Thesis title: "DECIPHERING ALLOSTERIC REGULATION OF THE SARS-COV-2 MAIN PROTEASE AND ITS MODULATION BY SMALL MOLECULES"

Abstract

The COVID-19 pandemic has highlighted the need for antiviral strategies that remain effective against viral evolution. The 3-chymotrypsin-like protease ($3CL^{pro}$) of severe acute respiratory syndrome coronavirus 2 is essential for viral replication and a major drug target. Most current inhibitors target the catalytic site, which is susceptible to resistance due to mutations. This study investigates both catalytic and allosteric mechanisms that regulate $3CL^{pro}$ activity, intending to identify alternative strategies for inhibition. We performed a comparative analysis of key catalytic residues (F140 and C145) and allosteric residues (N28 and R298) that contribute to enzyme activity and dimerization. The allosteric site affects not only enzymatic activity but also the oligomeric form of the protein. Mutational and computational analyses showed that changes in these residues disrupt critical interactions required for enzymatic function and oligomer stability. Both in-silico and in-vitro results confirmed that perturbation of these sites reduces proteolytic activity and alters the structural integrity of the enzyme. We further explored an allosteric pocket near residue Asn28 as a potential druggable site. Structure-based virtual screening identified novobiocin as a ligand for this region. Biophysical studies showed direct binding with sub-micromolar affinity ($K_d \sim 3 \times 10^{-7}$ M). Ligand binding reduced thermal stability and affected dimer formation. Enzymatic assays showed a strong decrease in catalytic turnover with minimal impact on substrate binding, indicating an allosteric mode of inhibition. Molecular docking and simulation analyses supported stable binding and revealed localized changes in protein dynamics. These findings establish that both catalytic and allosteric sites play essential roles in regulating $3CL^{pro}$ function. Targeting allosteric regions such as the Asn28-associated pocket offers a promising approach for antiviral development. This strategy may provide improved specificity and reduce the likelihood of resistance compared to active-site inhibitors.