A Rapid Matrix Approach for the Discovery of Potent IRAK4 Targeted Protein Degraders

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Abstract

IL-1 receptor–associated kinase 4 (IRAK4) plays an essential role in regulating innate immunity and has emerged as a significant drug target for inflammatory and immune conditions. Inhibitors targeting the enzymatic function of IRAK4 have shown limited utility, owing to the role IRAK4 plays in providing scaffold interactions essential for maintenance and signaling through the Myddosome.

To effectively block both the enzymatic and scaffolding functions of IRAK4, we aimed to develop an IRAK4 targeted protein degrader for the treatment of rheumatoid arthritis and other inflammatory diseases.

We initiated this effort by selecting three diverse IRAK4 binders, allowing us to explore three different vectors to access solvent. We synthesized a diverse library of degraders utilizing six different ligases. The resulting library screen identified potent CRBN- and VHL-based degraders for a single vector. Ternary complex modeling facilitated rapid optimization of VHL-based IRAK4 degraders with lower molecular weight, fewer rotatable bonds, and practically no linker between the IRAK4 and VHL binders.

We successfully developed potent and selective IRAK4 degraders that show improved inhibition of cytokine secretion compared to inhibitors.

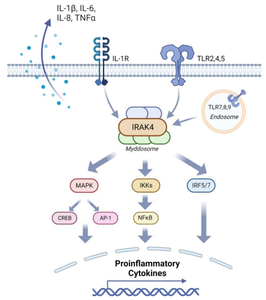
Introduction

IRAK4 Biology

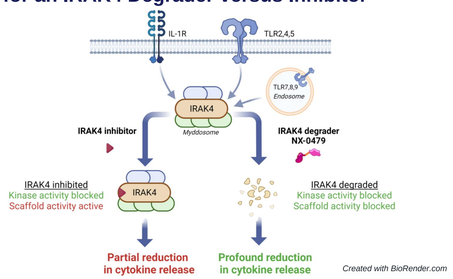
Rheumatoid arthritis and atopic dermatitis represent areas of high unmet clinical need with global adult prevalence rates of ~1% and 2%, respectively.

IRAK4 is a serine/threonine kinase that acts as a central mediator of TLR and IL-1R signaling.

In addition to its kinase function, **IRAK4 has a kinase-independent scaffolding function**, which is essential for formation of the Myddosome and subsequent production of proinflammatory cytokines.



Rationale for an IRAK4 Degrader versus Inhibitor

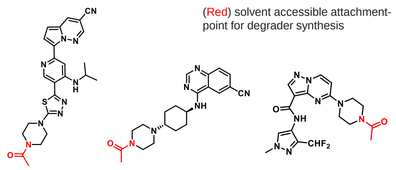


Full suppression of IRAK4 function can only be achieved by elimination of both enzymatic kinase and scaffolding activities.

Results

Figure 1. Chemical Structures of IRAK4 Target Binders Utilized for Synthesis of Initial Degrader Libraries

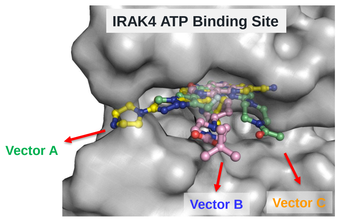
Different IRAK4 target binders were evaluated for potency and ADME properties. Three target binders, exemplifying different solvent exit vectors (A-C), were selected for synthesis of the initial degrader libraries which consisted of linkers of different lengths and binders for 6 ligases (CRBN, VHL, IAP, MDM2, β-TRCP and KEAP1).



Target Binder number (Vector)	1 (A)	2 (B)	3 (C)
IRAK4 biochemical IC_{50} (nM)	0.60	15	4.8
MW / LogD, pH 7.4	488 / 1.5	378 / 1.0	418 / 1.0
Solubility, Ksol PBS pH 7.4 (μM)	0.9	223	83
CACO-2 Papp(A-B) with Elacridar *10^{-6} cm/s	0.73	6.7	20

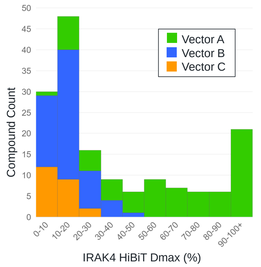
[1]Patent WO2020 036986

Figure 2. Selected Binders Provide Three Different Vectors to Solvent



X-ray crystal structure overlay of three target binders bound to IRAK4 showing vectors (red) to solvent from the ATP-binding site.

Figure 3. IRAK4 HiBiT Results



Vector A compounds led to more potent and complete degradation than Vectors B or C.

Figure 4. Optimization of Vector A Degraders

IRAK4 degradation potency for CRBN- and VHL-based compounds from Series A were optimized.

Reduction in rotatable bonds was an important design parameter and led to improved potencies.

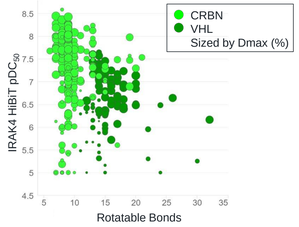
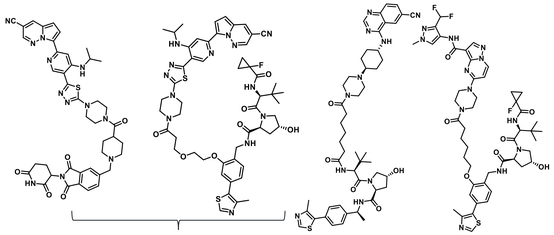
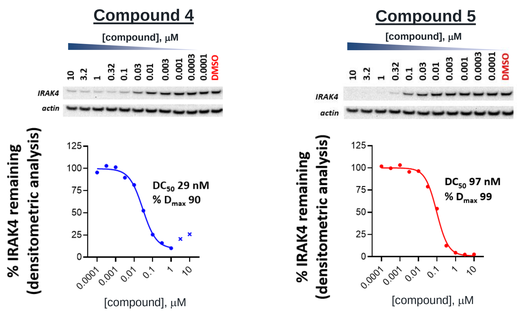


Figure 5. Examples from Initial Degrader Library Screens



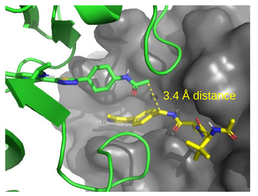
Compound number	4	5	6	7
Ligase recruited	CRBN	VHL	VHL	VHL
IRAK4 biochemical IC_{50} (nM)	0.6	6.2	14	4,045
MW / LogD, pH 7.4	827 / 1.4	1076 / 2.7	905 / nt	1005 / nt
IRAK4 HiBiT DC_{50}, nM (Dmax)	27 (100%)	144 (≥100%)	>10,000 (31%)	>10,000 (14%)

Figure 6. Potent CRBN- and VHL-based IRAK4 Degraders Orthogonally Confirmed by Western Blot



Compounds **4** and **5** showed no effect on viability with 72-hour treatment in a CellTiter-Glo assay.

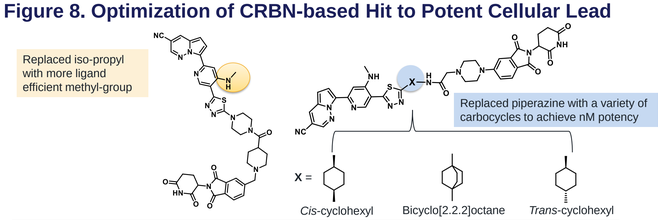
Figure 7. Ternary Complex Model Aided in Design of Alternate VHL Attachment-Point With a Very Short Linker



Potency improvement by utilization of alternative VHL attachment site and short rigid linker

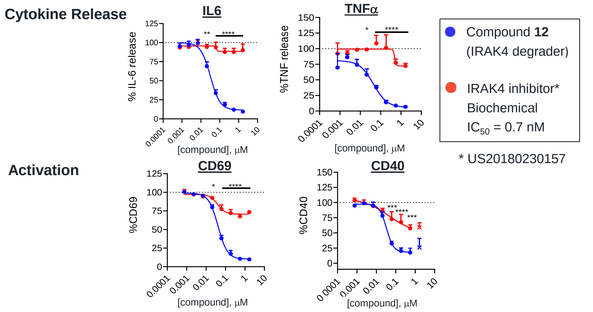
Compound 8
IRAK4 DC_{50} = 30 nM (≥100%)
MW = 987
LogD = 3.3

Figure 8. Optimization of CRBN-based Hit to Potent Cellular Lead



Compound number	9	10	11	12
IRAK4 biochemical IC_{50} (nM)	1.1	0.9	1.1	0.9
MW / LogD, pH 7.4	799 / <1.0	813 / nt	839 / -0.2	813 / 0.6
IRAK4 HiBiT DC_{50}, nM (Dmax)	19 (≥100%)	63 (≥100%)	29 (≥100%)	7 (≥100%)

Figure 9. IRAK4 Degradation, but not Inhibition, Prevents TLR9-induced B-cell Activation and Cytokine Release



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Conclusions

- Utilization of a library of diverse IRAK4 target binders, ligases binders, and linkers allowed rapid identification of potent CRBN- and VHL-based degraders.
- Ternary complex modeling aided the optimization of VHL-based IRAK4 degraders.
- A potent cellular tool compound was used to demonstrate the superiority of degradation over inhibition for IRAK4. Further research and development of IRAK4 degraders inspired by this work was conducted, including work that led to nomination of the development candidate **NX-0479**, for the treatment of inflammatory diseases.

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