1. Problem & Solution

The Problem

- ✗ **Autoimmune Diseases:** Immune system attacks body's own tissue (RA, MS, IBD).
- ✗ **Root Cause: RORγt overexpression** drives the IL-23/IL-17 axis and inflammation.
- ✗ **Current :** Existing treatments lack precision and cause systemic side effects.

Targeted Innovation

The Solution

- ✓ **ADC Technology**: Merges accuracy with potent drug payload.
- ✓ **Precision Delivery:** Delivers **RORγt inhibitor** directly to disease-causing cells.
- ✓ **Outcome:** Maximizes therapeutic effect while minimizing harm to healthy tissue.

2. Purpose & Objectives

Can we harness antibodies as intelligent delivery systems to stop inflammation at its source? This project investigates a novel ADC approach to Th17-mediated autoimmune disease.

Design & Construct	Target & Inhibit	Measure Efficacy
Synthesize an ADC linking a RORγt inhibitor to an IL-23/IL-17 receptor using site-specific chemistry.	Validate the ADC's ability to selectively bind target cells and effectively inhibit the RORγt transcriptional pathway.	Quantify significant reductions in pro-inflammatory IL-17 and IL-23 cytokine levels in treated cells.

3. Background Research

The Disease Context

Autoimmune diseases like RA, MS, and IBD are driven by **RORγt overexpression**.

This drives the IL-23/IL-17 axis, causing chronic inflammation, with **Th17 cells** as the key pathogenic drivers.

Current Challenges:

Small Molecule Inhibitor Limitations

- Small molecules (e.g., TMP778, GSK805) effectively inhibit cytokines.
- **BUT:** Clinical use is limited by low bioavailability.
- Systemic distribution causes off-target toxicity.

Linker Design Limitations

- Difficulty controlling drug-to-antibody ratio (DAR)
- Risk of premature payload release
- Limited stability in systemic circulation

4. Hypothesis and Variables

Research Hypothesis

If a RORγt inhibitor is conjugated to an via a cleavable linker, then it can enable more targeted delivery and reduce inflammatory signaling compared to drug by itself.

Independent Variables

- Treatment Type (Free Drug vs. ADC)
- Linker Composition (Val-Cit vs. Val-Ala)
- Drug Concentration

Dependent Variables

- Cell Viability (Cytotoxicity)
- IL-17 / IL-23 Cytokine Levels
- RORγt Transcriptional Activity

Controlled Variables

- Incubation Time & Temperature
- Molar Ratios
- Buffer pH & Composition

5. Methodology

01 Linker Synthesis

Phase I: Synthesis

- Engineered cleavable **Val-Cit/Val-Ala** linker
- Activated via **NHS/Carbonate** chemistry
- Conjugated to **TMP778** analog
- Purified via **HPLC & SEC**

02 Structural Analysis

Phase II: Characterization

- Validated via **^{1}H-NMR and FT-IR**
- Confirmed purity via **LC-MS/HRMS**
- Verified correct molecular mass
- Confirmed structural integrity

03 Stability & Release

Phase III: Evaluation (Planned)

- Incubate in PBS, serum & enzymes
- Monitor degradation via **LC-MS**
- Confirm intracellular release via **Cathepsin B** digestion

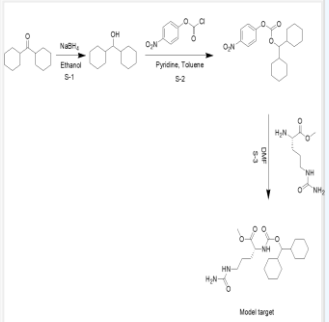
04 Biological Assays

Phase IV: Biologicals (Planned)

- Conjugate to via **Maleimide–Thiol**
- Evaluate RORγt inhibition
- Assays: **FRET** , Nuclear Receptor, & **IL-17F Promoter**

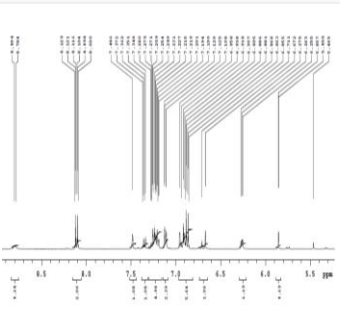
6. Data and Graphics

Model Reaction



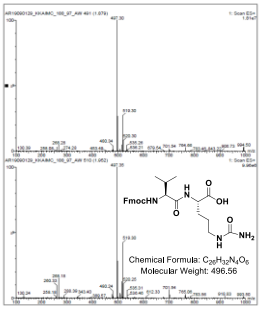
Generated by the student using Chem-Draw 7.

NMR of the Payload



Generated at KAIMRC.

LC-MS of the Linker



Performed at KAIMRC by the student.

High Purity
>95% confirmed by LC-MS

Structure Verified
Exact mass match in NMR/MS

Stability Confirmed
Stable under reaction conditions

Comparative Analysis

Feature	GSK805 (Small Molecule)	The ADC Platform
Off-Target Effects	High risk due to systemic exposure	Reduced due to targeted delivery ✓
Potency	Limited by Systemic Toxicity	Potentially Higher effective Potency ✓
Selectivity	Systemic Distribution	Targeted to RORγt-expressing immune cells ✓

8. Result

1 **Successful Synthesis**

Linker-payload synthesized with high yield and purity.

2 **Structural Validation**

^{1}H-NMR & MS analysis confirms correct molecular structure.

3 **Reproducible Chemistry**

Model reaction establishes a robust, repeatable protocol.

4 **Biological Stability**

Chemical stability ensures suitability for biological assays.

9. Conclusion

Successful Linkage

This model chemistry shows that it is possible to successfully link a biocompatible, cleavable linker to a RORγt inhibitor payload and confirm via testing.

Strategic Foundation

This gives us a solid base for building the full ADC targeting cells producing IL-17 and IL-23.

10. Applications & Impacts

Precise Immunomodulation

- Provides technology for precise immunomodulation.
- Controls Th17 differentiation via RORγt regulation.

Targeted Therapy & Safety

- Enables treatment of autoimmune diseases with targeted therapy.
- Reduces systemic toxicity compared to free small molecules

Platform Versatility

- Adaptable to work with other immune receptors or cytokines.
- Modular linker-payload design enables rapid adaptation for new targets.

11. Future work

Conjugation

Complete conjugation & determine DAR.

Functional Tests

Conduct FRET & RORγt functional assays.

Inhibition

Assess inhibition of IL-17/IL-23 cytokines.

Safety Profile

Perform MTT, ROS, & JC-1 safety assays.

In Vitro Models

Commence inflammatory model investigations.

12. References

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[3] Dixit, T., Vaidya, A. & Ravindran, S. Therapeutic potential of -drug conjugates possessing bifunctional anti-inflammatory action in the pathogenesis of rheumatoid arthritis. Arthritis Res. Ther. 26, 216 (2024).
[4] Xiao, S. et al. Small molecule RORγt antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. Immunity 40, 477–489 (2014).
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