MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

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

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

Student in Practice
Luis De Castilla (January-June) (*Universidad Rey Juan Carlos*, Madrid, Spain)

* *Titulado Superior* (Advanced Degree)
** *Plan de Empleo Joven –Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

The Medicinal Chemistry Section is part of the multidisciplinary Experimental Therapeutics Programme (ETP) that is focused on early drug discovery activities. Our aim is to generate advanced chemical compounds that could be further developed into drugs to treat cancer. The Section deals with the design, synthesis, and optimisation of compounds that are characterised in the Biology Section of ETP to evaluate their potency in biological targets, *in vitro* and *in vivo* drug-like properties and, finally, 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 proteolysis targeting chimeras (PROTACs). Additionally, we have entered the chemical biology field 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 high quality inhibitors that are to be used as chemical tools for target validation studies and to serve as the basis for the development of advanced lead compounds."

RESEARCH HIGHLIGHTS

In 2020, ETP was involved in several drug discovery projects (FIGURE). A summary of some of them is provided below.

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

This CNIO project yielded advanced optimised molecules with a "first-in-class" profile. Preliminary toxicity studies in rats were carried out using the leading product of our chemical series. The results showed a preclinical toxicity profile compatible with its potential development as a drug.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is led by Maria A. Blasco (CNIO Telomeres and Telomerase Group). During 2020, new biotinylated chemical probes that do not require "click chemistry" were used to identify the molecular targets of ETP-946. Unfortunately, no conclusive results were obtained. By contrast, studies performed in the ETP Biology Section and focused on the cellular level of the altered genes, after treatment with ETP-946, allowed us to identify the potential mechanism of action of ETP-946 (and its series, Series 2) by which the levels of TRF1 in telomeres are modulated. Currently, resynthesis of several compounds is required to validate this new hypothesis.

Additionally, during this year, the Biology Section developed an assay to measure the binding of TRF1 to telomeric DNA, and a wet screening campaign was run with the aid of virtual screening techniques. We identified potential "direct" inhibitors of TRF1 and we are currently involved in their validation by resynthesizing them to confirm the observed activity. Once we have confirmed the hits, we will start SAR activities around them.

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

This project is undertaken in collaboration with Marcos Malumbres (CNIO Cell Division and Cancer Group). In 2020, we characterised the selectivity (+ 460 kinases) of 3 previously explored chemical series. One of them showed quite acceptable selectivity, while the other 2 proved to be excessively promiscuous. The selective series was chosen for further development, and we significantly advanced its SAR exploration by synthesising more than 100 compounds. As a result, we obtained nanomolar MASTL inhibitors biochemically and in cells, and with good metabolic stability in liver microsomes. Fine optimisation of this series is currently ongoing. Additionally, we are exploring the development of PROTAC molecules to degrade MASTL and not only to inhibit its catalytic activity. We synthesised a collection of more than 80 PROTAC-like molecules using CRBN and VHL as E3 ligands. Two compounds were identified as moderate degraders in the MDAMB231 cell line. This exploration is still ongoing to identify more potent degraders. Other strategies are also being explored, for example, the use of hydrophobic tagging (HyT) in our molecules to induce degradation of MASTL.

Discoidin domain receptor (DDR) 1/2 inhibitors

This project was carried out in the context of a doctoral thesis by an FPI "*Severo Ochoa*" Fellowship student. In this thesis project a chemical series of DDR1/2 inhibitors was generated that led to the identification of potent compounds at a biochemical and cellular level, and with acceptable selectivity. The results will be protected by a patent application.

HistoneH4-lysine20 N-methyltransferase (SETD8) inhibitors

In collaboration with Óscar Fernández Capetillo (CNIO Genomic Instability Group), the aim of this project, recently internalised in the ETP, 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 has started and will help us to understand their binding mode and to design *de novo* SETD8-is, including intellectual property in their structures.

Finally, we also gave support to the Experimental Oncology Group by the synthesis of reference compounds needed for their projects. ■

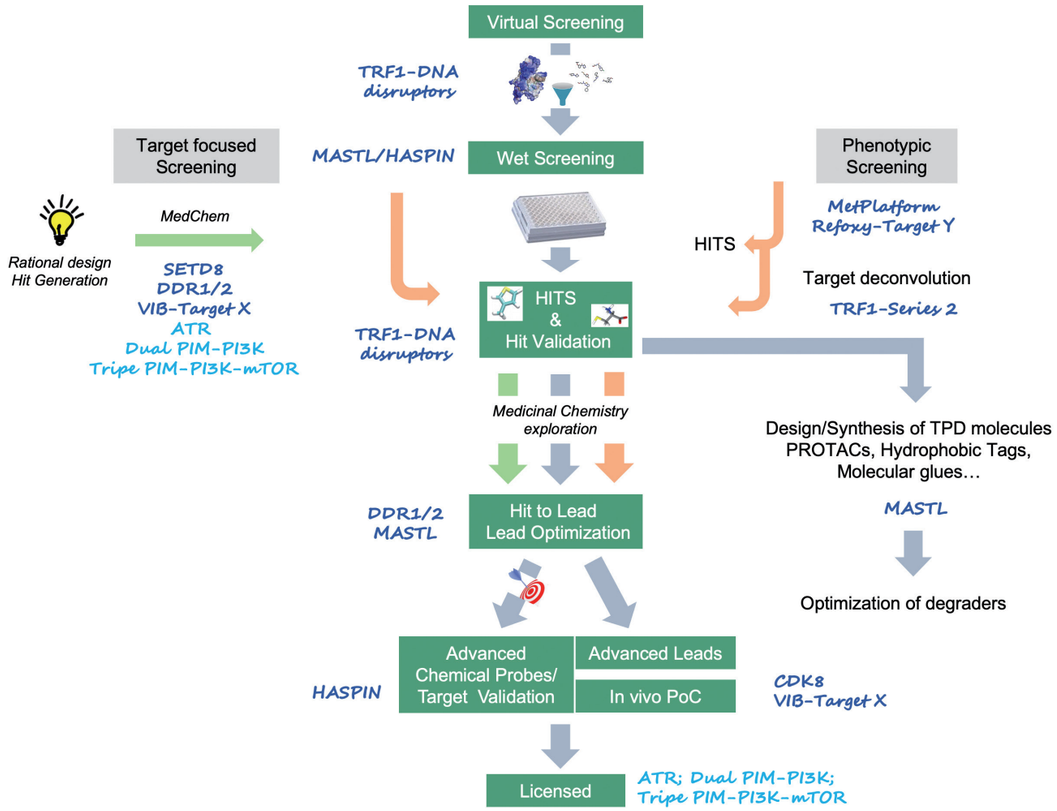

FIGURE Drug Discovery phases for the different projects investigated in ETP. The project is indicated in blue colour, close to its status (dark blue, current projects with activities in ETP; light blue past projects).

PUBLICATIONS

- Lynch CJ, Bernad R, Martínez-Val A, Shahbazi MN, Nóbrega-Pereira S, Calvo I, Blanco-Aparicio C, Tarantino C, Garreta E, Richart-Ginés L, Alcazar N, Graña-Castro O, Gómez-Lopez G, Aksoy I, Muñoz-Martín M, Martinez S, Ortega S, Prieto S, Simboeck E, Camasses A, Stephan-Otto Attolini C, Fernandez AF, Sierra MI, Fraga MF, Pastor J, Fisher D, Montserrat N, Savatier P, Muñoz J, Zernicka-Goetz M, Serrano M (2020) Global hyperactivation of enhancers stabilizes human and mouse naive pluripotency through inhibition of CDK8/19 Mediator kinases. *Nat Cell Biol* 22, 1223-1238.
- Esteban-Burgos L, Wang H, Nieto P, Zheng J, Blanco-Aparicio C, Varela C, Gómez-López G, Fernández-García F, Sanclemente M, Guerra C, Drosten M, Galán J, Caleiras E, Martínez-Torrecuadrada J, Fajas L, Peng S-B, Santamaría D, Musteanu M, Barbacid M (2020). Tumor regression and resistance mechanisms upon CDK4 and RAF1 inactivation in KRAS/P53 mutant lung adenocarcinomas. *Proc Natl Acad Sci USA* 117, 24415-24426.
- Mohlin S, Hansson K, Radke K, Martinez S, Blanco-Apiricio C, Garcia-Ruiz C, Welinder C, Esfandyari J, O'Neill M, Pastor J, von Stedingk K, Bexell D (2020). Anti-tumor effects of PIM/PI3K/mTOR triple kinase inhibitor IBL-302 in neuroblastoma. *EMBO Mol Med* 12, e11749.
- Kennedy SP, O'Neill M, Cunningham D, Morris PG, Toomey S, Blanco-Aparicio C, Martinez S, Pastor J, Eustace AJ, Hennessy BT. (2020). Preclinical evaluation of a novel triple-acting PIM/PI3K/mTOR inhibitor, IBL-302, in breast cancer. *Oncogene* 39, 3028-3040.
- Cash TP, Alcalá S, Rico-Ferreira MDR, Hernández-Encinas E, García J, Albarrán MI, Valle S, Muñoz J, Martínez-González S, Blanco-Aparicio C, Pastor J, Serrano M, Sainz B Jr (2020). Induction of lysosome membrane permeabilization as a therapeutic strategy to target pancreatic cancer stem cells. *Cancers (Basel)* 12, 1790.
- Martínez-González S, García AB, Albarrán MI, Cebriá A, Amezquita-Alves A, García-Campos FJ, Martínez-Gago J, Martínez-Torrecuadrada J, Muñoz I, Blanco-Aparicio C, Pastor J (2020). Pyrido[2,3-b][1,5]benzoxazepin-5(6H)-one derivatives as CDK8 inhibitors. *Eur J Med Chem* 201, 112443.