MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

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

Graduate Student
Francisco J. García

Technicians
Ivan Arribas (until June) (TS)*.
Lucía De Andrés (since July) (TS)*,
Carmen Fernández (until April) (TS)*. Miriam Pastor (since June) (TS)*

* *Titulado Superior* (Advanced Degree)

OVERVIEW

The Medicinal Chemistry Section is part of the interdisciplinary Experimental Therapeutics Programme that is dedicated to early Drug Discovery in the oncology field. Our aim is to discover new anticancer agents based on the hypotheses and targets generated by CNIO's Basic Research Groups; this is done in close collaboration with these Groups. Medicinal Chemistry activities start with the identification of hits through High Throughput Screening (HTS) campaigns from targeted or phenotypic assay or hits generated in our Section by applying Rational Drug Design Strategies; these are then optimised to obtain novel lead compounds with *in vivo* activity in different animal models. For hits obtained from phenotypic screenings, we help to decipher the mechanism of action responsible for the observed phenotype, synthesising affinity probes that will be used for cellular localisation (imaging techniques) and extracting the target/s (pull down experiments). We are also developing PROTACs (proteolysis targeting chimeras) as promoters of cell protein degradation to establish their applicability across diverse drug discovery projects.

"We have successfully designed and synthesised an irreversible affinity chemical probe of ETP-946 that is to be used for imaging and pull-down/proteomics analysis experiments; the aim is to decipher the mechanism of action of this TRF1 modulator."

RESEARCH HIGHLIGHTS

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

ETP-93, with demonstrated proof of concept studies (PoC) in mouse models, and ETP-18 were identified as potent, selective and orally bioavailable CDK8 inhibitors. We are involved in the multigram scale up of these compounds in order to perform toxicity studies in rats, as well as to determine if the compounds/inhibition of the target is safe enough to progress them to the next phases of drug development. With these results in hand, we will be able to initiate the transfer of our results to companies interested in developing our compounds into drugs. Additionally, we will also perform PoC studies (efficacy and biomarker modulation) with ETP-18.

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

In collaboration with the CNIO Cell Division and Cancer Group, we continue with the exploration around the chemical series identified to obtain potent and selective compounds. Strategies to generate MASTL-PROTACs are also contemplated in order to generate degrader compounds of the protein; the first PROTACs have been synthesised with moderate MASTL activity (FIGURE). We are initiating a hit generation phase to obtain novel Mastl inhibitor hits.

HASPIN inhibitors

Highly selective hits identified from 2 previously generated chemical series were scaled up to be characterised *in vivo* in order to determine their pharmacokinetics in mice as well as to be used in the biological characterisation to study the relevance of HASPIN in cancer, including their effect in antiproliferative experiments as single agents and in combination with other antitumour agents. We continue with the chemical exploration around the hits to conclude the SAR activities and to define the scope of their kinase activity.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group (TTG). ETP-946 was identified as a TRF1 modulator under screening assay conditions, and we are currently working on deciphering its mechanism of action. One of the approaches that we have taken is to use affinity chemical probes. We generated SAR information from the chemical exploration in the hit-to-lead phase (approximately 150 compounds were synthesised), and with this information we identified those parts of the hit molecule at which to install linkers and synthesise probes. During this year, we accomplished synthesis of an irreversible affinity chemical probe (ETP-093), which contains photoreactive and reporter groups, made as small as possible to minimise the interference upon binding to the target proteins. The aliphatic diazirine photoreactive group of ETP-093 enables, after incubation with cells, short irradiation to generate the highly reactive carbene species that will react with the binding protein/s. The terminal alkyne reporter of ETP-093 was then used for subsequent target identification by conjugation to suitable reporters (biotin-N_3) using biorthogonal click chemistry conditions, which enable pull-down experiments. So far, we have performed the first pull down experiment and the proteomic analysis, which will be further repeated 3 more times for a robust interpretation of the results. Pull down experiments with the reversible affinity probe ETP-455 were generated. This chemical probe lacks the photoreactive group, so its binding with affinity protein/s is not covalent and we may lose some relevant information during the washing steps phase. Nevertheless, once we have finalised all the experiments, we will compare results between reversible/irreversible affinity chemical probes. We have filed a patent application to cover the chemical series of ETP-946. This project was recently awarded a grant from the *CaixaImpulse* programme and we are currently working on the optimisation of the drug-like properties of ETP-946 together with the generation of novel chemical space for patent reinforcement.

Collaborations with other CNIO groups

We continue our collaborations with other researchers from the Centre, for instance, with Alejo Efeyan (Metabolism and Cell Signalling Group) performing stability, reactivity studies of the hits and synthesis of tools to help decipher their mechanism of action; and with Paco Real (Epithelial Carcinogenesis Group) for the synthesis of reference compounds. ■

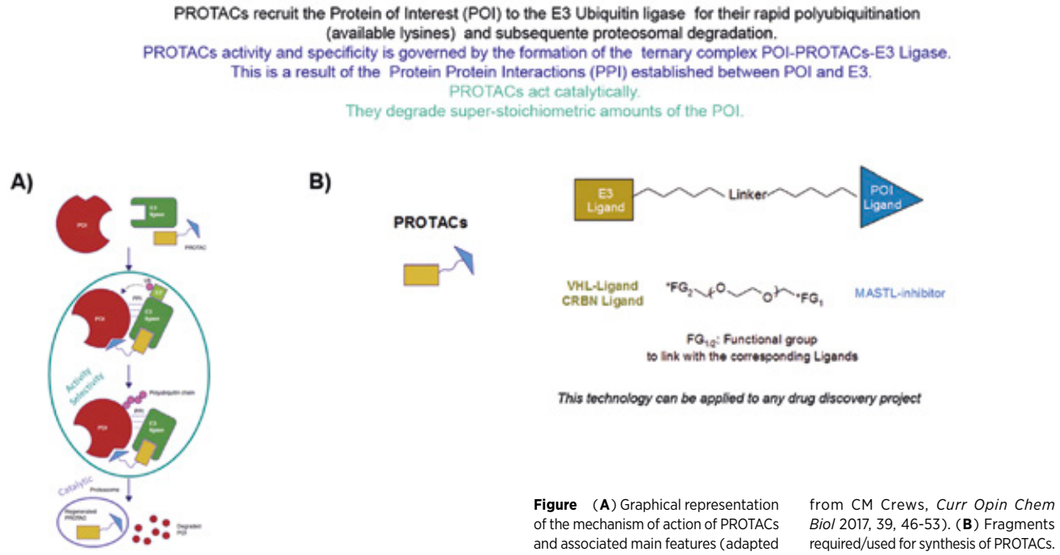

Figure (**A**) Graphical representation of the mechanism of action of PROTACs and associated main features (adapted from CM Crews, *Curr Opin Chem Biol* 2017, 39, 46-53). (**B**) Fragments required/used for synthesis of PROTACs.

- **PATENT**
- Pastor JA, Blasco MA, Martinez S, Blanco-Aparicio C, García AB, Gómez-Casero E, Bejarano, L, Méndez-Pertuz M, Martínez P, García-Beccaria M (2018). Novel TRF1 modulators and analogues thereof. *EP18382659.3*.