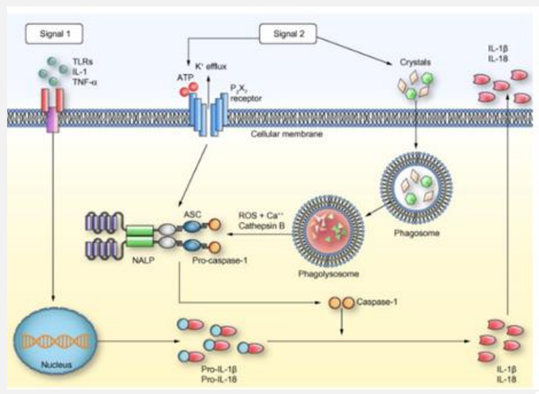
Discovery and Optimization of an mRNA Display Cyclic Peptide inhibitor of the IL1β/IL1R Interaction

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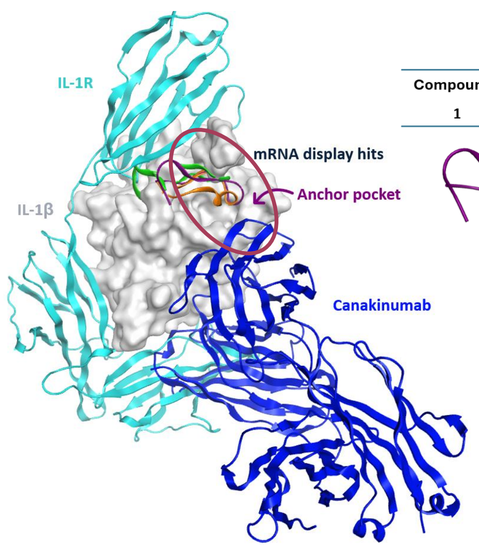
INTRODUCTION

Interleukin-1β (IL-1β), a member of the IL1 family of cytokines, is a key mediator of the inflammatory response[1]. Dysregulated IL-1β signaling plays a critical role in the pathogenesis of numerous diseases, including autoimmune, autoinflammatory, infectious, and degenerative disorders[2]. IL-1β is also involved as target in cardiovascular (CV) diseases, since inflammation drives the progression of atherosclerosis. Lowering inflammation through IL-1β pathway, provides atheroprotection, independent of lipid levels[3]. IL-1β activity can be inhibited by blocking the protein-protein interaction (**PPI**) between IL-1β and its cell-surface receptor IL1R. Clinically, IL-1β inhibition has been achieved using injectable anti-IL-1β monoclonal antibodies[3,4]. However, monoclonal antibody-based therapies are limited by challenges in fine-tuning cytokine modulation and achieving an optimal risk–benefit profile.

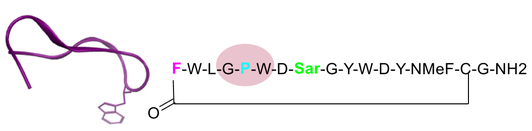


Macrocyclic Peptides Targeting IL-1β

- Macrocyclic peptides (**MCPs**) represent an innovative therapeutic modality for diseases involving protein-protein interactions (**PPI**)
- They offer potential advantages compared to large biological agents, such as increased tissue penetration and possibility for oral administration.
- mRNA display performed by Merck using PeptiDream platform identified a peptide hit (**Compound 1**) interfering with IL-1β signaling having a binding epitope distinct from mAB Canakinumab (about 450 Å^2 overlap)
- The peptide was binding close to the floppy N-terminal and C-terminal regions of IL1β.



Compound#	Biochem IC_{50} (nM)	Cell IC_{50} (nM)	Chymotrypsin $T_{1/2}$ (min)
1	324	1084	9



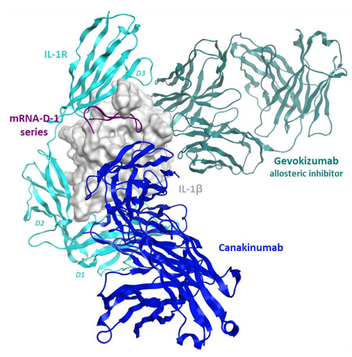
Assays → Disruption of IL-1β/IL-1R interaction

Biochemical: AlphaLISA assay

Cell: MRC-5 (human fibroblasts); measuring effect on downstream IL-6 release

Fig. 1 Initial Lead from mRNA Display

mRNA display hit (**Compound 1**) recapitulates Canakinumab pharmacology, inhibiting IL-6 expression induced by human IL-1β in fibroblast cells



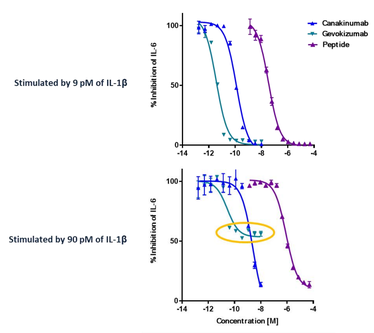
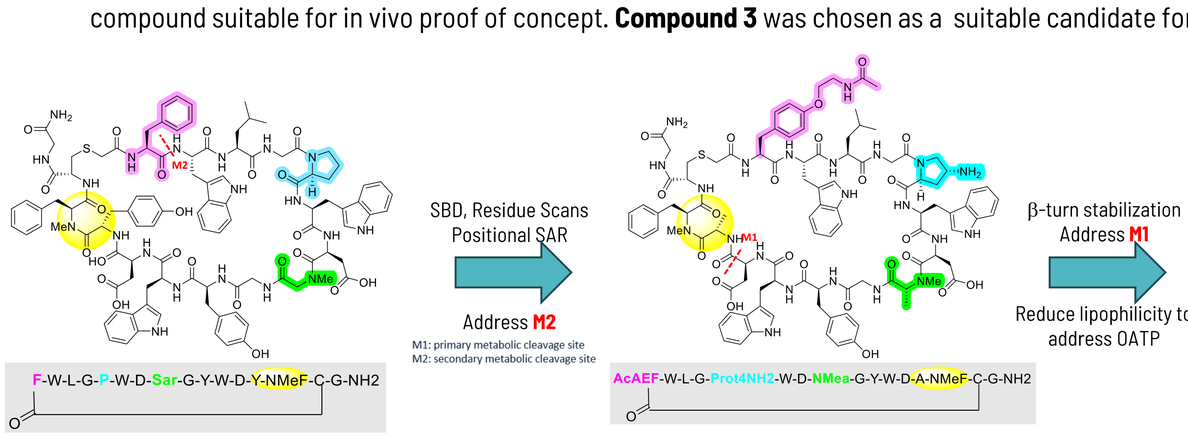


Fig. 2 Pharmacology of Lead from mRNA Display

RESULTS AND DISCUSSION

SAR Evolution

- Residue scanning and structure-based initial design allowed the evolution of **Compound 1** through potency improvement due to target engagement and conformational stabilization
- Further SAR studies helped addressing GI tract instability and OATP transporter issues achieving sufficient potency to develop a compound suitable for in vivo proof of concept. **Compound 3** was chosen as a suitable candidate for in vivo PK/PD study



Compound 1
Biochem IC_{50}: 324 nM
Cell IC_{50}: 1084 nM
Chymotrypsin $T_{1/2}$: 9 min
ALogP98: -1.05; OATP IC_{50}: 100 nM

Compound 2
Biochem IC_{50}: 30 nM
Cell IC_{50}: 75 nM
Chymotrypsin $T_{1/2}$: 106 min

Compound 3
Biochem IC_{50}: 46 nM
Cell IC_{50}: 211 nM
Chymotrypsin $T_{1/2}$: >12 h
ALogP98: -7.02; OATP IC_{50}: >10 μM

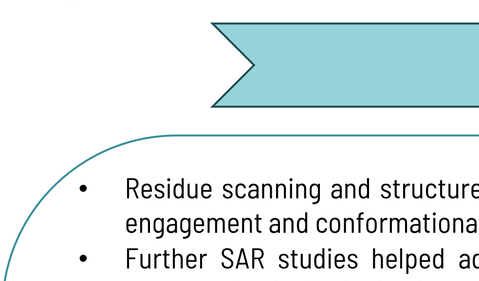
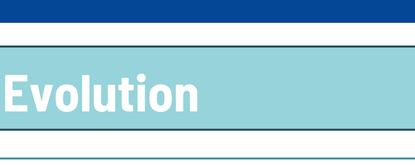


Fig.3 X-ray co-crystal of IL-1β and **Compound 1**

Residue scans
- Alanine
- D-amino acid
- *N*-methylation
- Identify which residues are essential and which are amenable to change



AA1	Biochemical (nM)	Cell (nM)
F (**Cpd 1**)	324	1084
ChA	676	2017
YCH2COOH	304	83
AcAEF	42	249

AA5	Biochemical (nM)	Cell (nM)
P (**Cpd 1**)	324	1084
4RNH2Pro	28	848
4F2Pro	193	1179
aMeP	na	Na

AA13	AA14	Biochemical (nM)	Cell (nM)
Y(**Cpd 1**)	NMeF(**Cpd 1**)	324	1084
A	NMeF	36	102
Aib	G	314	4517
Aib	Sar	332	2169

Fig.4 Scan results and examples of relevant single point positional SAR on Compound 1

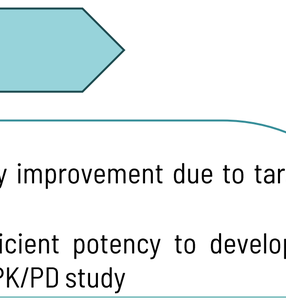
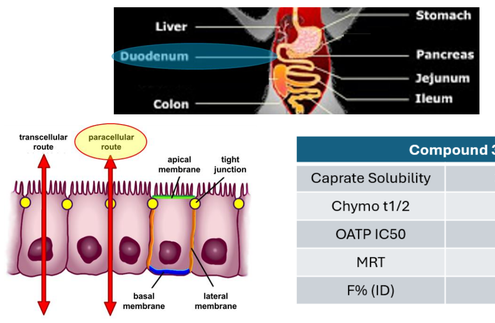


Fig.5 Crystal of **Compound 3** illustrates extension from F1 into space occupied by Arg4

In Vivo data

✓ **Compound 3** demonstrated oral bioavailability when administered to intraduodenally cannulated rats in the presence of 30% Na Caprate as permeation enhancer



Compound 3	
Caprate Solubility	>4 mg/mL
Chymo t1/2	>750 min
OATP IC50	>10 uM
MRT	0.4 h
F% (ID)	24 %; 4 %

✓ **Compound 3** was used to benchmark the MCP modality with Cana and test the specificity of cyclic peptides with IL-1RKO mice in air pouch model

✓ It demonstrated in vivo reduction in IL-6 in air pouch model with a MOA similar to Canakinumab

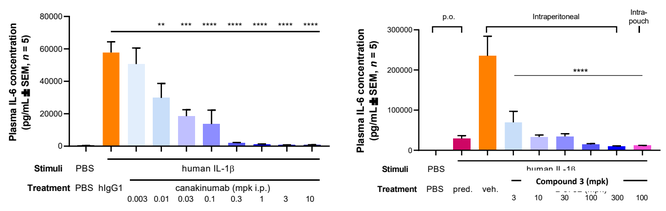


Fig.5 **Compound 3** blocks IL-6 induced by human IL-1b in the mouse air pouch modelIL1R

CONCLUSIONS

✓ An mRNA Display macrocyclic peptide lead inhibiting IL1β/IL1R PPI was evolved through structure-based design and data-driven multi-parameter optimization to a proof-of-concept molecule demonstrating formulation-enabled oral exposure (>2% F) in rodent ID PK study with a permeation enhancer (30% Na Caprate) and PD effect in in vivo with a profile which compares favorably with benchmark antibody Canakinumab and retains a similar MOA

✓ This work demonstrated the tractability of macrocyclic peptides as alternative chemical modality to efficiently tackle the PPI between IL1β and IL1R, and opens the path to future development of peptide-based oral therapies for diseases involving this pathway

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