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Design and discovery of novel Granzyme B ligands and evaluation as diagnostics tools in CAR T-cell therapy assessment

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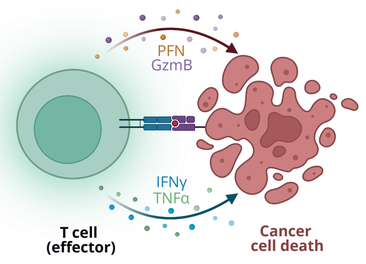
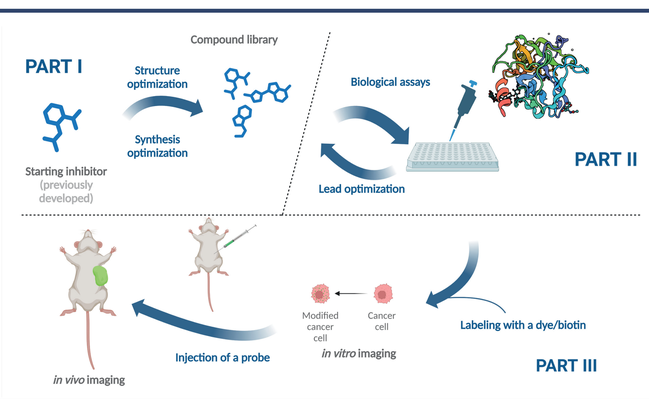
INTRODUCTION

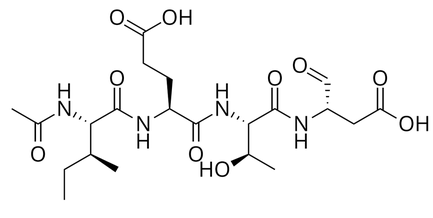
- **Granzyme B (Grnz B) is a serine protease** expressed in cytotoxic T-cells and natural killer (NK) cells that targets virus-infected cells and tumor cells.[1]
- Grnz B activates the **apoptotic pathway** by cleavage of caspases 8, 10, 3, 7.[1,2]
- Grnz B acts as a **signal factor in CAR T-cell immunotherapy.**
- Grnz B represents an intra-tumoral and extra-tumoral **target for molecular imaging** for **early cancer diagnostics and immunotherapy efficiency.**[3]





AIMS AND OBJECTIVES

- Synthesis of a library of novel Grnz B inhibitors with a more drug-like profile to **overcome metabolic stability** issue.
- In vitro biological evaluation of the compounds against **human Grnz B, caspases 3, cathepsins B, L, and S.**
- **Accumulating SAR data.**
- Selection of the **most suitable** (most potent & selective) **inhibitor** to be converted into a **probe** (attachment of a linker with a tag).

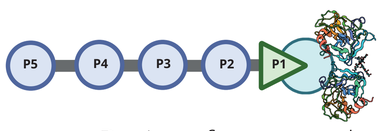


IEDT inhibitor[3]

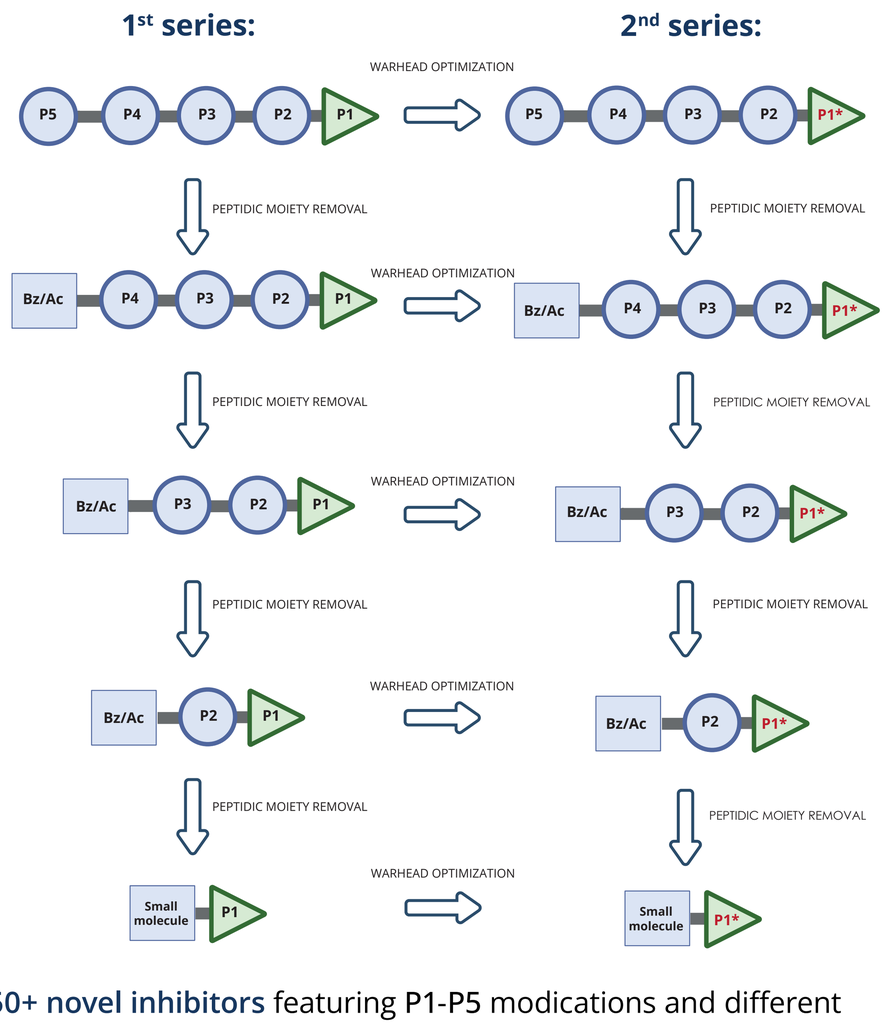
K_I = 80 nM

METHOD AND RESULTS

1. COMPOUNDS LIBRARY SYNTHESIS



Design of compounds



- **50+ novel inhibitors** featuring **P1-P5** modications and different linkers are synthesized and characterized.

LEAD OPTIMIZATION

2. BIOLOGICAL ASSESSMENT

Granzyme B expression: (performed by M.Sc. Emile Verhulst)

Source: HEK293 T-cells (transient transfection)

Activity tests:

Substrate: **Ac-IEPD-pNA** at the KM value
Enzyme: **recombinant Granzyme B**

- **~ 10 hit inhibitors observed (IC50 < 500 nM).**

Reference molecules

Compound	IC50 (nM)	Note
M1	>2245	Main structural motif
M2	125.1 ± 4.9	Ref. molecule 1
M3	45.54 ± 2.96	Ref. molecule 2

Polarity changes

Compound	IC50 (nM)	Note
M4	35.98 ± 7.18	Polar
M5	279.7± 2.9	Non-polar

Linker series

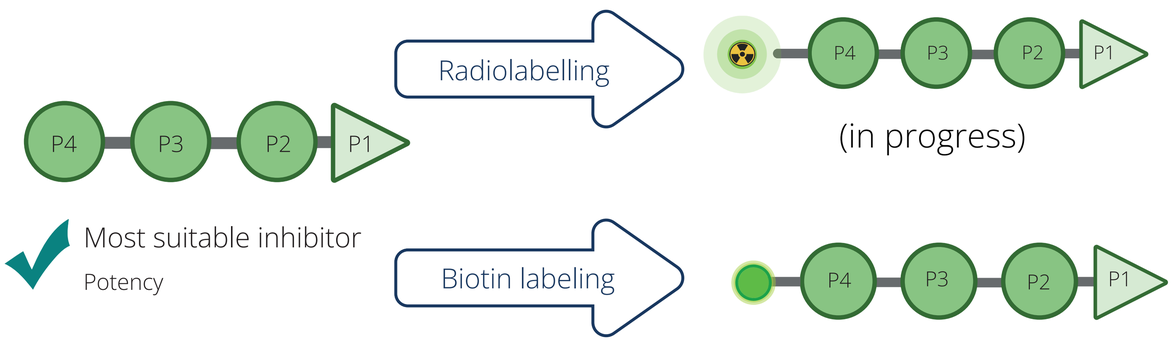
Compound	IC50 (nM)	Note
M6	451.8 ± 4.0	Linker 1
M7	50.74 ± 3.22	Linker 1 ↑ polarity
M8	54.59 ±1.30	Linker 2 ↑ polarity
M9	**27.72 ± 2.91**	**Linker 3 ↑ polarity**
M10	160.0 ± 33.2	Linker 4
M11	93.2 ± 15.2	Linker 4 - cold PET-probe
M12	187.2 ± 14.0	Linker 2 - Biotin probe

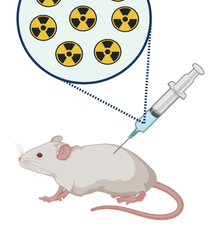
Selectivity tests:

- **High selectivity** (SI >> 100) against Cathepsins B, L and S.
- **Moderate selectivity** (SI ≈ 10) against Caspase-3.

- **Compounds library screened against Granzyme B, Caspase-3, and Cat B/L/S.**

3. PROBE DEVELOPMENT





- 2 precursors featuring different linkers are designed and charachterized.
- 1 probe evaluated with SPR.

REFERENCES

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Funded by the European Union

OncoProTools

This project has received funding from the European Union's Horizon Europe Research and Innovation programme under the Marie Skłodowska-Curie Action No 101073231.