Unraveling the diversity of molecular glue degraders

By investigating the structure–activity relationship of molecular glue degraders that target cyclin K, we discovered that a wide range of compounds, including known kinase inhibitors, possess this gain-of-function activity. These findings provide insights that might enable more rational design and optimization of molecular glue compounds.

This is a summary of:
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The problem

Molecular glue degraders induce cooperative interactions between a target protein and a ubiquitin ligase, leading to ubiquitination and degradation of the target[1]. The clinical success of lenalidomide (Revlimid)[2] demonstrated the therapeutic potential of this approach and led to extensive research efforts in the field. However, we still have limited understanding of how to discover, design and optimize molecular glue compounds, which hinders their broader applicability. So far, only a few examples of molecular glue degraders have been discovered and there have not been any large systematic studies that structurally characterize glue-induced complexes and evaluate their structure–activity relationship. Thalidomide analogues (immunomodulatory imide drugs, IMiDs) are a notable exception and studies show that very slightly modifying these compounds can lead to the recruitment of new neosubstrates or loss of glue activity[3]. The chemical diversity observed among published cyclin K degraders[4] suggests that this sensitivity to small structural changes is not generalizable to all molecular glues. We aimed to address this question and uncover the unifying and differentiating characteristics of different classes of molecular glue degraders, to facilitate more rational design and optimization efforts.

The discovery

Our study focused on cyclin K degraders, starting with the purine-based kinase inhibitor CR8, which we have shown to glue together DDB1 and CDK12–cyclin K, leading to cyclin K ubiquitination[5]. We derivatized CR8 based on the crystal structure of this ternary complex, focusing mainly on the 'gluing moiety' that protrudes from the active site of CDK12 into DDB1. By virtual screening and mining the literature, we also identified other scaffolds with putative cyclin K degradation activity. In total, we comprehensively evaluated more than 90 compounds, examining their ability to form ternary complexes in vitro, assessing kinase inhibition, cellular cyclin K degradation and cytotoxicity (with or without a neddylation inhibitor). We also determined the structures of 28 glue-induced ternary complexes and conducted proteomics and RNA sequencing analyses on selected degraders.

All the compounds we tested acquired cyclin K degradation activity by simultaneous CDK12 binding and engagement of DDB1 interfacial residues through aromatic, polar or hydrophobic interactions (Fig. 1). Despite the diverse nature of these glues, the overall architecture of the complexes remained remarkably similar. We identified over 40 potent degraders that triggered the formation of CDK12–DDB1 complexes in vitro with an affinity of less than 100 nM, and we rationally designed compounds that are more potent and selective than CR8. Furthermore, we defined a common structural fingerprint for cyclin K degraders and identified cryptic degraders among published kinase inhibitors. Notably, the activity of cyclin K degraders does not seem to require pronounced kinase inhibitory properties, and the balance between CDK12 inhibition and cyclin K degradation can be tuned by compound modifications. Finally, our study reveals distinct transcriptional signatures for cyclin K degraders compared with CDK12 inhibitors or a CDK12-targeting proteolysis-targeting chimera (PROTAC) molecule that does not degrade cyclin K.

The implications

The correlation observed between complex formation in vitro and cellular degradation suggests that the biochemical affinity of the ternary complex is predictive of the extent of target degradation in cells. Moreover, our results highlight that low-affinity protein–protein interfaces containing a defined cavity on one of the interactors provide attractive opportunities for prospective glue design. Therefore, these findings have broader implications for other classes of molecular glues. Comparing cyclin K degraders to IMiDs, we also propose that the interface size, and the compound's relative contribution to it, drive both the structure–activity relationship and substrate specificity of the degrader.

Although we have identified cyclin K molecular glue degraders that are more potent and selective than CR8 and provided many starting scaffolds for medicinal chemistry optimization, further work is needed to fully understand the distinct phenotypic effects of cyclin K degradation. The relevant disease contexts for these interface-leveraging scaffolds also need to be identified and their therapeutic potential assessed.

An interesting question is whether other kinases can be recruited to DDB1 using suitable chemical matter, given the structural complementarity between DDB1 and the kinase domain fold. The endogenous relevance of the DDB1–CDK12–cyclin K interaction is also yet to be described.

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EXPERT OPINION

"The combination of approaches together with the vast array of chemical matter makes this a superb study that will be of great interest to the broad fields of chemical biology, targeted protein degradation, induced-proximity therapeutics, and regulation by the ubiquitin system. The 28 new crystal structures will be a remarkable contribution to our understanding of molecular glue-mediated ternary complex formation." **An anonymous reviewer.**

FIGURE

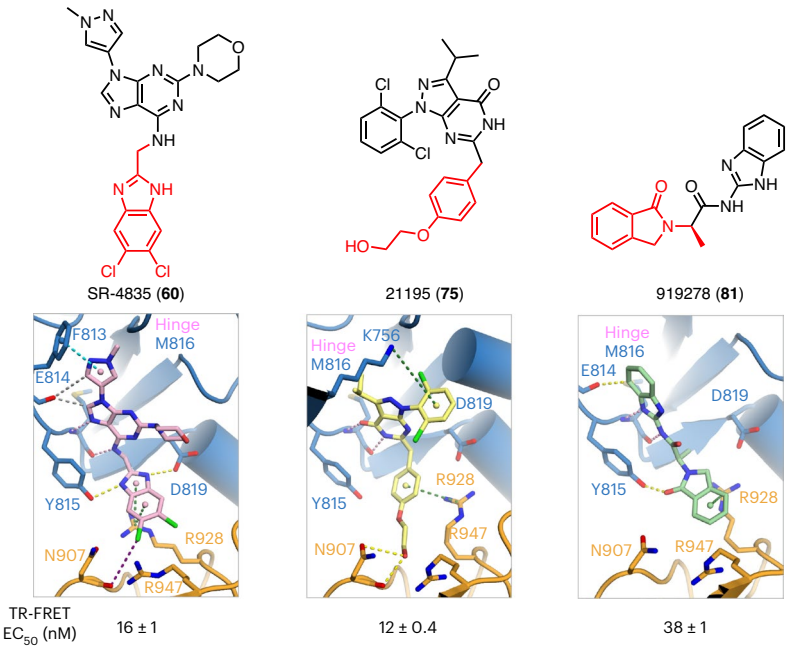

Fig. 1 | Diverse small molecules have cyclin K molecular glue degrader activity. Crystal structures of the DDB1–CDK12–cyclin K complex induced by the compounds SR-4835 (left), 21195 (middle) and 919278 (right). CDK12 is shown in blue and DDB1 in orange. Half-maximum effective concentration (EC_{50}) values from a time-resolved Förster resonance energy transfer (TR-FRET) ternary complex formation assay are listed for each compound. The 'gluing moieties' that bridge the interface and contact DDB1 are indicated in red on the chemical structures.

BEHIND THE PAPER

In our previous study, we were puzzled by the ternary complex formation observed in vitro with CR8-related inhibitors such as roscovitine[4]. As we encountered diverse compounds that could degrade cyclin K, we questioned whether they operated through the same mechanism and interface, driven by the false assumption that all glues follow IMiD-like structure–activity relationships. A deep dive into cyclin K degrader-induced complexes solved this mystery and clearly showed that glues can come in many different flavors! However, assembling this manuscript posed some challenges, mostly because of the sheer number of compounds and structures we evaluated. With a wealth of data to analyze and many possible stories emerging, it was initially daunting to establish a clear narrative. Nevertheless, we persevered, and we are very excited to now present what is hopefully a useful resource for the targeted protein degradation community. **Z.K.**

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FROM THE EDITOR

"This work provides a comprehensive examination of the chemical structural diversity that enables proper molecular glue functioning for small molecule degraders." **Editorial Team, *Nature Chemical Biology*.**