A high-quality small-molecule probe for target validation has to be cell permeable and demonstrate target engagement and selectivity, as well as pharmacological and phenotypic response. PROTACs (PROteolysis Targeting Chimeras) have emerged as new promising pharmacological modalities. Moreover, PROTACs represent the chemical equivalent of small interfering RNA (siRNA), albeit allowing removal of a protein at a post-translational level. Parameters such as the maximum level of target degradation (Dmax), confirmation of a proteasome dependent degradation mechanism, and kinetic parameters of POI degradation and selective degradation have to be taken into account to use PROTACs for target validation. In collaboration with Marcos Malumbres, we started an early drug discovery project to develop MASTL inhibitors and PROTACs, as non-advanced inhibitors have already been described. We have been able to develop both types of molecules, generating a set of PROTACs that meet the requirements to be used as chemical tools for target validation and to define their clinical niche.

“We identified selective and potent MASTL PROTACs with in vivo levels needed to perform PK/PD and proof of concept studies.”
RESEARCH HIGHLIGHTS

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

This project is undertaken in collaboration with the CNIO Genomic Instability Group. Our main objective is to generate and optimise novel SET8 inhibitors as new therapeutic agents. After 2 different screening campaigns, we identified both reversible and irreversible possible hits with micromolar activity. The covalent mechanism of action of the hits was validated by time dependent biochemical assays and the formation of adducts by proteomics with purified SETD8. In order to identify the reactive amino acid in SETD8, we are going to perform biochemical assays with a mutant protein and proteomics studies in cells to evaluate their selectivity. In addition, all possible hits have been tested in a cellular assay that measures monomethylation of H4K20 in order to prioritise chemical serials to improve their biochemical activity.

Collaborations with other CNIO Groups

The ETP-Biology Section performed in vivo studies of selected compounds and drugs such as pharmacokinetics and distribution studies in collaboration with the Microenvironment and Metastasis Group, the Brain Metastasis Group, and the Genomic Instability Group. Furthermore, we performed screening campaigns with the Topology and DNA Repair Group and the DNA Replication Group, and the Chromosome Dynamics Group giving support to perform cellular screenings.

Refoxo collaboration: We gave logistics and data analysis support.

Collaboration with other institutions

Collaborations with other CNIO Groups

SET domain containing lysine methyltransferase 8 (SETD8)

This project is conducted in collaboration with the CNIO Telomeres and Telomerase Group. We are working to identify disruptors of TRF1 binding to ds telomeric DNA, and so far we have identified several hits from different chemical series after virtual screening and wet assays, and screening of a collection of 1500 molecules selected from our ETP-library and analogue searching. We confirmed the specific disruption of the binding of TRF1 to dsTelDNA with screen and counter screen alpha assays, and a fluorescent displacement assay to discard the binding of the compounds to dsTelDNA. Now we are validating these hits by applying orthogonal assays against TRF1 and the dsTelDNA probe, such as EMSA and thermofluor assay with freshly prepared and/or resynthesised samples. Compounds that disrupt the binding of TRF1 to ds telomeric DNA by binding to TRF1 will be tested in a TRF1 phenotypic assay.

Collaborations with other CNIO Groups

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Collaboration with other institutions