BSc and MSc project description
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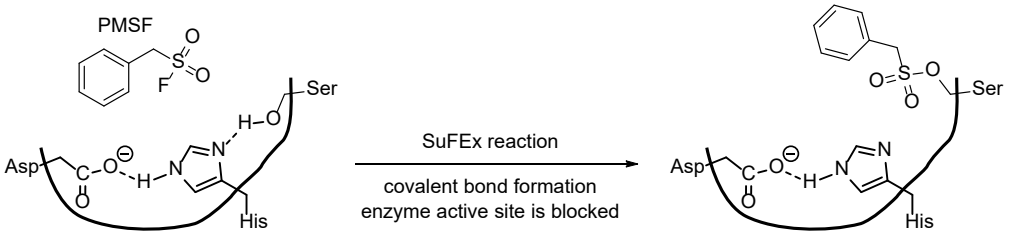
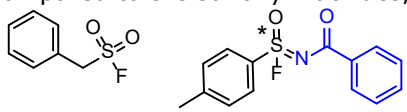
Sulfonimidoyl fluorides as novel chiral, selective serine protease inhibitors

Introduction. Proteases are one of the most abundant classes of enzymes. They cleave peptide bonds by hydrolysis, yielding smaller peptide fragments and are e.g. essential in digestion. We are particularly interested in serine proteases, of which the defining feature is the nucleophilic serine in the active site of the enzyme. The flanking residues determine the substrate specificity of the enzyme. Normally, serine proteases are well regulated by a complex mechanism that (in)activates them. However, this mechanism can malfunction, and then intervention is needed; hence the development of serine protease inhibitors. One of the best known serine protease inhibitors is phenyl methyl sulfonyl fluoride (PMSF).[1] It reacts with the serine of the active site, and covalent inhibition occurs. This is an example of a SuFEx reaction – a Sulfur-Fluoride Exchange reaction, a click-type reaction.[2,3] Important characteristics of click reactions are their high specificity and yield in benign conditions. PMSF undergoes the SuFEx reaction very efficiently, however, due to its reactivity, there is no selectivity in the inhibition – all serine proteases will react with PMSF.



In our lab, we have developed SuFEx hubs, similar to the sulfonyl fluoride of PMSF.[4] These so-called sulfonimidoyl fluorides have several advantages. 1) sulfonimidoyl fluorides are a bit less reactive compared to the sulfonyl fluorides, which means they might be more selective in reactivity. 2) the nitrogen atom can be functionalized (here with Ph-C=O, but also with e.g. amino acids, smaller or bigger moieties, etc.), which offers new opportunities to tune selectivity towards a specific enzyme. 3) perhaps most importantly, the sulfonimidoyl fluoride core is inherently *chiral*, which offers the opportunity for *chiral* inhibitors to be studied.During this project, novel sulfonimidoyl fluorides will be synthesized. Chiral material can be obtained by either a chiral synthesis approach, or by resolution after synthesis, by e.g. preparative chiral HPLC separation. Next, the synthesized sulfonimidoyl fluorides will be tested on their inhibitory effect of serine proteases.[5] Currently, we evaluate the activity on chymotrypsin, but other enzymes, such as subtillisin, trypsin and elastase can also be evaluated. After performing initial assays, further investigations in, for example, enzyme inhibitory constants (such as K_i, etc.) can be determined, using high-throughput assays.



Topics to be studied. Research projects regarding this topic can combine organic 'small molecule' synthesis with a variable amount of enzyme assays. Projects typically include the synthesis of starting materials (or focus on further methodological advances in making these), evaluation of the chirality of these materials, and enzyme activity assays. YOUR drive and expertise are important in co-determining the direction of the project!
Techniques to be used. Multistep organic synthesis, purification and characterization methods, such as chromatography (column, HPLC, chiral HPLC), NMR, mass spectrometry, IR. Enzyme assays, monitored by UV-Vis spectrophotometry.

Literature

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(4) Liang, D.-D.; Streefkerk, D. E.; Jordaan, D.; Wagemakers, J.; Baggerman, J.; Zuilhof, H. *Angew. Chem. Int. Ed.* **2020**, *59*, 7494.
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