Synthesis of Potential Small-Molecule Inhibitors for *S.commune* Metacaspase

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Introduction

- Metacaspases are enzymes involved in the cell death pathway of fungi. A better understanding of metacaspase function can be helpful in the development of the antifungal drugs.
- The final goal of the project is to elucidate the structures and the properties of five types of metacaspase enzyme in the simple multicellular *S. commune* fungus.
- The Fox Lab wants to study the properties of five types of metacaspase in the *S. commune* fungus. In order to learn about this, we need to know the structures of purified proteins. The Fox Lab has managed to clone all five genes, among which, Scp 1, 2, and 3 have been expressed successfully. Scp 1 and 3 have been purified.
- The Kehlbeck Lab is developing inhibitors for this *S. commune* metacaspase. The project aims to produce compound 11 (Figure 1), which is expected to serve as the inhibitor for all five metacaspases.

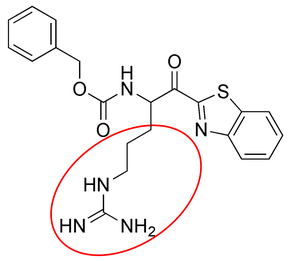


Figure 1. Berg's *Trypanosoma brucei* metacaspase inhibitor (TbMCA inhibitors #11)

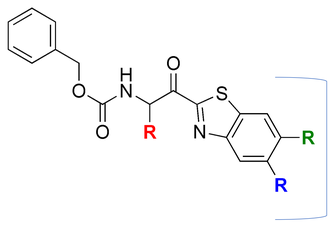
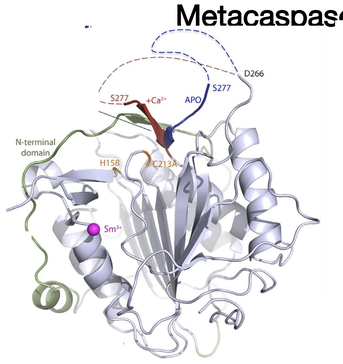


Figure 2. Amino acid analogues in order to explore the molecular features required for inhibition

Metacaspase Enzymes



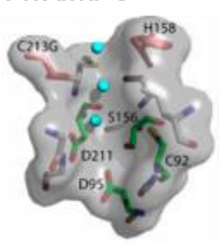


Figure 3. *Trypanosoma brucei* metacaspase (left) and the zoom in view (right) of the binding site where the inhibitor will bind to the enzyme and block the activity of C213 and H158.

Benzothiazole synthesis

- Demkiw has developed a method that will allow us to explore the molecular space of the benzothiazole. Different benzothiazole compounds with different substituent groups on the benzene ring will be used to generate different inhibitors.
- Therefore, the first part of the project is to create and scale up different benzothiazole compounds as starting materials for the future synthesis of a library of different inhibitors.

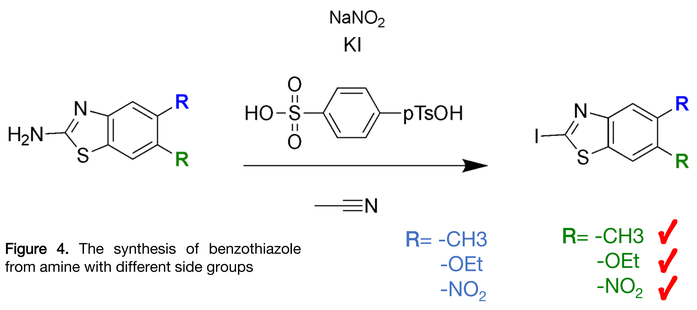


Figure 4. The synthesis of benzothiazole from amine with different side groups

Experimental Approach

- The synthesis of several amino acid benzothiazole analogues will confirm that Demkiw's synthesis pathway can be applied to our system to generate a library of potential inhibitors.
- These analogues will be evaluated for their ability to inhibit *S. commune* metacaspase enzyme activity.
- Analogues with R groups with differing electronic and steric features will allow us to identify the best inhibitors for *S. commune* metacaspase.

Metacaspase Inhibitor Synthesis

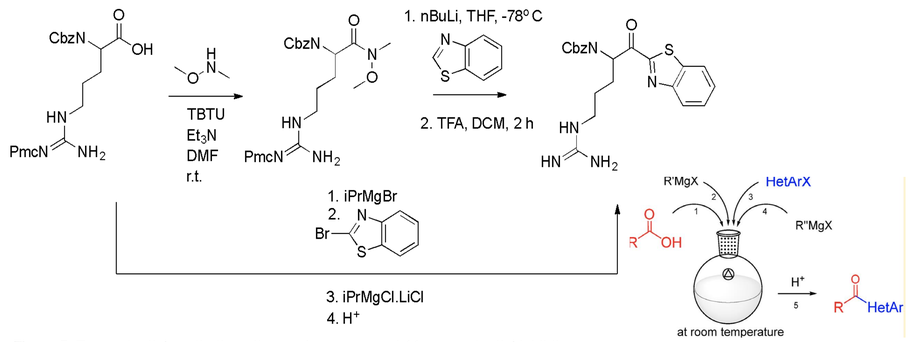


Figure 5. Two potential synthetic pathways for compound 11 as a potential inhibitor for S. commune metacaspase represented by the synthesis mechanism for Berg's first hit T. brucei metacaspase inhibitor (above) and our proposed use of Demkiw's synthesis pathway from carboxylic acid and heteroaryl halides. (below)

Synthetic Routes

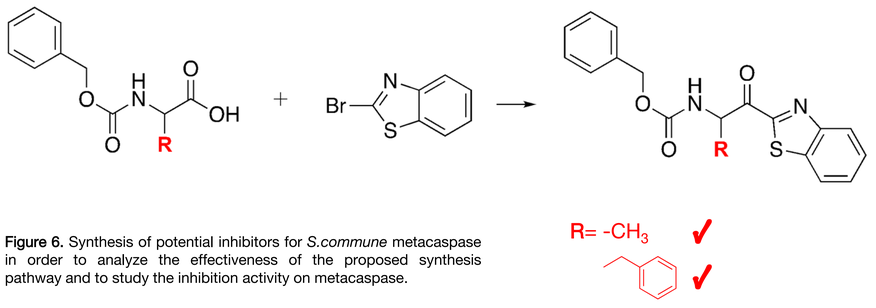


Figure 6. Synthesis of potential inhibitors for *S.commune* metacaspase in order to analyze the effectiveness of the proposed synthesis pathway and to study the inhibition activity on metacaspase.

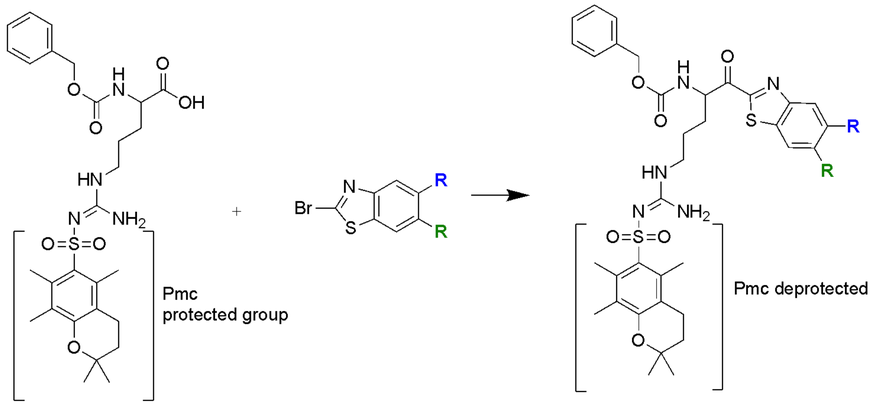


Figure 7. Synthetic pathway for potential S. commune metacaspases inhibitors based on Demkiw's systhesis method from Carboxylic Acid and Heteroaryl Halides.

H-NMR Analysis

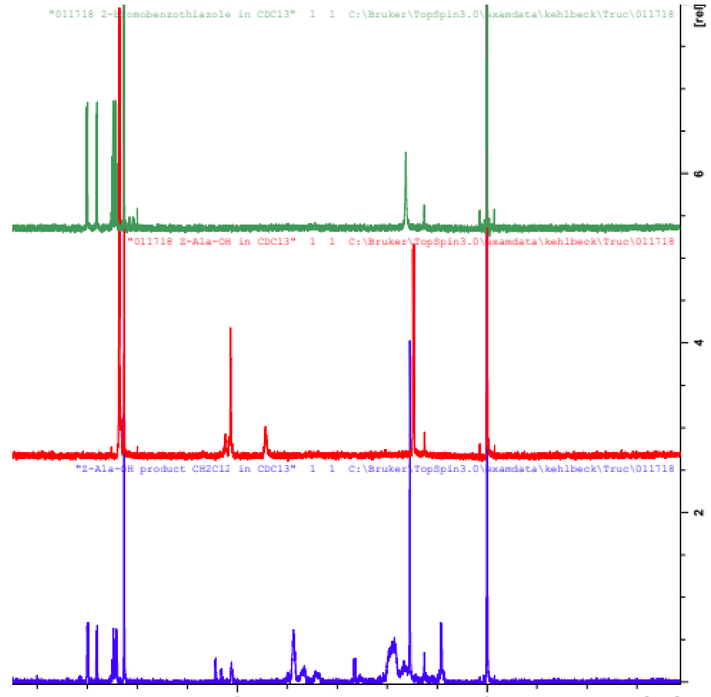


Figure 8. Comparison of H-NMR data of two starting materials 2-bromobenzothiazole (green) and Z-Ala-OH (red) and of the potential inhibitor product (blue) revealed the shift in benzene H and the appearance of new peaks in the product H-NMR and confirmed the effectiveness of the proposed synthesis pathway.

Conclusions and Future Plans

- From the promising H-NMR data, I aim to keep altering the synthesis method gradually to gain a higher yield. The synthesis method will be studied using different model reactions to compare how it works on structures with different R-groups.
- The synthesized products from model reactions are then used to test the activity of metacaspase enzyme in the Fox Lab.
- The final goal of the project is to make a library of different inhibitors for *S.commune* metacaspase. The amended pathway will be applied to make compound 11 and several different inhibitors with different R-groups.
- Different potential inhibtors will be tested in order to propose the most effective inhibitor for *S.commune* metacaspase.

References

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