Innovative probes for imaging tumor-associated cathepsins through Positron Emission Tomography (PET)

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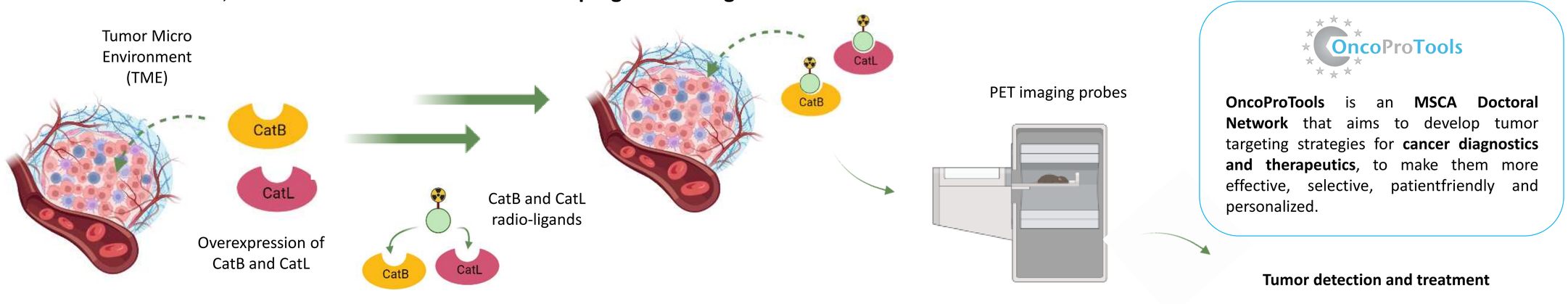
Introduction

Cathepsins play pivotal roles in diverse physiological and pathological processes. **Increased cathepsin levels** have been observed **in both cancer cells and cancer-associated stromal cells**, which contribute to **different tumor progression stages**.[1]

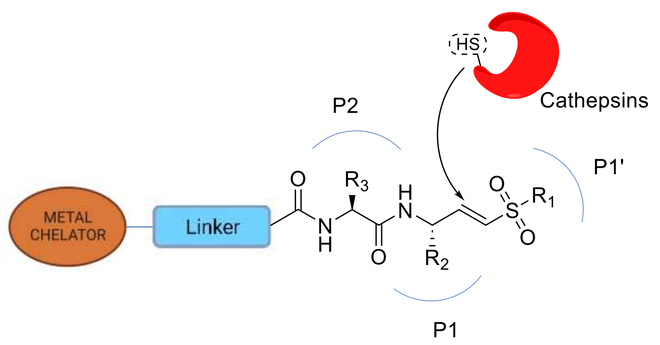


Given their role in tumor growth, cathepsins look promising as **biomarkers** for **cancer diagnosis**. Therefore, the main goal of this research project is to synthesize, develop and evaluate cathepsin inhibitors with a linker capable of connecting to different types of cargos, such as diagnostic radionuclides, holding potential for creating **efficient imaging probes** which can serve as valuable tools for **molecular imaging for tumor detection and treatment** monitoring.[2]

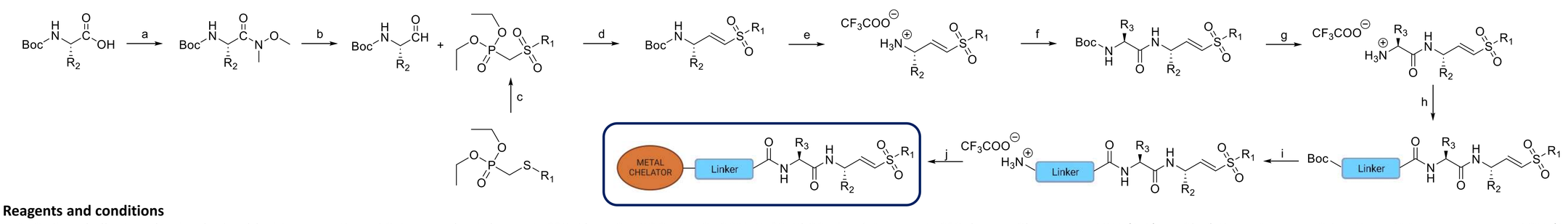
Background and Rationale

Even though several classes of cathepsin inhibitors have been developed, there are still some problems that have limited their use in clinical routine, such as **off-target inhibition** which has led to **side effects in clinical trials**.

Different warheads, designated to covalently link the catalytic residue acting as cathepsin inhibitors, have been identified. Among them, vinyl sulfones stand out as one of the most promising ones, undergoing **irreversible thio-Michael addition** to the active site Cys25 of both Cathepsin B and Cathepsin L.[3]

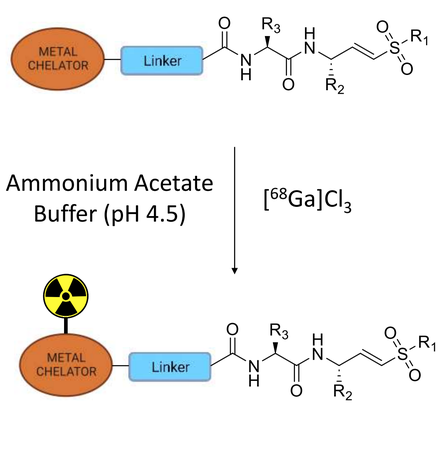


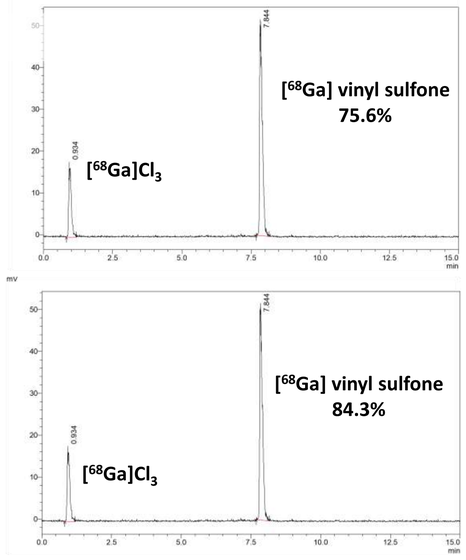
Synthesis of Cathepsin Inhibitors



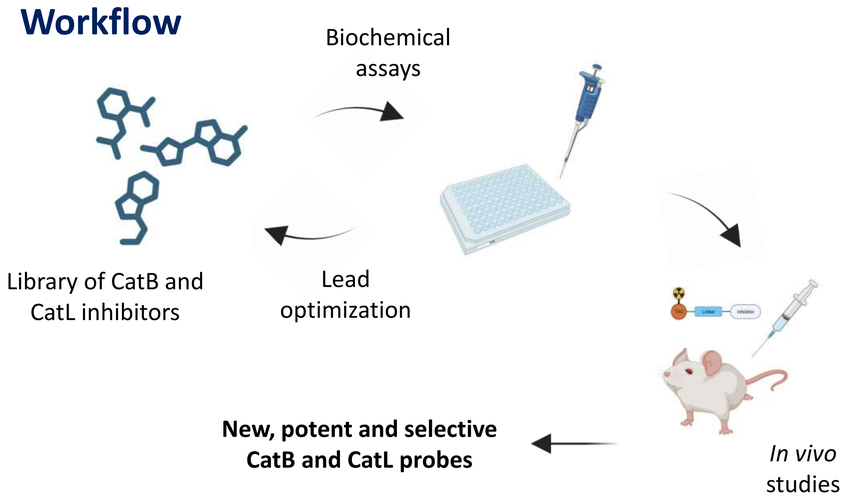
Reagents and conditions
a. N,O-dimethylhydroxylamine hydrochloride, TEA, TBTU, DCM, rt, ov. **b**. $LiAlH_4$, THF, 0°C, 1h. **c**. CH_3COOH, H_2O_2 30%, 85°C, 2h30min. **d**. NaH, THF, 0°C then rt, 3h. **e**. TFA : DCM (1:1), rt, 2h. **f**. Boc-AA, TEA, HOBt, TBTU, DMF, rt, ov. **g**. TFA : DCM (1:1), rt, 2h. **h**. Boc-Linker, TBTU, DIPEA, DMF, rt, ov. **i**. TFA : DCM (1:1), rt, 2h. **j**. METAL CHELATOR, DIPEA, DMF, rt, ov.

^{68}Gallium radiolabeling





Workflow



Conclusion and Outcomes

Expanding upon the established scaffold of known inhibitors, modifications were introduced to the **R_1, R_2 and R_3 positions** of the vinyl sulfone moiety. These alterations aimed to create **novel active inhibitors** specifically targeting Cathepsin B and Cathepsin L enzymes.

Additionally, modifications were implemented at the **R_4 position**, incorporating a **linker** that enables the use of a **chelator agent** enabling the synthesis of **new radiolabeled ligands**.

The biological characterization of these newly developed inhibitors is currently underway through *in vitro* assays to assess their inhibitory activity and selectivity

A comprehensive characterization of these compounds is in progress to ensure its effectiveness and suitability for subsequent investigations

References
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2. *Schleyer KA, Cui L. Molecular probes for selective detection of cysteine cathepsins. Org Biomol Chem. 2021, 19, 6182.*
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