MEDICINAL CHEMISTRY SECTION

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OVERVIEW

The Medicinal Chemistry 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 that are then characterised in the Biology Section of ETP, in order to evaluate their potency in biological targets *in vitro* and *in vivo* and ultimately to demonstrate their efficacy and mechanism of action in animal models (*in vivo* proof-of-concept). As a complementary strategy to the classic inhibitors, we also contemplate the degradation of particular targets using different chemical approaches such as the use of PROTACs. Additionally, we have entered the field of Chemical Biology in order to discover and identify novel drugs and targets from phenotypic screenings. In this regard, we contribute by synthesising high quality chemical tools needed for interrogating the observed phenotype.

"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 Section's activities focus mainly on the following projects:

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is led by Maria A. Blasco (Telomeres and Telomerase Group). In previous years, the ETP Biology Section developed an assay to measure the binding of TRF1 to telomeric DNA. After virtual and wet screening campaigns, we identified some disruptors of such binding that do not interfere with the assay system nor with DNA. During 2022, we analytically characterised the hits, resynthesised fresh batches, and synthesised some analogues to establish Structure Activity Relationships (SAR). The compounds are currently being evaluated in orthogonal assays by ETP's Biology Section and by a pharmaceutical company that is interested in the target, under an MTA agreement.

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

This project is being undertaken in collaboration with Marcos Malumbres (Cell Division and Cancer Group). We have been involved in the fine optimisation of ETP-715, a potent cell active, selective compound without cardiotoxic alerts but with low exposure levels after oral administration in pharmacokinetic (PK) studies performed in BALBC mice. Seventy six new analogues have been synthesised so far, and the most promising ones, in terms of potency and *in vitro* ADMET, are being evaluated in PK studies. Additionally, we have started to work on back-up series, to reinforce the intellectual property and to determine the impact of different scaffolds on drug-like properties. Fifty seven new compounds from different chemical series have been synthesised, and we have identified new series with potent compounds that will be characterised in terms of *in vivo* PK. In addition, we continue with our activities developing PROTACs (Proteolysis Targeting Chimeras) to degrade MASTL protein. Previously, we identified ETP-823 as our first MASTL PROTAC that potently degrades MASTL protein via E3 ligase and proteasome recruitment. PK studies showed *in vivo* levels above its DC50 in cells in plasma after IP administration. We performed a fine optimisation of ETP-823, and 62 new PROTACs were synthesised by exploring different linkers, different functional groups in the growing vectors, and different E3 ligase ligands (FIGURE 1). So far, we have identified several new PROTACS with good degradation profiles in different cell lines.

HistoneH4-lysine20 N-methyltransferase (SETD8) inhibitors

In collaboration with Óscar Fernández Capetillo (Genomic Instability Group), we explored one of the initial hits identified in a cellular assay in Capetillo's laboratory, but the chemical exploration of this series was put on hold due to lack of activity in the biochemical assay. After 2 screening campaigns, we identified new hits (covalent and non-covalent), and we are currently working on their validation by re-synthesising the hits and synthesising some analogues to establish SARs.

Foxo activators (collaboration with Refoxy Pharmaceuticals GmbH)

We have been involved in the selection of new analogues of the hits identified in screening campaigns, as well as in the quality control analyses. Several Foxo activators have been identified, and negotiations for licensing the compounds to Refoxy are underway.

NUDT5 inhibition

We are collaborating with GRG-IUC to optimise a hit that inhibits the ATP generating activity of NUDT5 in a biochemical assay. Several analogues have been obtained, and we are currently characterising the compounds.

RANK antagonists as a novel therapeutic approach for the treatment of TNBC patients

We are collaborating with Eva González-Suárez (CNIO) to develop small molecules that specifically target the RANK receptor. The activities in 2022 focused on acquiring the virtual hits and assessing the quality control to validate them in wet assays (SPR, cells, etc.) and to generate robust data.

Apart from the drug discovery activities, we give support to several Groups by synthesising reference or tool compounds. During 2022, we carried out such work for the following Groups: Brain Metastasis, Genomic Instability, and Telomeres and Telomerase. ■

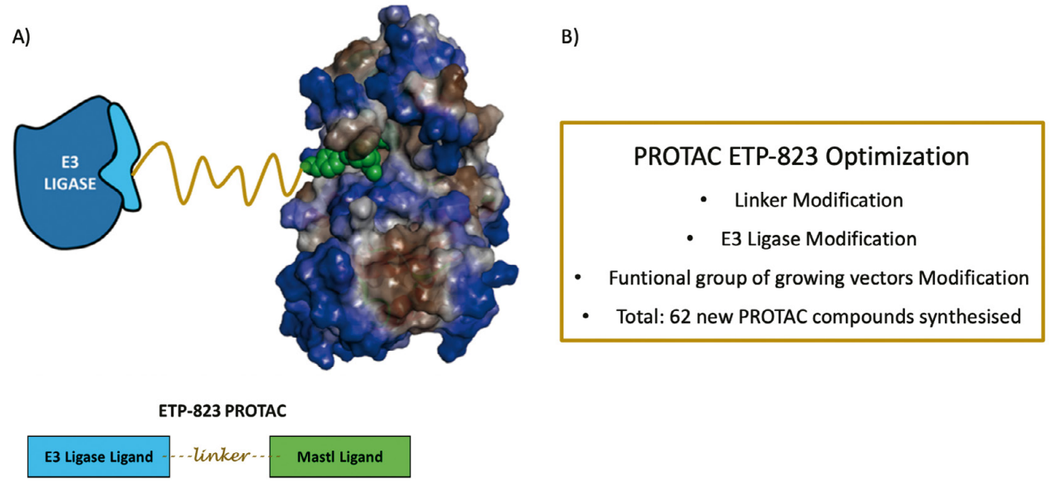

FIGURE 1 (**A**) Representation of PROTAC ETP-823 binding to the kinase domain of human MASTL protein (PDB 5LOH) through MASTL Ligand, and to E3 Ligase through the corresponding Ligand. (**B**) Strategies for the optimisation of ETP-823.

PUBLICATION

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