Title: Design and synthesis of proteolysis targeting chimeras (PROTACs) directed to trypanothione reductase enzyme

Supervisor: Prof. Maria Laura Bolognesi

Research Project and Training Plan

The planned activity for this research project is part of the FISR2019_03796 project entitled: "PROLEISH (Proteolysis targeting chimeras (PROTACs) to treat leishmaniasis)" - CUP B34G19000680008 - Scientific Coordinator and holder of the funds: Prof. Maria Laura Bolognesi.

PROLEISH aims at developing innovative therapeutic tools for the treatment of leishmaniasis. Leishmaniasis is a vector-borne disease with high morbidity and mortality rates [1]. The disease is endemic in tropical and subtropical areas, but also in the Southern Europe and Italy with an increasing incidence [2]. This situation is worsened by the absence of effective drugs. Main limitations of current therapies include limited efficacy, high toxicity, and the development of resistance. Thus, novel anti-leishmania drugs are urgently needed [3].

The ambitious goal of PROLEISH is the development of first-in-class Proteolysis-Targeting Chimeras (PROTACs) to be potentially applied to leishmaniasis. PROLEISH aims to develop PROTACs directed at the trypanothione reductase (TR) enzyme, which is a validated antileishmanial target [4]. PROTACs are heterobifunctional compounds comprising three elements: a ligand for a protein of interest (POI), an E3 ligase recruiting element, and a linker that connects the aforementioned scaffolds. Through the formation of the ternary POI-PROTAC-E3 ligase complex, the PROTAC induces E3 ligase-mediated ubiquitination of the POI, and its consequent selective degradation by the proteasome system [5].

The research project is aimed at developing a novel set of PROTACs, which will be then optimized through iterative medicinal chemistry. In silico, physicochemical and biological profiling will drive iterative design-make-test cycles. The project is highly multidisciplinary, complements ongoing lab efforts, and expands upon our recent discoveries. In particular, the hired post-doc will design and synthesize PROTACs with different structural and physicochemical properties by exploiting innovative synthetic strategies. She/he will perform structure-activity relationship studies to combine the POI ligand to the E3 ligase recruiting element through a suitable linker. Thanks to the collaboration with the Medicinal Computational Chemistry group of the University of Bologna, the hired research fellow will identify the putative structural modifications of PROTAC's elements to enhance targets' recognition, and degradation activity. Moreover, the hired fellow will collaborate with PROLEISH team members from Italian National Research Council and Istituto Superiore di Sanità, with diverse and complementary expertise, spanning from biochemistry and structural biology to parasitology. The biological evaluation of the newly synthesized PROTACs will include: (i) characterization of the binding to TR and E3 ligase through different techniques (e.g., SPR and X-ray crystallography), (ii) assessment of the *in vitro* anti-Leishmania activity (promastigote, amastigote, amastigote-macrophage assays), and (iii) evaluation of TR degradation by the proteasome system (Western blot).

The hired research fellow is expected to actively participate to the different aspects of the project by managing interdisciplinary research tasks. She/he will also contribute to the dissemination of the obtained results in meetings, publications, and conferences.

References

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