treatment cycles [1]. In this context, an important advance has been the recent demonstration that an oral treatment with miltefosine (MIL, figure 1a) can bring to complete recovery in over 90% of patients with anthroponotic *Leishmania visceralis* (LV), but with severe side effects [2].

Thus, the aim of our drug discovery study is to identify new leads, which are more active than MIL, and allow an oral administration. During our studies on trypanosomatidic diseases, we have identified a new lead, named **H80** (figure 1b)**,** showing low micromolar EC50 in macrophages infected with *Leishmania infantum*, *L. donovani* and *L. major.* It also showed low propensity to develop drug resistance when compared to MIL [3]. Therefore, **H80** is considered an interesting antileishmanial agent active against both visceral and cutaneous Leishmaniasis in vitro and displays an activity like that of MIL (Figure 1c) [3].

The objective of the present work is to study the mechanism of action and the target of H80, that are so far unknown, making it difficult to improve the compound activity by rational design. To achieve this aim, we have developed imaging studies based on the intrinsic fluorescence of the compound, to follow its internalization behaviour inside promastigote cells. Fluorescence studies with two different sources of lights (DAPI, and CFP as control) revealed that **H80** is up taken through vesicles with an endocytosis mechanism. The distribution of the compound in the cells is predominantly cytoplasmic, lateral to the nucleus, which remains vesicle-free. These results were confirmed by a second fluorescence-based immunoassays with Rabit-α-luciferase and A21244 Alexa647 probe (Figure 1d).

In conclusion, by exploiting different fluorescent imaging studies we have demonstrated thatour compound **H80** is internalized by the amastigotes through vesicles, via endocytosis, and localizes in the cytoplasm. Next steps will include a LC-MS/MS proteomics study on fractionated amastigotes to map the biochemical and functional pathways differentially modulates by H80, with respect to Miltefosine, and to allow a target/off-target interactome description.

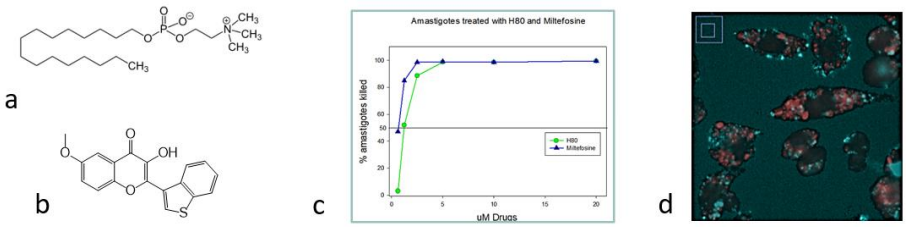

Figure 1. (a) Miltefosine structure. (b) H80 structure. (c) EC50 of Miltefosine and H80 in L *infantum* amastigotes. (d) fluorescence-based immunoassay for internalization study of H80.

References

[1] Kaye, P et al. Leishmaniasis: complexity at the host–pathogen interface. Nat Rev Microbiol 9, 604–615 (2011).

[2] Dorlo TP et al. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J Antimicrob Chemother. 2012 Nov;67(11):2576-97.

[3] Borsari, C. *et al.* Discovery of a benzothiophene-flavonol halting miltefosine and antimonial drug resistance in Leishmania parasites through the application of medicinal chemistry, screening, and genomics. *Eur. Med. Chem.* **183**, (2019).