In Silico and *In Vitro* Screening for Anti SARS-CoV-2 Main Protease Inhibitors

([1]*Chulalongkorn University,* [2]*Khon Kaen University,* [3]*Shanghai University*)
○ Piyatida Pojtanadithee,[1] Kamonpan Sanachai,[2] Phornphimon Maitarad,[3] Kittikhun Wangkanont,[1] Thanyada Rungrotmongkol[1]
Keywords: SARS-CoV-2; 3C-like protease; Pharmacophore; Molecular docking; MD simulation

The outbreak of the potentially fatal coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is rapidly contributing to public health concerns across the world. The viral 3-chymotrypsin-like protease ($3CL^{pro}$) enzyme cleaves the polyproteins to generate individual functional proteins and is significant for its life cycle. Therefore, it is considered an attractive antiviral target protein. This study employed *in silico* and *in vitro* methods to identify new antiviral candidates targeting SAR-CoV-2 $3CL^{pro}$ from an in-house database. Drug-likeness properties were used for early screening to collect a set of compounds with highly successful drug discovery. The chemical features, binding modes and binding affinities, enzymatic activity, intermolecular interactions, and system stability of the entire system were then investigated using pharmacophore-based virtual screening, molecular docking, enzyme inhibition assays, and molecular dynamics (MD) simulations. This virtual screening was explored based on the pharmacophore model of three peptidomimetic inhibitors (11a, 13b, and N3) and approved drug (Nirmatrelvir) complexes with $3CL^{pro}$. Afterwards, they served as a reference compound during the docking process. There were 75 from a set of 1,052 compounds possessed a characteristic of drug-likeness, and 60 of them were selected as hit compounds from the pharmacophore-based virtual screening. As a result, three docked compounds (SWC422, SWC423, and SWC424) provide stronger binding affinity than Nirmatrelvir and suggested as antiviral candidates inhibiting $3CL^{pro}$ with key residues M49, G143, C145, M165, L167, and A191. The results revealed that their inhibitory efficiency was comparable. Thus, a candidate compound SWC423 was selected to determine the system stability through MD simulation, which will serve as a guide for further *in vitro* and *in vivo* experiments in the COVID-19 drug development.