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

Contact info (LinkedIn)

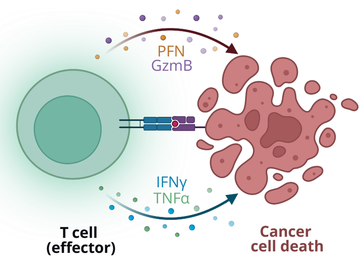
University of Antwerp
Medicinal Chemistry

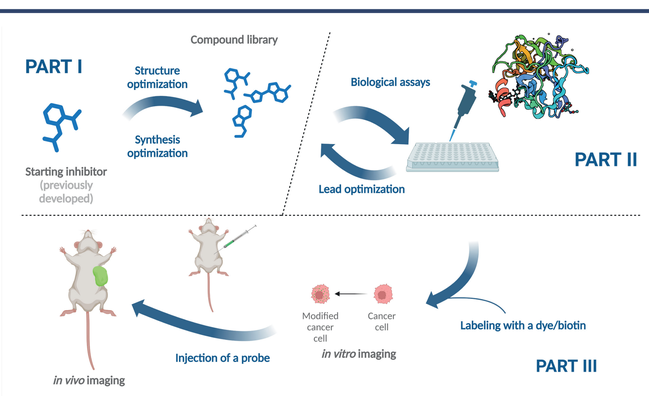
Nikita Shikut[1], Magdalini Thanopoulou[1], Fernanda dos Reis Rocho[2], Roberta Pacifico[1], Emile Verhulst[2], Gayathri S[3], Maarten Vanermen[3], Imane Laktit[4], Maya Berg[4], Ingrid de Meester[2], Filipe Elvas[3], and Pieter Van Der Veken[1]

[1]Laboratory of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Antwerp, Wilrijk, Belgium
[2]Laboratory of Medicinal Biochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Wilrijk, Belgium
[3]Laboratory of Molecular Imaging and Radiology, Department of Nuclear Medicine, University of Antwerp, Wilrijk, Belgium
[4]Department of Pharmaceutical Sciences, University of Antwerp, Wilrijk, Belgium

INTRODUCTION

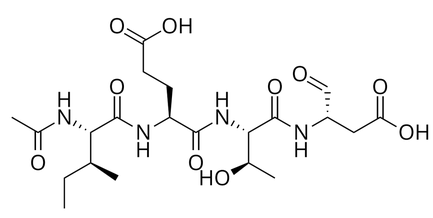
- **Granzyme B (GzmB) is a serine protease** expressed in cytotoxic T-cells and natural killer (NK) cells that targets virus-infected cells and tumor cells.[1]
- GzmB activates the **apoptotic pathway** by cleavage of caspases 8, 10, 3, 7.[1,2]
- GzmB acts as a **signal factor in CAR T-cell immunotherapy**.
- GzmB 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 GzmB inhibitors with a more drug-like profile to **overcome metabolic stability** issue.
- *In vitro* biological evaluation of the compounds against **human GzmB, caspase-3, cathepsins B, L, and S.**
- **Generation of structure-activity relationship (SAR) data.**
- Selection of the **most suitable** (most potent & selective) **inhibitor** to be converted into a **probe** (attachment of a linker with a tag).

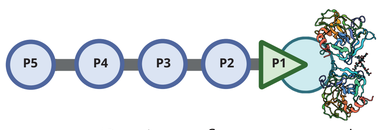


IEDT inhibitor[3]

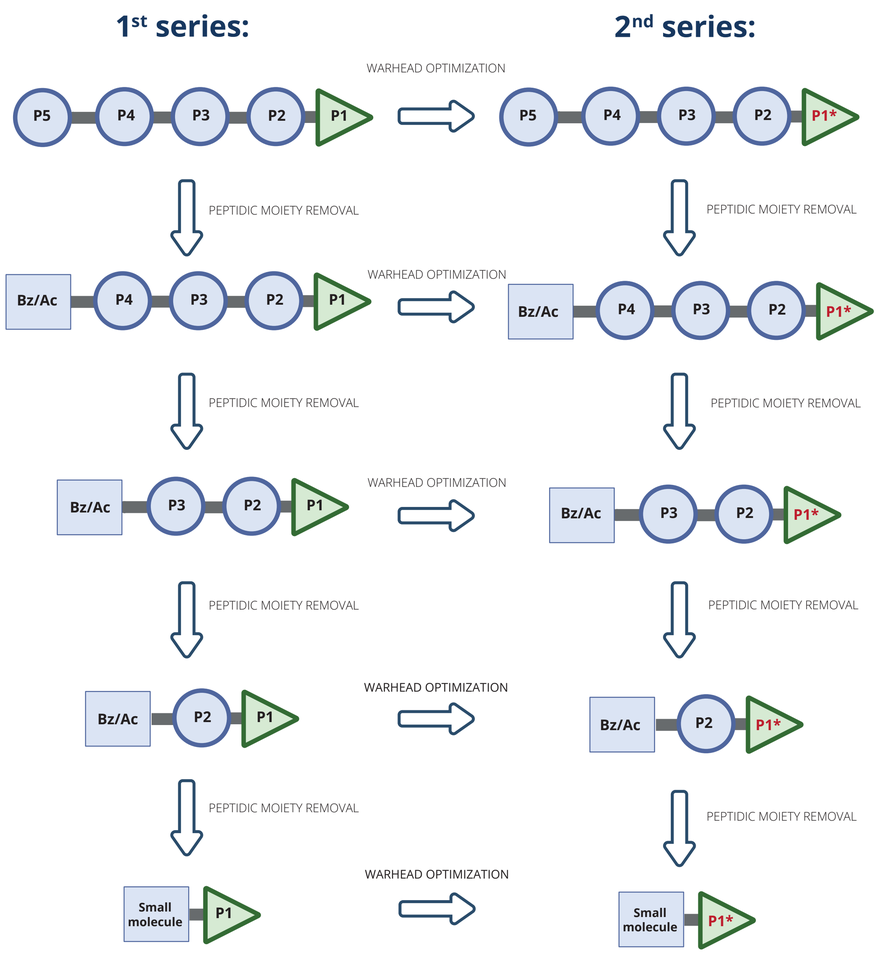
K_I = 80 nM

METHOD AND RESULTS

1. COMPOUNDS LIBRARY SYNTHESIS



Design of compounds



- **50+ novel inhibitors** featuring **P1-P5** modications and diverse linker design are synthesized and characterized.

LEAD OPTIMIZATION

2. BIOLOGICAL ASSESSMENT

Granzyme B expression: (performed by dr. Emile Verhulst & dr. Fernanda Rocho)

Source: HEK293 T-cells (transient transfection).

Activity tests:

Substrate: **Ac-IEPD-pNA** at the K_M value.
Enzyme: **recombinant human Granzyme B.**

- **~ 10 hit compounds observed (IC_{50} < 500 nM).**

Reference molecules

Compound	IC_{50} (nM)	Note
M1	>2245	Main structural motif
M2	125 ± 5	Ref. molecule 1
M3	46 ± 3	Ref. molecule 2

Polarity changes

Compound	IC_{50} (nM)	Note
M4	36 ± 7	Polar
M5	280 ± 3	Non-polar

Linker series

Compound	IC_{50} (nM)	Note
M6	452 ± 4	Linker 1
M7	51 ± 3	Linker 1 ↑ polarity
M8	55 ± 1	Linker 2 ↑ polarity
M9	**28 ± 3**	**Linker 3 ↑ polarity**
M10	160 ± 33	Linker 4
M11	93 ± 15	Linker 4 - cold PET-probe
M12	187 ± 14	Linker 2 - Biotin probe

Selectivity tests:

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

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

3. PROBES DEVELOPMENT

- **One ^{18}F-PET-tracer** is evaluated *in vitro* and in healthy mice.
- **Two biotin probes** are evaluated with SPR.

REFERENCES

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2. J. Biol. Chem. 2020. 295 (28), 9567–9582.
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