MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

Staff Scientists
Ana Belén García
Cristina Gómez
Esther González
Sonsoles Rodríguez
Carmen Varela

Graduate Student
Javier Sánchez (since August) (PEJ, CAM)*

Technicians
Lucía De Andrés (TS)* (PEJ) **
Bruno Di Geronimo (TS)* (PEJ) **
Franciso Javier García (until August) (TS)* (PEJ) **

* *Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

** *Titulado Superior* (Advanced Degree)
*** *Plan de Empleo Joven* (Youth Employment Plan)

OVERVIEW

The Medicinal Chemistry (MedChem) Section is part of the multidisciplinary Experimental Therapeutics Programme (ETP) focused on early drug discovery activities. ETP is integrated into the CNIO's structure, and acts as a bridge between basic research groups in cancer biology and the pharmaceutical industry, with the aim of transferring the results obtained in basic research laboratories to products, potential drugs that help to understand the biology of cancer, or the development of new therapies. The Section deals with the design, synthesis, and optimisation of compounds, classical inhibitors, and degraders such as PROTACs, which are then characterised by ETP's Biology Section, in order to evaluate their potency in biological targets *in vitro* and *in vivo* and ultimately to demonstrate their efficacy and mechanisms of action in animal models (*in vivo* proof-of-concept). The Section is also involved in the synthesis of high-quality chemical tools that help to decipher the mechanism of action of an observed phenotype in cellular assays, as well as in the synthesis of reference compounds that assist basic researchers in their investigations.

"In our MASTL project, we generated the first MASTL PROTAC (ETP-823) that potently degrades MASTL protein via E3 ligase and proteasome recruitment."

RESEARCH HIGHLIGHTS

Our MedChem activities in 2021 mainly focused on the following projects:

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is led by Maria A. Blasco (Telomeres and Telomerase Group). During 2020, the Biology Section developed an assay to measure the binding of TRF1 to telomeric DNA, and different wet screening campaigns were run. To date we have identified potential "direct" inhibitors of TRF1 that do not interfere with the assay system nor with DNA. We are currently involved in their validation by re-synthesising them to confirm the observed activity with pure compounds, as well as by synthesising some direct analogues to establish a preliminary SAR of the series.

Microtubule-associated serine/threonine protein kinase-like (MASTL) inhibitors

This project is being carried out in collaboration with Marcos Malumbres (Cell Division and Cancer Group). We are following 2 different chemical strategies in this project; on the one hand, we are immersed in the fine optimisation of the main chemical series of MASTL inhibitors, in which we have identified a potent, cell active, and selective compound without cardiotoxic alerts (ETP-715). Our objective is to obtain an orally bioavailable compound to carry out efficacy studies in mouse models. On the other hand, we are exploring the development of PROTACs (Proteolysis Targeting Chimeras) molecules to degrade MASTL, not only to inhibit its catalytic activity. Chemically induced degradation of proteins, based on hijacking the natural protein degradation machinery present in cells as a mechanism to degrade proteins of interest (POI) linked to disease, has emerged as an innovative approach in drug discovery (DD) and development. This concept has been put into practice by designing and synthesising hetero-bifunctional small molecules, the so-called PROTACs. Unlike traditional drugs, PROTACs aim to reversibly eliminate the aberrantly functioning protein rather than inhibiting its (catalytic) function. PROTACs follow an event-driven rather than an occupancy-driven pharmacological paradigm and, importantly, act catalytically to degrade super stoichiometric amounts of the target protein. The mechanism of action of PROTACs implies more robust and durable pharmacological effects than just inhibition, providing some advantages, such as the use of lower and/or intermittent dosing regimens, which in turn can lead to improvement of the therapeutic index for PROTACs based drugs, due to reduction or elimination of dose-limiting toxicity adverse events related to off-targets. These molecules consist of 3 components: a target protein-binding moiety, a degradation machinery-recruiting unit (typically an E3 ubiquitin ligase), and a linker that couples these 2 functionalities. During 2021, we continued our activities in this field by increasing the number of PROTACs synthesised along with the negative probes required to determine their mode of action. As a result of this exploration, we identified 1 PROTAC (ETP-823, FIGURE 1) with a degradation concentration 50 of 0.63 µM in the MM1.S cell line that will help us to better understand MASTL biology. We are currently involved in the fine optimisation of this PROTAC to improve its potency and confer it with *in vivo* properties.

Histone H4-lysine 20 N-methyltransferase (SETD8) inhibitors

In collaboration with Óscar Fernández Capetillo (Genomic Insability Group), the aim of this project, incorporated into the ETP in 2021, is to generate and optimise SETD8 methyltransferase inhibitors as new therapeutic agents. In 2020, we started our activities by synthesising and acquiring some reference compounds. The initial chemical exploration around some identified hits from Capetillo's Lab rendered several compounds, but in the micromolar range. On the other hand, the screening of irreversible molecules identified hits with low micromolar activity. The hits were synthesised in good purity to validate their mechanisms of action, and in parallel we initiated a chemical strategy based on the synthesis of covalent compounds.

Apart from the drug discovery activities, we give support to several Groups by synthesising reference compounds or purifying compounds to help them with their projects. During 2021, we carried out these activities for the following Groups: Epithelial Carcinogenesis, Microenvironment & Metastasis, Brain Metastasis, Telomeres and Telomerase, Experimental Oncology, Topology and DNA Breaks. ■

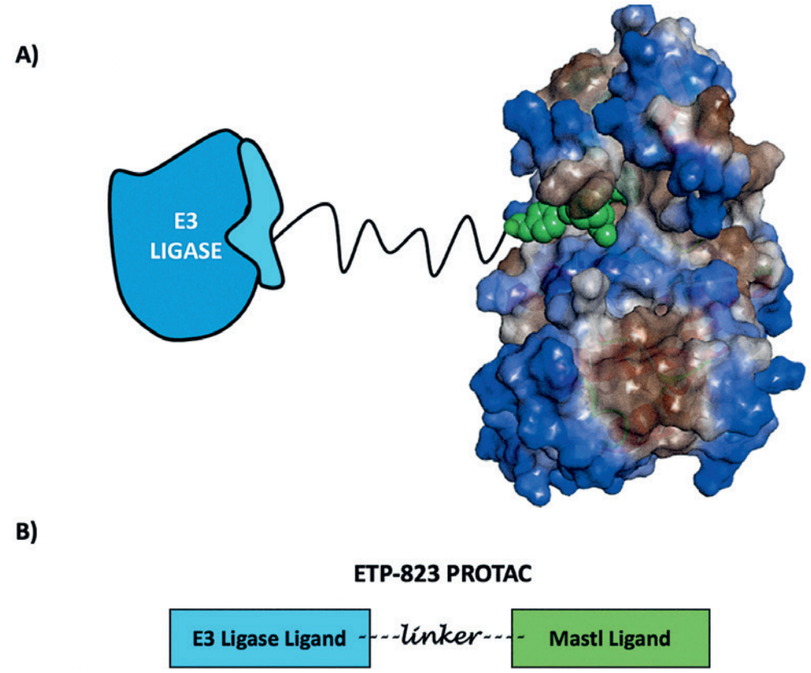

FIGURE 1 (**A**) Representation of PROTAC ETP-823 binding to the kinase domain of human MASTL protein (PDB 5LOH) through MASTL Ligand, and to E3 Ligase through the corresponding Ligand. (**B**) Components of ETP-823 PROTAC.

PUBLICATIONS

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