NOVEL PROBES FOR IMAGING OF TUMOR-ASSOCIATED CATHEPSINS VIA POSITRON EMISSION TOMOGRAPHY (PET) AND FLUORESCENCE MICROSCOPY

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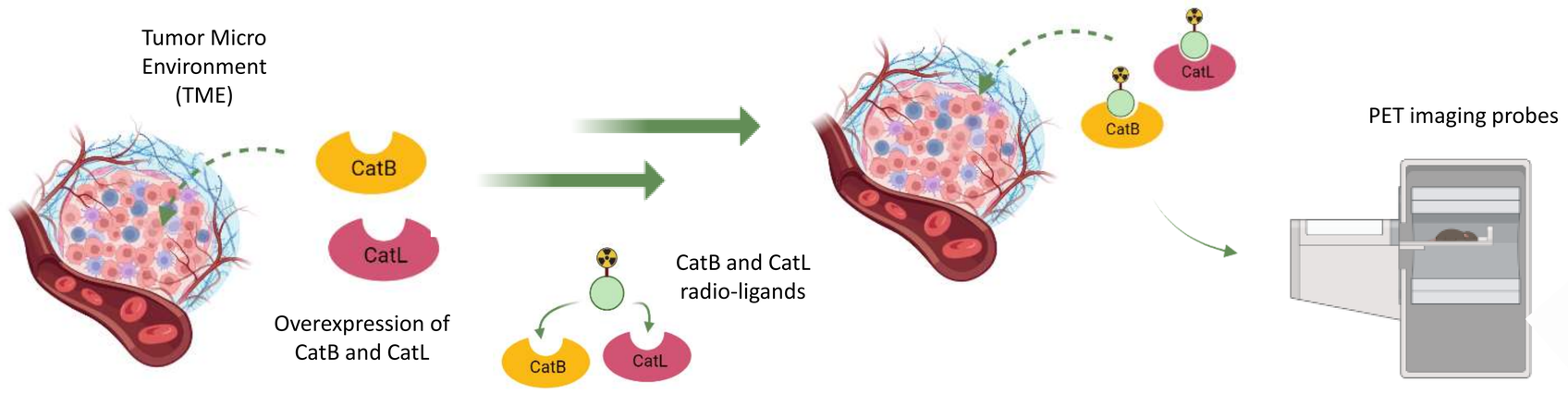
Introduction

Cathepsins play crucial roles in various physiological and pathological processes. **Upregulation of cathepsins** has been observed **in both cancer cells and cancer-associated stromal cells**, which contributes to **tumor development**.[1]



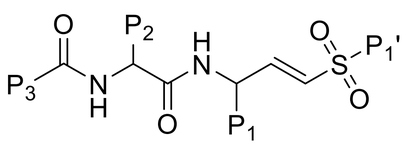
OncoProTools is an **MSCA Doctoral Network** that aim to develop tumor targeting strategies for **cancer diagnostics and therapeutics**, to make them more effective, selective, patientfriendly and personalized.

Due to these characteristics, cathepsins look promising as **biomarkers** for **cancer diagnosis**. Thus, developing cathepsin inhibitors with a linker that allows the connection to different type of cargos, as diagnostic and therapeutic radionuclides, will allow to obtain **efficient imaging probes**, which represent an appropriate tool for molecular imaging and an interesting **biomarker for tumor detection and treatment**.[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 clinics, such as **off-target inhibition** which has led to **side effects in clinical trials**.

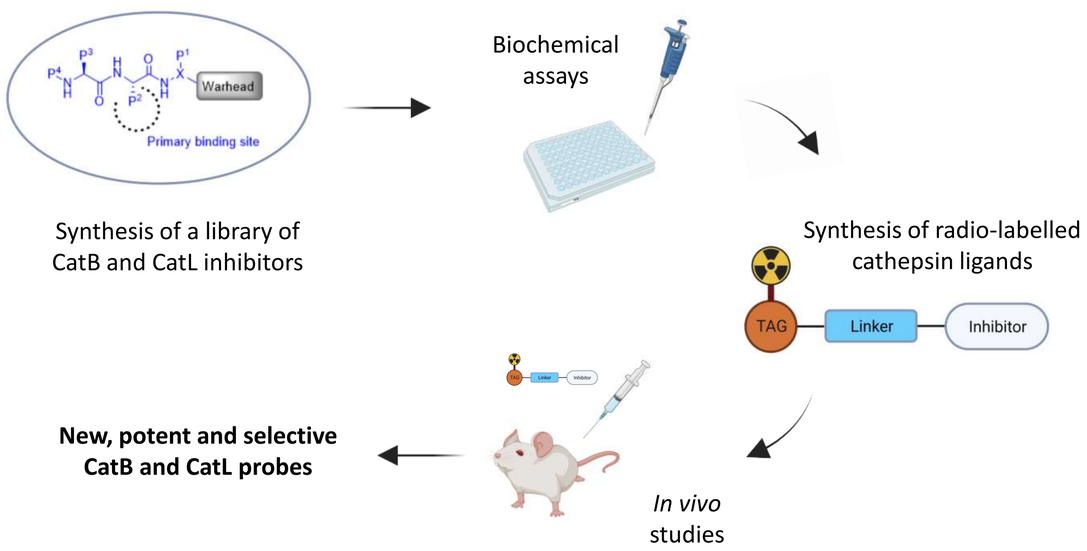
Several warheads, designated to covalently link the catalytic residue acting as cathepsin inhibitors, have been identified to target proteases as activated ketones, epoxides, azanitriles or Michael acceptors like **vinyl sulfones**.



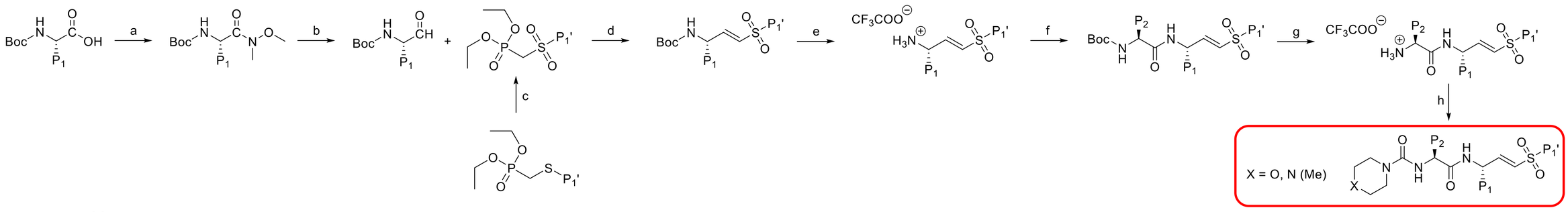
General vinyl sulfone scaffold

One of the most promising inhibitors are represented by **vinyl sulfones**, which undergoes **irreversible thia-Michael addition** to the active site Cys25 of both Cathepsin B and Cathepsin L.[3]

Workflow

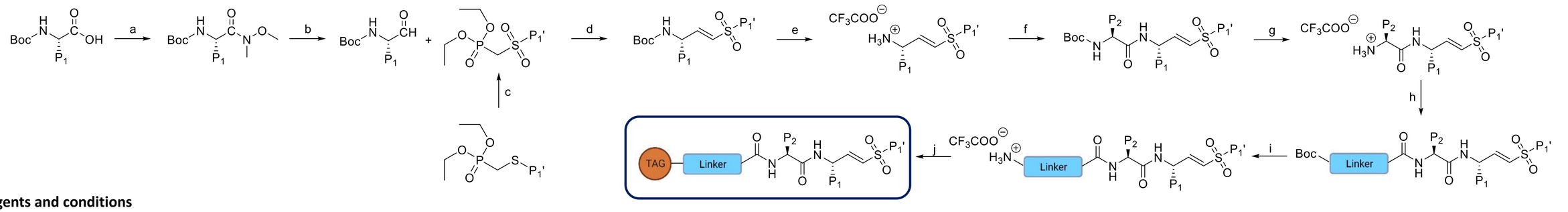


Synthesis of Cathepsin Inhibitors



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, 3h. **h**. morpholine-4-carbonyl chloride / 4-methylpiperazine-1-carbonyl chloride hydrochloride, DIPEA, THF, -15°C then rt, ov.



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Conclusion and outcomes

Building upon the existing scaffold of known inhibitors, modifications were made to the **P1', P1 and P2 positions** of the vinyl sulfone moiety. These modifications aimed to create **novel active inhibitors** targeting Cathepsin B and Cathepsin L enzymes.

These newly developed inhibitors are currently undergoing biological characterization through in vitro assays to evaluate their activity

Modifications were also introduced at the **P3 position**, incorporating a **linker** that allowed the link of a **chelator agent** which will enable the synthesis of **new radio-labeled ligands**.

Extensive characterization of this compound is currently underway to ensure its effectiveness and suitability for further study

References

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2. *Schleyer KA, Cui L. Molecular probes for selective detection of cysteine cathepsins. Org Biomol Chem. 2021, 19, 6182.*
3. *Santos MMM, Moreira R. Michael Acceptors as Cysteine Protease Inhibitors. Mini Rev. Med. Chem. 2007, 7, 1040.*

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