# ABSTRACT

Rhomboids are intramembrane serine proteases that belong to the evolutionarily widespread rhomboid superfamily. Rhomboids developed a slightly different catalytic mechanism compared to classical serine proteases; they utilise a catalytic dyad (Ser/His) instead of the common triad (Ser/His/Asp), and the rhomboid active site is buried in the membrane. This, coupled with their hydrophobicity, makes them quite difficult to study. Therefore, even though they are known to be involved in several important biological processes it is still not clear how exactly most of them are involved in the regulation of or in the pathologies of diseases related to these processes (such as malaria, Parkinson's disease or cancer). Our understanding is hindered by the lack of tools for their characterisation both *in vitro* and *in vivo*. In my thesis I present new fluorogenic substrates based on the LacYTM2 sequence, which is hydrolysed by several different rhomboid proteases. Using Förster resonance energy transfer (FRET)-based methods, these substrates are suitable for continuous monitoring of rhomboid activity *in vitro*. Modifications in the P5-P1 residues can improve selectivity for a specific rhomboid, the choice of FRET pair of fluorophores that absorbes light of longer wavelengths makes them suitable for high throughput screening (HTS).

Selective and potent inhibitors are a valuable tool for studying the molecular mechanisms underlying enzyme function. However, such inhibitors have been lacking for rhomboid proteases. The inhibitors developed in my thesis are non-toxic and easily synthetically accessible and modifiable. The inhibitors based on the N-methylen saccharin or the benzoxazin-4-one scaffold are not potent enough for direct cell biological use, but further derivatisation could lead to improvement in potency. The peptidyl ketoamides are based on the structural understanding of rhomboid specificity and mechanism and they are the most promising class. They combine a substrate-derived peptidyl part with the electrophilic reactive group of the ketoamide, extended by a hydrophobic substituent. The resulting inhibitor scaffold is potent, selective, covalent and reversible. With low nanomolar potency *in vivo*, peptidyl ketoamides are by far the most effective rhomboid inhibitors available. Modifications of the peptidyl part and the C-terminal hydrophobic substituent will enable tuning of inhibitor selectivity to diverse rhomboid proteases.