BIOLOGY SECTION

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OVERVIEW

The early Drug Discovery (eDD) process encompasses screening campaigns for hit identification, hit generation and hit lo lead and lead optimisation phases, in order to end up with a lead compound able to demonstrate *in vivo* proof-of-concept. ADME – an acronym for absorption, distribution, metabolism and excretion – describes the disposition of a pharmaceutical compound within an organism. ADME properties influence the drug levels and the kinetics of drug exposure to the tissues, and hence influence the performance and pharmacological activity of the compound as a drug. It is fundamental to assess the parameters for ADME properties early on during the discovery stage, since they provide critical information that can help to better interpret the screening results and to design new molecules. Drug-like properties should be optimised in parallel to pharmacological activity against the target. For that reason, we perform ADME characterisation of the compounds during the initial steps of eDD projects and we carry out PK, PK/PD and distribution studies to validate the drug properties of our advanced molecules.

"The screening of the ETP-antitumour library in the phenotypic TRF1 assay has enabled us to identify new signalling pathways modulating TRF1 levels that will contribute to a better understanding of TRF1 biology."

RESEARCH HIGHLIGHTS

During 2018, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

We started proof-of-concept studies in mouse models with ETP-18, a selective advanced orally bioavailable lead compound that demonstrated both plasma and tumour levels as well as biomarker modulation (pSTAT1), in a dose dependent manner up to 8 h after oral administration in PK/PD studies in MOLM13 xenografts. Tolerance and efficacy studies will be performed. In parallel, after pharmacokinetics studies of 3 more selective CDK8 inhibitors, we have identified ETP-24 as a backup of ETP-18.

To determine if the inhibition of the target by our compounds is safe enough to progress them to the next phases of drug development, we are running toxicity studies in rats with the leads ETP-93 (dual CDK8/HASPIN-i) and ETP-18 (selective CDK8-i), in comparison with known inhibitors.

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

These projects are undertaken in collaboration with the CNIO Cell Division and Cancer Group. For MASTL, we tested 85 new compounds in our biochemical assay with active human full length MASTL protein; 11% of them were tested as part of the hit generation phase and we have also tested our ETP-antitumour library to identify novel hits. One drug is under validation as a putative MASTL-inhibitor. For HASPIN, we tested in biochemical and cellular assays, 42 compounds to complete the SAR exploration of the chemical series. We have evaluated the antiproliferative activity of highly selective HASPIN-inhibitors (S(35) of 0.025 and 0.007) from 2 different chemical series in a panel of 40 cell lines covering the more relevant tumour types. Now, we are evaluating their effect in combination treatments. We have also characterised in ADME assays representative compounds for the 2 chemical series. We have performed a preliminary pharmacokinetic study with 1 selected compound with good ADME properties and we are running a distribution study to validate it as a good tool compound for i*n vivo* proof-of-concept.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres has been used to test 30 compounds, which include ETP-946 analogues and its corresponding irreversible chemical probes. We have identified an active irreversible chemical probe, ETP-093, and we are running pull-down experiments with it. Several nuclear targets have been identified as potential targets and we want to validate them with 2 more pull-down experiments. In the meantime, we have used ETP-455, a reversible chemical probe, to perform pull-down experiments in triplicate. After comparison of the 3 experiments, we have identified 2 putative targets that we are trying to validate by orthogonal assays. We will compare the pull-downs with both reversible and irreversible chemical probes in order to select the best candidates. On the other hand, we have performed distribution studies with ETP-946 and have observed that the compound is distributed in tissues. Furthermore, by using a chemical biology approach, we have validated 3 more signalling pathways that were identified in the screening of the ETP-antitumour library that modulates TRF1 levels at telomeres; Maria Blasco's laboratory is deciphering the molecular mechanism behind this. These results are part of a patent application PCT/EP2018/074832. Finally, we have started a virtual screening with the aim of identifying disruptors of TRF1 dimerization (FIGURE).

Collaborations with other CNIO Groups

ETP-Biology continued providing support to follow-up on the results obtained from the screenings performed by the Brain Metastasis Group and the Metabolism and Cell Signalling Group. Moreover, we have provided support by testing and analysing the ETP-antitumour library, either alone or in combination, in order to identify: i) novel treatments of NSCLC mouse cell lines mutant in KRas with and without C-RAF and CDK4, in collaboration with the Experimental Oncology Group; and ii) novel modulators of Midkine expression, in collaboration with the Melanoma Group. ■

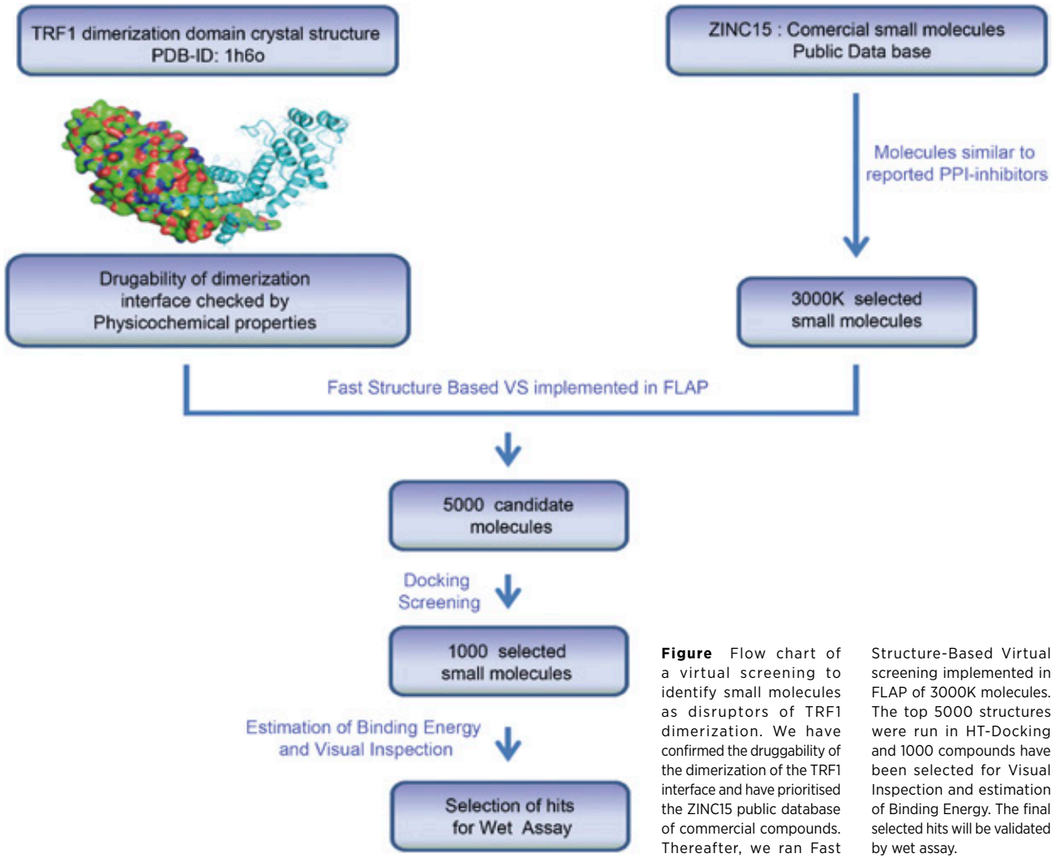

Figure Flow chart of a virtual screening to identify small molecules as disruptors of TRF1 dimerization. We have confirmed the druggability of the dimerization of the TRF1 interface and have prioritised the ZINC15 public database of commercial compounds. Thereafter, we ran Fast Structure-Based Virtual screening implemented in FLAP of 3000K molecules. The top 5000 structures were run in HT-Docking and 1000 compounds have been selected for Visual Inspection and estimation of Binding Energy. The final selected hits will be validated by wet assay.

PUBLICATIONS

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- Toledo RA, Garralda E, Mitsi M, Pons T, Monsech J, Vega E, Otero Á, Albarran MI, Baños N, Durán Y, Bonilla V, Sarno F, Camacho-Artacho M, Sanchez-Perez T, Perea S, Álvarez R, De Martino A, Lietha D, Blanco-Aparicio C, Cubillo A, Domínguez O, Martínez-Torrecuadrada JL, Hidalgo M (2018). Exome sequencing of plasma DNA portrays the mutation landscape of colorectal cancer and discovers mutated VEGFR2 receptors as modulators of antiangiogenic therapies. *Clin Cancer Res* 24, 3550-3559.

PATENTS

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- Blasco MA, Pastor JA, Bejarano L, Méndez-Pertuz M, Martínez P, Blanco-Aparicio C, Gómez-Casero E, García-Beccaria M (2018). Modulation of TRF1 for brain cancer treatment. *EP18382658.5*.