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Novel E3 Ligase Ligand Libraries for Degradation of Proteins Implicated in Malignant Diseases

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1. Introduction and Objectives

Proteolysis-targeting chimeras (PROTACs) are emerging tools for therapeutic intervention by eliminating disease-causing proteins. PROTACs are bifunctional heterodimers that bind simultaneously to an ubiquitin E3 ligase, and a protein to be degraded (POI), where the two small molecule ligands are connected by a chemical linker. The close vicinity of the POI and the E3 ligase caused by the PROTAC triggers the polyubiquitination of the target protein (POI) leading to its degradation by the ubiquitin-proteasome pathway [1]. By knocking-down the target proteins directly rather than simply blocking them PROTAC provides multiple advantages over the small molecule inhibitors: 1. PROTAC technology could also address the undruggable protein targets by binding to any cleft on their surface (more than 80% of the disease related proteins are considered as undruggable); 2. only sub-stoichiometric amounts are needed for potent activity; 3. PROTAC acts effectively with a low systemic exposure, which reduces the off-target problems and toxic side effects. Presently, fewer than 10 of the more than 600 E3 ubiquitin ligases have been exploited for targeted protein degradation. The most commonly targeted E3 ligases are: VHL (Von-Hippel-Lindau ubiquitin ligase) and CRBN (Cereblon). In the present poster we propose targeting additional E3 ubiquitin ligases: cIAP (cellular inhibitor of apoptosis protein 1), and β-TRCP (β-transducing repeat-containing protein) by designing and selecting a small molecule library that preferably interacts with the above E3 ligases. Such ligands could also be appended with appropriate linkers forming partial PROTAC libraries or with POI binding ligands full PROTAC libraries. (***Figure 1***.)

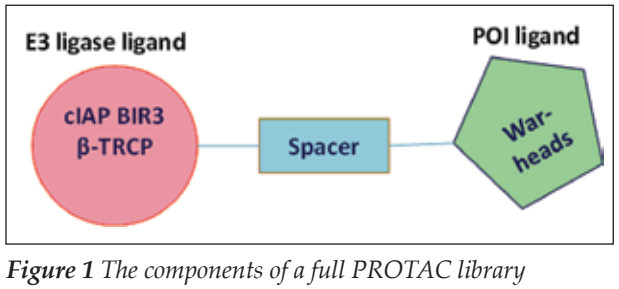

Figure 1 *The components of a full PROTAC library*

2. Results

Applying inhibitor of apoptosis proteins (IAPs). Generation of AVPI-mimetics-based E3 ligase ligand library

IAP family contains various proteins with high similarity (XIAP: X-linked IAP, cIAP1/2: cellular IAP1/2). XIAP inhibits initiator (Caspase-9) and effector caspases (Caspase-3 and -7), through the interaction of its BIR domains (BIR3 and BIR2) with the N-terminal tetrapeptide (AVPI) of the SMAC protein (Second Mitochondria-derived Activator of Caspases). Similarly, SMAC/AVPI is able to bind to the BIR3 domain of cIAPs and this interaction induces auto-degradation of the protein through ubiquitination [2]. However, if SMAC mimetics (compounds having AVPI-like structural motifs) linked to specific binders ("warhead") of a POI the conjugate could induce ubiquitination and degradation of this protein. Auto-ubiquitination and targeted ubiquitination can be balanced by achieving binding selectivity towards XIAP vs. cIAPs.

AVPI (Ala-Val-Pro-Ile) is the minimum peptide sequence that is able to bind BIR3 domains of IAPs with various activities. (XIAP BIR3, cIAP1 BIR3 and cIAP2 BIR3 proteins with Ki = 3.6 μM, 184 nM, and 316 Nm) [3]. Based on the structure of this tetrapeptide systematic research has been initiated

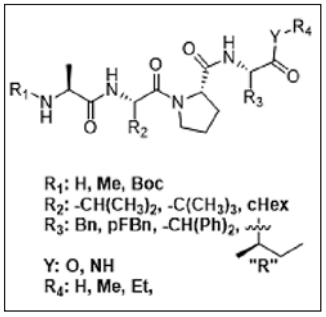

Figure 2 *General structure of the AVPI-mimetics library*

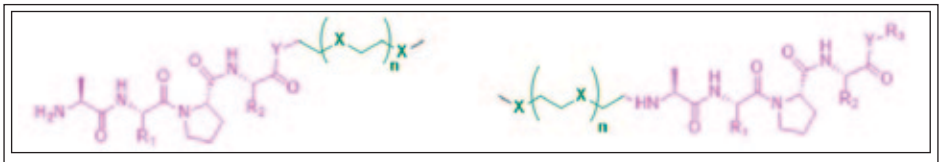

Figure 3 *General structure of partial PROTAC libraries: AVPI-mimetics libraries plus having various spacers and different attachment points*

and led to the discovery of potent apoptosis inducing IAP antagonists as well as E3 ligase triggering small molecule portion of various PROTACs (or SNIPERs: Specific and Non-genetic Inhibitor of apoptosis protein-dependent Protein ERasers) capable of degrading various POIs (kinases, estrogen and retinoic acid receptors etc.) [4].

The general structure of the AVPI-mimetics-based E3 ligase ligand library is shown in ***Figure 2***.

The spacer has additional key importance in the formation of a stable ternary complex (including E3 ligase and the POI) responsible for the effective ubiquitination [5]. The spacer length, flexibility, its hydrophobic or hydrophilic character as well as the attachment points are similarly critical for the efficiency of the PROTAC and avoiding auto-ubiquitination of cIAP1 ("suicide" degradation). Considering the above facts we extended our E3 ligase library to a partial PROTAC library, that also contains various linkers and holding various attachment points (***Figure 3***).

Virtual screening of the AVPI-mimetics library

A virtual E3 ligase ligand like compound library was generated and then *in silico* screened resulting in a 148 membered focused library. Good resolution cIAP1 BIR3 structures were collected from the PDB database (4kmn, 3uw4, 4hy4, 4hy5, 4mti, 4mu7). A cross docking calculation was performed by docking all ligands present in these structures, into all receptors followed by MM-GBSA ΔG_{bind} calculations. The 4LGE structure provided the highest average binding free energy, therefore, this structure was used for Induced Fit Docking calculations. The natural AVPI peptide showed -67.4 kcal/mol binding free energy, while replacement of Ile to Phe (AVPF) resulted in a stronger binding (-71.2). ***Figure 4*** displays the interaction between AVPF and the 4LGE.

In fact, AVPF-related architecture appeared in many potent SMAC mimetics and the lowest free energy (<-65 kcal/mol) members of our focused library are also mimicking this motif. The Induced Fit Docking and the subsequent MM-GBSA calculations were performed the Schrödinger Small- molecule Drug Discovery suite (Schrödinger Release 2019-4; Glide, Schrödinger, llc, New York).

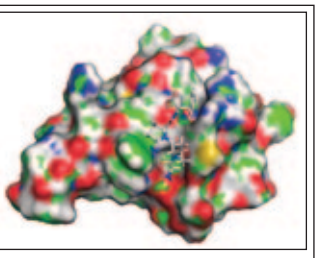

Figure 4 *3D modelling, the binding of AVPF to 4LGE.*

4. Conclusions and Future Plans

In the present poster we reported the generation of a AVPI-mimetics-based E3 ligase ligand library. We extended this focused library to a partial PROTAC library by the variable attachment of different linkers. Based on the above findings full PROTAC libraries could be generated either by connecting diverse small molecules or known ligands binding to specific POIs. The first scenario would allow to discover novel degraders of formally undruggable protein targets.

5. Acknowledgements

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