BIOTROPIC The Journal of Tropical Biology

Vol 7. No 2. Agustus 2023

ISSN 2580-5029

The Ability of Secondary Metabolites from *Actinomadura* sp. as COVID-19 Protease Inhibitor: In Silico Method

Yuriza Eshananda[1*], Sri Martina Wiraswati[1], Sri Lestari[1], Afifah Mariana[1] , Tia Erfianti[2], Hermin Pancasakti Kusumaningrum[3]

[1]Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia

[2]Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3]Biotechnology Study Program, Faculty of Natural Sciences and Mathematics, Universitas Diponegoro, Semarang, Central Java, Indonesia

**Corresponding author*: yuriza.eshananda@unsoed.ac.id

ABSTRACT

The pandemic of COVID-19 disease in the late of 2019 resulted in the massive screening for drug discovery purpose. However, there is still no reports about the ability of natural products from bacterial group of class *Actinobacteria* as COVID-19 inhibitor. The aim of this research is to identify the potential ability of natural compounds from *Actinomadura* sp., the member of class *Actinobacteria*, against two receptors of COVID-19 protease with PDB ID 6LU7 and 5R7Y. The eleven natural compounds were docked using AutoDock Vina and the interaction between receptor and ligands were analysed using LIGPLOT. The most potential compound was simulated for its interaction stability using Yet Another Scientific Artificial Reality Application (YASARA) dynamics. The result of molecular docking by AutoDock Vina showed that Sagamilactam become the most potential inhibitor for viral protease as it had lower binding affinity (6LU7:-12 and 5R7Y:-10.4) compared to the both of native ligand (6LU7:-11.4 and 5R7Y:-4.6). Furthermore, the interaction of the most potential ligand showed the low number of Root Mean Square Deviation (RMSD) deviation in molecular dynamic simulations. This result validated the docking method that used and indicated that secondary metabolites produced from rare actinobacteria of *Actinomadura* sp. have promising possibility to inhibit COVID-19 protease.

Keywords: *COVID-19 Protease, Actinomadura, Molecular Docking*

INTRODUCTION

Phylum *Actinobacteria* is the spore-forming and aerobic Gram-positive bacteria. It comprises 5 classes, 19 orders, 50 families, and 221 genera (Amin et al., 2020). Also, *Actinobacteria* are well-known as the antibiotic and enzyme producers. *Actinobacteria* can be divided into two groups namely, *Streptomyces* and non-*streptomyces* or also known as rare actinobacteria (Azman et al., 2015). Interestingly, many novel species from both are continuing to be discovered which also increase the possibility to find potential compounds.

The genus *Actinomadura* is one of rare Actinobacteria which has been defined as aerobic, Gram-positive, non-motile, and chemoorganotrophic soil actinomycetes. These microbes characterised with extensively branched, non-fragmenting substrate mycelium, and mostly aerial hyphae appearance matured into straight, curled, hooked or spiralled spore chains. Although *Actinomadura* strains are mostly mesophilic and non-pathogenic, some thermophiles have also been identified and strains that are pathogenic to both men and domestic/farm animals are known(Tarantini et al., 2021). Most of the members of *Actinomadura* are the antimicrobial producers against various pathogenic bacteria (Badji et al. 2006).

The spreading of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in Wuhan City of China in the year of 2019(Singhal, 2020). Currently, the Johns Hopkins University data showed that there are more than six hundred million cases with six million of deaths across the country (https://systems.jhu.edu/). Although some vaccines are already available, they have the limitation according to the efficacy duration which decreased from 1 month to 6 months after full vaccination(Feikin et al., 2022). Therefore, the screening of drug candidates, mainly from natural compounds, is still undergone by many researchers. The main protease (Mpro) is one of drug targets to inhibit viral replication (Huff et al., 2022). It intersects the viral polypeptide pp1a and pp1ab into functional proteins that are significant for the replication process. This enzyme also showed a low mutation ratio on its active site which did not widely impact the interaction with the Mpro inhibitor (Glaser et al., 2022).

Many of the compounds that potentially combat the SARS-CoV-2 Mpro are obtained from plants (Kusumaningrum et al., 2022; Puttaswamy et al., 2020). Previously there was no information regarding the ability of natural compounds from *Actinomadura* to inhibit the SARS-CoV-2 replications. Thus, the aim of this research is to identify the potential ability of natural compounds from *Actinomadura sp.* to inhibit COVID-19 protease.

METHODS

Data Mining

The protein structure of COVID-19 protease (PDB ID: 6LU7 and 5R7Y) were downloaded from the Protein Data Bank (PDB: https://www.rcsb.org/) The ligands used in this research were based on the previous research by (Ding et al. 2019) and obtained through PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The ligands which are used in this research comprises of below.

Ligand	References
Native	Protein Data Bank
Actinomadurol	(Ding et al., 2019)
Anthracene	
Azanthromicin A	
Bendigole D	
Bendigole E	
Bendigole F	
Hemi-Oxanthromicin D	
Oxanthromicin E	
Oxanthromicin F	
Oxanthromicin G	
Sagamilactam	

Protein and Ligand Preparation

The native ligands from both receptors were used as control and the active site of the grid box is adjusted by its position through Autogrid in Autodock 4.0 Software (Morris et al., 2009). The grid size for 6LU7 was 28 x 54 x 32 while for 5R7Y was set to 26 x 30 x 28 xyz points. The dimension of grid centre for 1IYL was 12.397(x), 47.683 (y), 0.272 (z) while for 5R7Y was 10.301 (x), -2.386 (y), 24.735 (z).

Molecular Docking Analysis

The COVID-19 Protease receptors were docked using AutoDock Vina (AV) software (Trott & Olson, 2009) operated in Windows subsystem Linux. The binding affinity value and RMSD used as the main parameter. In addition, the molecular interaction between ligands and receptors were analyzed using LIGPLOT.

Lipinski Rule of Five Test, Toxycology Analysis, and Antiviral Prediction

The physicochemical test and toxicology analysis of secondary metabolites that tested against COVID-19 main proteases affinity was carried out by entering the canonical SMILES into pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction). Meanwhile, the antiviral properties of each compound are predicted through the PassOnline server also by entering its canonical SMILES.

Molecular Dynamics Analysis

The Receptor-ligand complex is simulated using Yasara Dynamics (Krieger & Vriend, 2015) with AMBER force field (Case et al. 2005) for 7 nanoseconds. The root mean-square deviation (RMSD) was observed to measure the molecule's stability.

RESULTS AND DISCUSSION

In the present research, the binding affinity between natural compounds from genus *Actinomadura* and SARS-CoV-2 viral protease were studied in Table 1. The present study was proposed to identify the ability of secondary metabolites from *Actinomadura sp.* against SARS-CoV-2 viral protease (Mpro). Here we used two different Mpro, composed of 5R7Y and 6LU7. The current docking study was validated by re-docking the native ligand from the crystallographic protein-ligand complex. The binding affinity between the natural compounds and native ligands were compared to obtain compounds with the lowest binding energy.

Table 1. Molecular docking results with Lipinski rule of five and toxicity analysis

Ligand	Binding affinities (kcal/mol)		RMSD	Mass	Hydrogen bond donor	Hydrogen bond acceptors	LogP	Antiviral Activity Prediction	Ames Toxicity	LD50 (mol/kg)
	6LU7	5R7Y								
Native	-11.4	-4.6	0.0	-	-	-	-	-	-	-
Actinomadurol	-9.0	-7.9	0.0	318	3	4	3.515	33%	Yes	2.184
Anthracene	-8.8	-8.0	0.0	306	2	4	1.516	40%	Yes	2..307
Azanthromicin A	-8.3	-7.4	0.0	283	1	4	2.539	45%	Yes	2.375
Bendigole D	-10.3	-8.8	0.0	374	3	5	3.05	42%	No	2.208
Bendigole E	-10.3	-8.5	0.0	372	2	4	3.65	44%	No	1.957
Bendigole F	-9.4	-8.3	0.0	312	5	6	-0.05	58%	No	2.403
Hemi-Oxanthromicin D	-9.4	-9.1	0.0	446	5	9	2.91	50%	Yes	2.375
Oxanthromicin E	-9.4	-9.4	0.0	444	5	8	3.35	53%	No	2.599
Oxanthromicin F	-8.3	-7.6	0.0	314	3	6	1.63	42%	No	3.227
Oxanthromicin G	-11.0	-10.1	0.0	540	3	9	3.52	40%	No	2.857
Sagamilactam	-12.0	-10.4	0.0	659	3	4	7.54	47%	No	2.376

Regarding the docking result for 5R7Y receptor in Table 1, all of the compounds from *Actinomadura sp*. have lower binding affinity compared to the native ligand (Z45617795) with Sagamilactam becoming the most promising ligand. Furthermore, Sagamilactam also showed the lowest number of binding affinities energy in 6LU7 receptor. The more negative binding affinities using AutoDock Vina software analysis indicate the stronger binding affinity between ligand and receptor (Xue et al., 2022). Therefore, we use Sagamilactam for further investigation.

Pertaining the physicochemical properties, all the compounds from *Actinomadura* used in this study meet all the criteria of Lipinski rule of five. Lipinski's rule of 5 states that a molecule is considered drug-like when it satisfies: molecular weight < 500 Dalton, number of H-bonds donors < 5, number of H-bonds acceptors < 10 and LogP < 5(Lipinski, 2004). Although Sagamilactam has more than 500, it is still under the tolerance limit of 600 as there are several exceptions about Lipinski's rule of 5(Ivanović

et al. 2020). Thus, Sagamilactam has drug-likeness properties that might be utilized to inhibit viral protease.

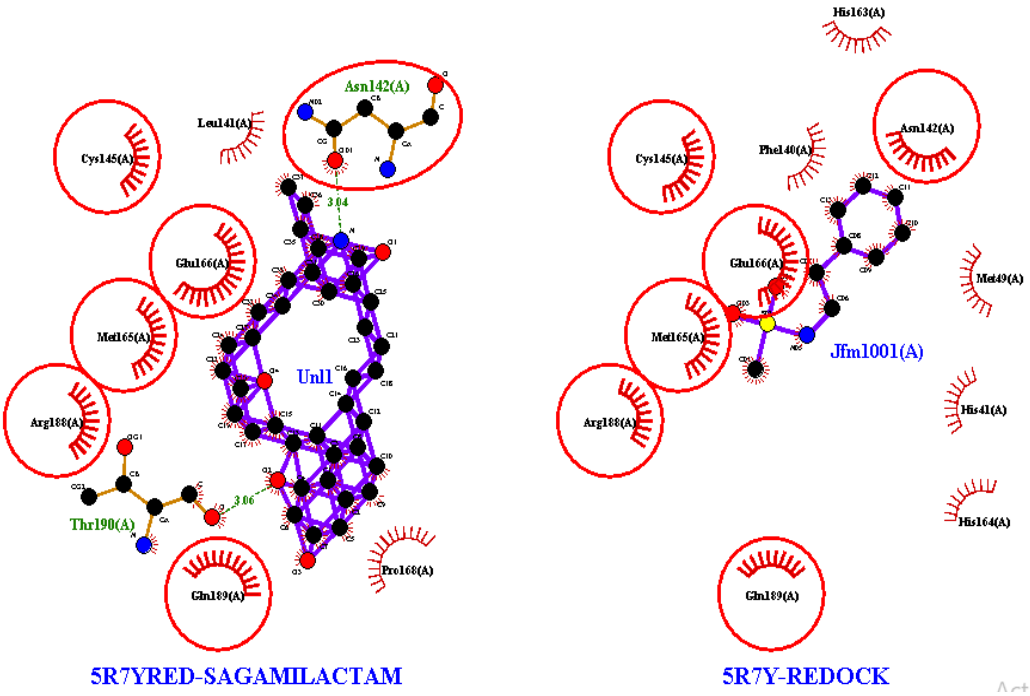

Figure 1. Molecular interaction between ligands and receptors 5R7Y.

Among all compounds found in *Actinimadura* sp., the potentially active of these compounds as antifungal based on Prediction of Activity Spectra for Substances (PASS) server analyses (Druzhilovskiy et al., 2017) are between 33% until 58% with Bendigole F as the highest. Meanwhile, the toxicity measurement showed that more than half compounds were not categorized as carcinogenic effects based on AMES toxicity measurement (Zeiger, 2019). The LD50 value also showed that almost all of the compounds were in Class V of toxicity (except Bendigole E) based on ProToxII webserver criteria. Class V means that the compounds may be harmful if swallowed.

Previously, Sagamilactam was known as the antibiotic with the main ability as the Anti-trypanosomal compound (Kimura et al., 2016). The Prediction Sagamilactam compound based on Activity Spectra for Substances (PASS) server analyses showed the number of 47% in potentially active as antiviral. The potentially active number less than 70% means that the opportunity to find the antiviral activity in experimental research is not too high (Ramadhan et al., 2020). However, we could validate our docking results through molecular interactions and molecular dynamic analysis before going into experimental approach.

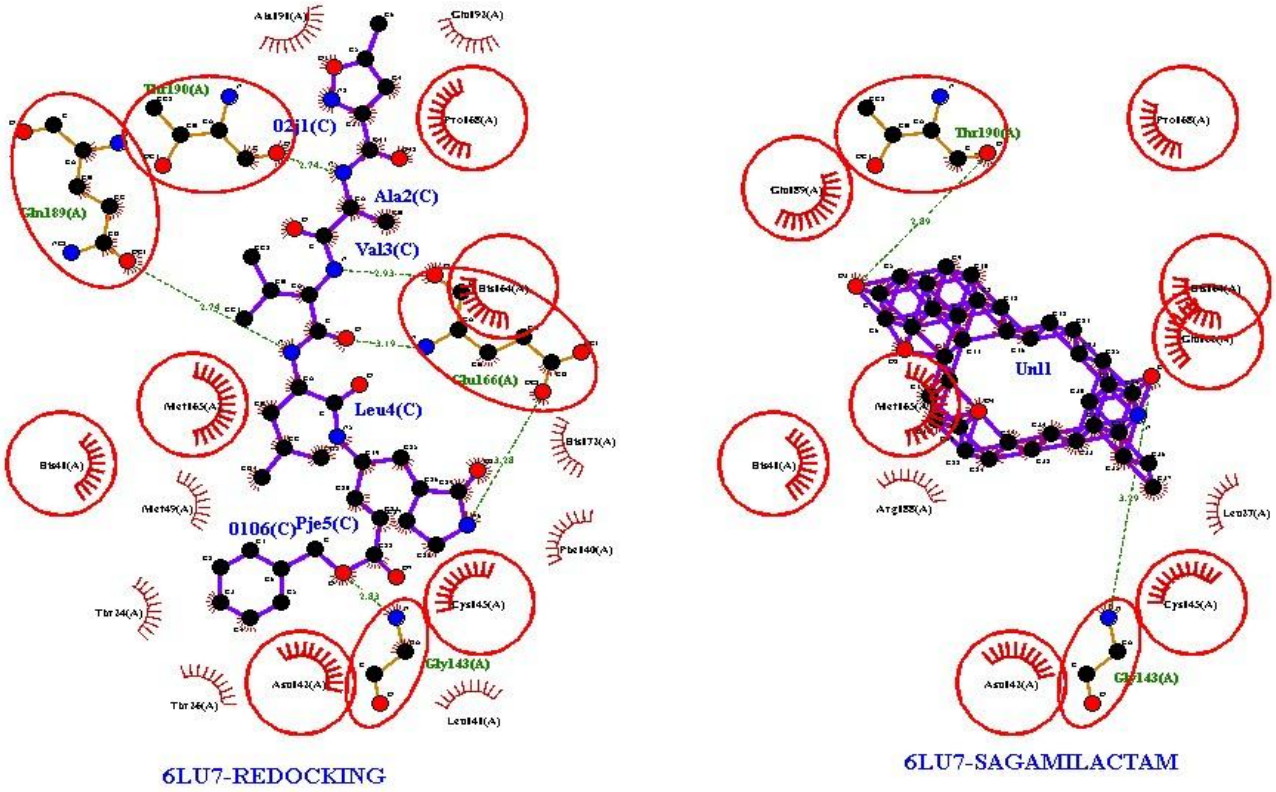

Figure 2. Molecular interaction between ligands and receptors 6LU7.

The Z45617795 (5R7Y) and N3(6LU7) were used as reference ligands to compare the interaction between ligands and receptors. As Sagamilactam has the lowest binding energy for both receptors in the previous analysis, it continued to interpret the molecular interactions. Beside the binding affinity, the hydrogen bonds and hydrophobic interactions are also interpreted as we can see in Figure 1 and Figure 2.

Structural analysis of the SARS-CoV-2 Mpro active site revealed several amino acid residues, namely THR-25, THR-190, THR-26, HIS-41, HIS-163, LEU-27, LEU-141, LEU-166, SER-46, PHE-140, PHE-185, PRO-168, MET-49, MET-165, TYR-54, ASN-142, ALA-191, GLY-143, GLN-189, GLN-192, GLU-166, ASP-187, and CYS-145 (Zhang et al., 2020). Based on the Figure 1, The native ligand of 5R7Y form only hydrophobic interaction (Interaction data). Meanwhile, the Sagamilactam form hydrogen bond with THR-190 and ASNL-42. Both of ligands showed five hydrophobic bond similarities which comprises of CYS-145, GLU-166, MET-165, ARG-188, and GLN 189. These interactions confirmed that all of the ligands have interacted in the active sites of SARS-CoV-2.

The redocking result of N3 ligand to 6LU7 receptor showed four hydrogen bond interactions which comprises of GLY-143, GLU-166, GLN-189, and THR-190. Meanwhile, the interactions between Sagamilactam and 6LU7 only have two

hydrogen bonds in GLY-143 and THR-190. Both of these ligands have similarity in five hydrophobic bond. All ligands also attached on the amino acid residues that located at the SARS-CoV-2 Mpro active site. The previous research showed that the amino acid of Gly143 is the strongest residue to create hydrogen interaction with ligands. Besides, the interactions in Gly143 and Glu166 have significant role in maintaining the ligand-receptor complex (Kusumaningrum et al., 2022).

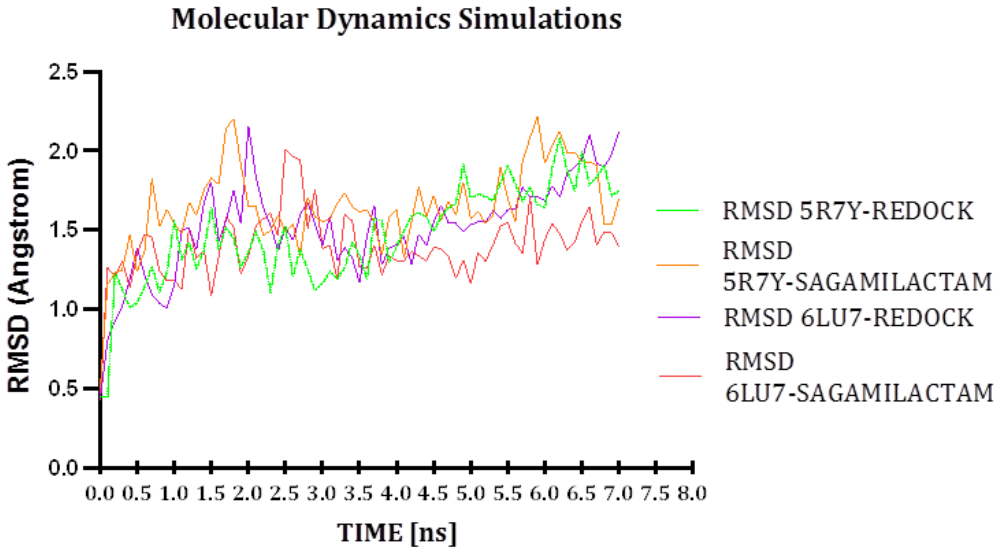

Figure 3. The results of root mean square deviation (RMSD) analysis in Molecular Dynamics.

Molecular Dynamics analysis provided important information regarding the dynamic behaviour of the protein-ligand complex. The root mean square deviation (RMSD) value provides the deviation value for a group of atoms in the protein-ligand complex (Al-Karmalawy et al., 2021). The RMSD values were measured for 7 nanoseconds (ns) time simulation. The result of MD simulation can be observed through Figure 3.

RMSD value is utilized to show the difference between protein's backbones final position from its initial confirmation. The smaller the deviation, the more stable protein structure (Aier et al., 2016). During simulations of Sagamilactam in both receptors, the RMSD values of the ligand-receptor was observed between 0 and 2.2 Angstrom. Thus, there were no significant deviations found in any ligan-receptor complexs which indicated protein stability. In addition, the presence of hydrogen bond between ligand and receptor might have significant key in the RMSD result as the higher RMSD value mostly correlated with lower number of hydrogen bond (Kony et al., 2007).

CONCLUSION

Based on our study, Sagamilactam was the most promising compound from *Actinomadura* sp. that can inhibit SARS-CoV-2 viral protease based on molecular docking and molecular dynamics analysis. Further in vitro are needed to strengthen this study.

REFERENCES

Aier, I., Varadwaj, P. K., & Raj, U. 2016. Structural insights into conformational stability of both wild-type and mutant EZH2 receptor. *Scientific Reports*, *6*. https://doi.org/10.1038/srep34984

Al-Karmalawy, A. A., Dahab, M. A., Metwaly, A. M., Elhady, S. S., Elkaeed, E. B., Eissa, I. H., & Darwish, K. M. 2021. Molecular Docking and Dynamics Simulation Revealed the Potential Inhibitory Activity of ACEIs Against SARS-CoV-2 Targeting the hACE2 Receptor. *Frontiers in Chemistry*, *9*. https://doi.org/10.3389/fchem.2021.661230

Amin, D. H., Abdallah, N. A., Abolmaaty, A., Tolba, S., & Wellington, E. M. H. 2020. Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective. *Bulletin of the National Research Centre*, *44*(1). https://doi.org/10.1186/s42269-019-0266-8

Azman, A. S., Othman, I., Velu, S. S., Chan, K. G., & Lee, L. H. 2015. Mangrove rare actinobacteria: Taxonomy, natural compound, and discovery of bioactivity. In *Frontiers in Microbiology* (Vol. 6, Issue AUG). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2015.00856

Badji, B., Zitouni, A., Mathieu, F., Lebrihi, A., & Sabaou, N. 2006. Antimicrobial compounds produced by Actinomadura sp. AC104 isolated from an Algerian Saharan soil. *Canadian Journal of Microbiology*, *52*(4), 373–382. https://doi.org/10.1139/W05-132

Case, D. A., Cheatham Iii, T. E., Darden, T., Gohlke, H., Luo, R., Merz, K. M., Onufriev, A., Simmerling, C., Wang, B., & Woods, R. J. 2015. *The Amber Biomolecular Simulation Programs*. http://amber.scripps.edu.

Ding, T., Yang, L. J., Zhang, W. D., & Shen, Y. H. 2019. The secondary metabolites of rare actinomycetes: Chemistry and bioactivity. In *RSC Advances* (Vol. 9, Issue 38, pp. 21964–21988). Royal Society of Chemistry. https://doi.org/10.1039/c9ra03579f

Feikin, D. R., Higdon, M. M., Abu-Raddad, L. J., Andrews, N., Araos, R., Goldberg, Y., Groome, M. J., Huppert, A., O'Brien, K. L., Smith, P. G., Wilder-Smith, A., Zeger, S., Deloria Knoll, M., & Patel, M. K. 2022. Duration of effectiveness of vaccines against SARS-CoV-2 infection and COVID-19 disease: results of a systematic review and meta-regression. *The Lancet*, *399*(10328), 924–944. https://doi.org/10.1016/S0140-6736(22)00152-0

Glaser, J., Sedova, A., Galanie, S., Kneller, D. W., Davidson, R. B., Maradzike, E., del Galdo, S., Labbé, A., Hsu, D. J., Agarwal, R., Bykov, D., Tharrington, A., Parks, J. M., Smith, D. M. A., Daidone, I., Coates, L., Kovalevsky, A., & Smith, J. C. 2022. Hit Expansion of a Noncovalent SARS-CoV-2 Main Protease Inhibitor. *ACS Pharmacology and Translational Science*, *5*(4), 255–265. https://doi.org/10.1021/acsptsci.2c00026

Huff, S., Kummetha, I. R., Tiwari, S. K., Huante, M. B., Clark, A. E., Wang, S., Bray, W., Smith, D., Carlin, A. F., Endsley, M., & Rana, T. M. 2022. Discovery and Mechanism of SARS-CoV-2 Main Protease Inhibitors. *Journal of Medicinal Chemistry*, *65*(4),

2866–2879. https://doi.org/10.1021/acs.jmedchem.1c00566

Ivanović, V., Rančić, M., Arsić, B., & Pavlović, A. 2020. Lipinski's rule of five, famous extensions and famous exceptions. In *POPULAR SCIENTIFIC ARTICLE* (Vol. 3, Issue 1).

Kimura, T., Iwatsuki, M., Asami, Y., Ishiyama, A., Hokari, R., Otoguro, K., Matsumoto, A., Sato, N., Shiomi, K., Takahashi, Y., Omura, S., & Nakashima, T. 2016. Anti-trypanosomal compound, sagamilactam, a new polyene macrocyclic lactam from Actinomadura sp. K13-0306. *Journal of Antibiotics*, *69*(11), 818–824. https://doi.org/10.1038/ja.2016.28

Kony, D. B., Hünenberger, P. H., & van Gunsteren, W. F. 2007. Molecular dynamics simulations of the native and partially folded states of ubiquitin: Influence of methanol cosolvent, pH, and temperature on the protein structure and dynamics. *Protein Science*, *16*(6), 1101–1118. https://doi.org/10.1110/ps.062323407

Krieger, E., & Vriend, G. 2015. New ways to boost molecular dynamics simulations. *Journal of Computational Chemistry*, *36*(13), 996–1007. https://doi.org/10.1002/jcc.23899

Kusumaningrum, H. P., Ferniah, R. S., Jannah, S. N., Kurniawati, M. B., Afifah, A., Sumbodo, Y. M., Hanif, S. S., Erfianti, T., & Eshananda, Y. 2022. Relationship Between Phylogenetic of Apium and Foeniculum Plants from Central Java, Indonesia, and Their Secondary Metabolites Potency against COVID-19 Protease. *Open Access Macedonian Journal of Medical Sciences*, *10*(A), 1234–1241. https://doi.org/10.3889/oamjms.2022.9852

Lipinski, C. A. 2004. Lead- and drug-like compounds: The rule-of-five revolution. In *Drug Discovery Today: Technologies* (Vol. 1, Issue 4, pp. 337–341). https://doi.org/10.1016/j.ddtec.2004.11.007

Morris, G. M., Ruth, H., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. 2009. Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Puttaswamy, H., Gowtham, H. G., Ojha, M. D., Yadav, A., Choudhir, G., Raguraman, V., Kongkham, B., Selvaraju, K., Shareef, S., Gehlot, P., Ahamed, F., & Chauhan, L. 2020. In silico studies evidenced the role of structurally diverse plant secondary metabolites in reducing SARS-CoV-2 pathogenesis. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-77602-0

Ramadhan, D. S. F., Fakih, T. M., & Arfan, A. 2020. Activity Prediction of Bioactive Compounds Contained in Etlingera elatior Against the SARS-CoV-2 Main Protease: An In Silico Approach. *Borneo Journal of Pharmacy*, *3*(4), 235–242. https://doi.org/10.33084/bjop.v3i4.1634

Singhal, T. 2020. A Review of Coronavirus Disease-2019 (COVID-19). In *Indian Journal of Pediatrics* (Vol. 87, Issue 4, pp. 281–286). Springer. https://doi.org/10.1007/s12098-020-03263-6

Tarantini, F. S., Brunati, M., Taravella, A., Carrano, L., Parenti, F., Hong, K. W., Williams, P., Chan, K. G., Heeb, S., & Chan, W. C. 2021. Actinomadura graeca sp. nov.: A novel producer of the macrocyclic antibiotic zelkovamycin. *PLoS ONE*, *16*(11). https://doi.org/10.1371/journal.pone.0260413

Trott, O., & Olson, A. J. 2009. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, NA-NA. https://doi.org/10.1002/jcc.21334

Xue, Q., Liu, X., Russell, P., Li, J., Pan, W., Fu, J., & Zhang, A. 2022. Evaluation of the binding performance of flavonoids to estrogen receptor alpha by Autodock, Autodock Vina and Surflex-Dock. *Ecotoxicology and Environmental Safety*, *233*. https://doi.org/10.1016/j.ecoenv.2022.113323

Zeiger, E. 2019. The test that changed the world: The Ames test and the regulation of chemicals. In *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* (Vol. 841, pp. 43–48). Elsevier B.V. https://doi.org/10.1016/j.mrgentox.2019.05.007

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., & Hilgenfeld, R. 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved a-ketoamide inhibitors. *Science*, *368*(6489), 409–412. https://doi.org/10.1126/science.abb3405