CASE STUDY

Designing chemical probes for DCAF1 using MatchMaker™

Overview

- Cyclica partnered with the Structural Genomics Consortium (SGC) to design a chemical probe for DCAF1, a low data WDR protein.
- DCAF1 is part of a E3 ubiquitin ligase complex, a critical component of the ubiquitin proteasome system, making it a potentially novel target for targeted protein degradation.
- MatchMaker identified hit molecules for DCAF1 that were verified by the SGC in several follow on experiments.
- The SGC solved the first co-crystallized structure of DCAF1 with one of our molecules, CYCA-117-70, which has been deposited in the RCSB Protein Data Bank : 7SSE.
- Follow up hit expansion experiments tested 20 analogues and resulted in a 25-fold boost in potency: 70 uM to ~3uM

DCAF1 has been identified as a putative antiviral host target and more broadly as a promising target to facilitate proteasome-mediated degradation of therapeutic targets[1]. It has a complex domain architecture, containing a WD40 repeat (WDR) domain, which is one of the most abundant protein-protein interactions (PPIs) domains in the human proteome. Given the significant role that PPIs play in many cellular processes and diseases[2] there has been renewed interest in exploring WDR domain proteins, including DCAF1. Acting through the WDR domain, DCAF1 recruits substrate proteins to the CUL4A-RBX1-DDB1-DCAF1 E3 ubiquitin ligase complex for subsequent proteasomal degradation, which is the key process we aimed to modulate or exploit using small molecule probes.

To discover novel DCAF1 probes, the interaction profiles of approximately 3 million commercially available compounds with ~8,500 proteins, including DCAF1, were rapidly predicted using Cyclica's MatchMaker technology. Briefly, MatchMaker is a deep learning approach capable of assessing small molecule-protein interactions across the proteome. MatchMaker predictions informed the nomination of compounds based not only on predicted binding for DCAF1, but also on the lack of interaction with undesirable off-targets. Using MatchMaker predictions, alongside traditional CADD tools, we predicted and tested experimentally 100 compounds for DCAF1 with our collaborators at the SGC. Compounds were assessed through Surface Plasmon Resonance (SPR) to determine binding affinity to DCAF1 as well as an unrelated WDR protein (WDR5). Hits were declared when binding to DCAF1 was observed without indiscriminate binding to WDR5. A top compound, CYCA-117-70, showed selective binding for DCAF1 with a KD= 70µM.

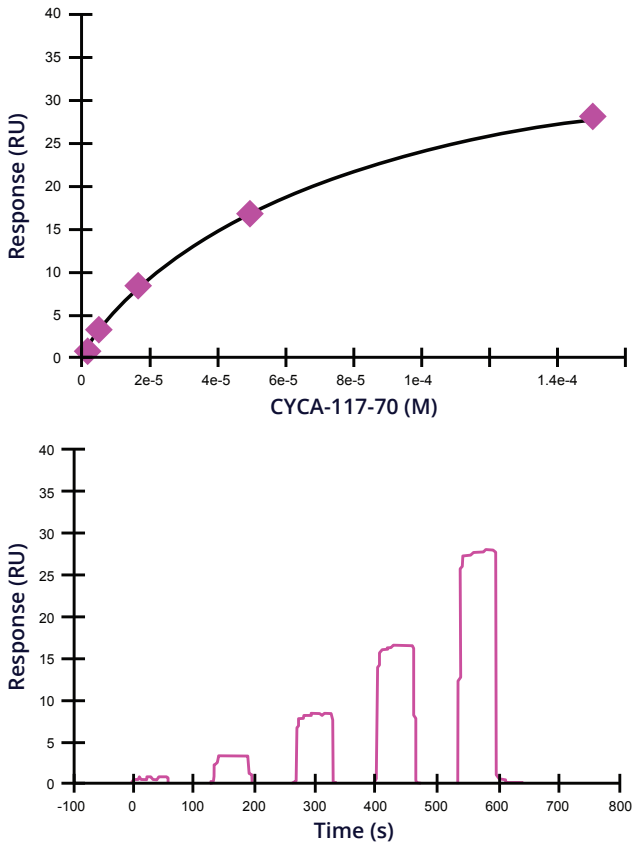

Figure 1. SPR experiment with DCAF1 and CYCA-117-70 reveals binding with KD = 70µM

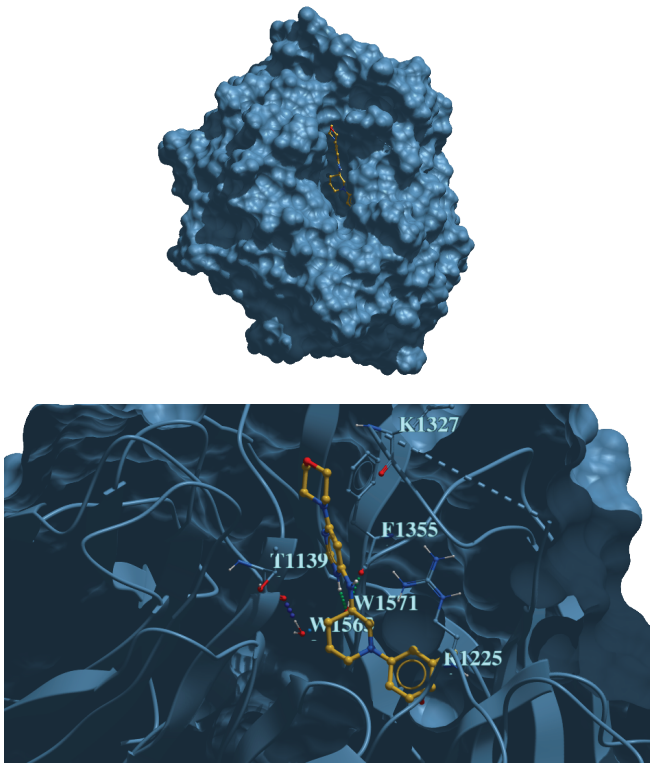

Figure 2. Co-crystal structure of CYCA-117-70 bound to the WDR-domain of DCAF1

CYCA-117-70 was subsequently co-crystallized and deposited as the first ligand-bound structure of DCAF1 in the PDB [PDB ID : 7SSE[3]] (Figure 2).

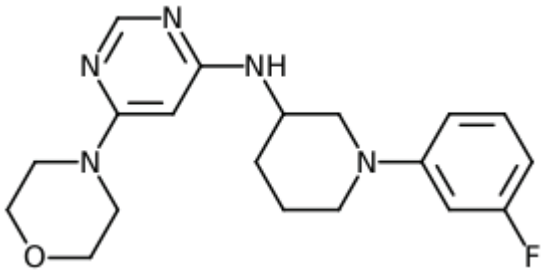

Figure 3. Initial hit compound (CYCA-117-70) Kd = 70 uM.

Prior to determination of the co-crystal structure, a hit expansion exercise was conducted exploring the nearby chemical space of CYCA-117-70. Testing these 20 compounds provided some foundational SAR, with the most potent molecule, CYCA-117-113, showcasing a 25-fold boost in potency. Cyclica is using the series information and the co-crystal structure to propose additional structures, with the SGC testing these compounds to further drive down the tool compound potency.

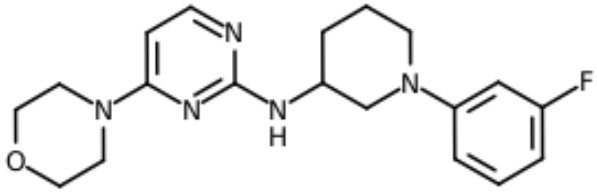

Figure 4. Hit expansion compound (CYCA-117-113) 3 uM

References

1. Schapira, M., Tyers, M., Torrent, M. *et al. Nat Rev Drug Discov* 16, 773–786 (2017)
2. Lu, H., Zhou, Q., He, J. *et al. Sig Transduct Target Ther* 5, 213 (2020)
3. Kimani, S., Owen, J. *et al. To be published* (https://www.rcsb.org/structure/7SSE)