ARTIKEL

MOLECULAR DOCKING OF XIAMYCIN DERIVATIVES ON RNA-DEPENDENT RNA POLYMERASE AS A SARS-COV-2 VIRUS REPLICATION INHIBITOR

Hamnah Al Atsariyah[1], Dwi Utami[2*], Andika[3]
[1]Undergraduate Program, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[2]Departement of Analytical and Medicinal Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[3]Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin, South Kalimantan, Indonesia

ABSTRACT

Xiamycin is a pentacyclic indolosesquiterpenoid derived from mangrove endophytes was studied in vitro to inhibit the replication of porcine epidemic diarrhea virus and human immunodeficiency virus. This study aimed to examine the potential pharmacological activity of xiamycin derivatives as an inhibitor of SARS-CoV-2 virus replication through molecular docking and analyze their pharmacokinetic and toxicity profiles.

The selection of comparison pharmaceutical agents used ChemDes. Xiamycin derivatives as test compounds and molnupiravir as inhibitors were screened for Lipinski's Rule of Five. The identification of RdRp as a molecular target was confirmed through the PASSonline program. The RdRp receptor was selected based on an assessment of receptor quality via the RCSB PDB and PDBsum. Minimization energy used Avogadro and Swiss PDB programs. Receptor and ligand were docked by Autodock. Visualization of molecular docking results with Biovia and Pymol. ADMET profile assessment using the ADMETlab 2.0 program.

This study shows that xiamycin E had the highest pharmacological potential compared to other xiamycin derivatives and molnupiravir with an energy affinity of -6.92 kcal/mol, an inhibitory constant of 8.41 M, and knows three key amino acid residues in the NiRAN domain, namely amino acid ASN 209, LYS 50, and ASP 218. ADMET prediction shows that xiamycin E is ideal for certain parameters and not ideal for certain parameters.

The xiamycin derivatives have the potential to be developed as an antiviral. Optimization of xiamycin E as a candidate for the RdRp inhibitor of SARS-CoV-2 requires further studies related to the structure tissue exposure/selectivity activity relationship.

Keywords: molecular docking, xiamycin, RdRp, SARS-CoV-2

INTRODUCTION

Currently, COVID-19 in the world has reached more than 501 million cases and caused more than 6 million deaths (Worldometer, 2022). In Indonesia, COVID-19 cases have reached more than 6 million cases and caused more than 155,000 deaths (SATGAS COVID-19, 2022). Various drugs are being investigated for COVID-19 treatment regimens, including molnupiravir.

Molnupiravir is a ribonucleoside analog that has been reported to have the potential as an inhibitor of SARS-CoV-2 replication through inhibition of RNA-dependent RNA polymerase (RdRp).

Various medicinal compounds based on natural ingredients are being investigated for COVID-19 drugs. Indonesia has an abundance of marine natural products, including mangroves. The Ministry of Environment and Fores in 2021 has released the National Mangrove Map, it is known that the total area of Indonesia's mangroves is 3,364,076 Ha (KKP, 2021).

*Corresponding author. Email: dwi.utami@.pharm.uad.ac.id

Mangroves have a variety of endophytic microbes in their tissues. Endophytic microbes can produce various secondary metabolites (Radji., 2005). The bioactive secondary metabolites are useful as anti microbial, antiviral, anticancer, and anti-inflammatory (Shan et al., 2018).

Xiamycin is a pentacyclic indole sesquiterpenoid compound from marine actinomycetes, such as Streptomyces sp. GT20021503 isolated from *Bruguiera gymnorrhiza* (Ding et al., 2010). Xiamycin C-E isolated from *Streptomyces sp*, HK18 has been studied in vitro to inhibit the replication of the porcine epidemic diarrhea virus (PEDV) (Kim et al., 2016).

Structural protein and non-structural protein (Nsp) SARS-CoV-2 are important targets for COVID-19 therapy. The RNA-dependent RNA polymerase (RdRp) or Nsp12 receptor is one of the targets in the research and development of COVID-19 drugs.

In this study, the isvestigation of xyamicin as the potential antivirus through inhibition of RNA-dependent RNA polymerase has been conducted by molecular docking. The results of this study are expected to provide a scientific basis for the development of mangrove endophyte bioactiveves through molecular pharmacology tests in the development of new antiviral drugs. The results of this study are expected to provide a scientific basis for the development of mangrove endophyte bioactives through molecular pharmacology tests in the development of new antiviral drugs.

MATERIALS AND METHODS

Materials

The tools used in this research are hardware and software. The hardware used is a laptop with an Intel(R) Core(TM) i7-6500U CPU @2.50GHz 1336x908p monitor, 4GB RAM, NVIDIA GeForce VGA, Windows 10 Education 64-bit. The various programs used are Chemdes, PDBsum, PASSonline, Avogadro, Swiss PDB, Notepad, AutoDock 4.2, Biovia Discovery Studio Visualizer, Open Babel, Pymol, and ADMET lab 2.0.

The materials used in this research are ligand and receptor structures. The structure of the test and comparison ligands used were taken from https://pubchem.ncbi.nlm.nih.gov/ in the form of xiamycin A (CID: 38358410), xiamycin B (CID: 53469155), xiamycin C (CID: 127034219), xiamycin D (CID: 127034220), xiamycin E (CID: 127034221), xiamycin methyl ester (CID: 50898452), 19-Carbonyl-Xiamycin (CID: 156581618), 19-Methoxyl-Xiamycin (CID: 1565811619), molnupiravir (CID: 145996610). RNA-dependent RNA polymerase (RdRp) protein structure (PDB ID: 7AAP) downloaded from https://www.rcsb.org/.

Ligand and Receptor Preparation

Ligand preparation was carried out to remove water molecules and add hydrogen atoms. Receptor preparation was carried out to separate the receptor and native ligand, remove water molecules, and add hydrogen atoms. Ligand and receptor preparation was carried out using the Biovia program.

Energy minimization

After preparation, energy minimization was carried out using the Avogadro application with parameters MMFF94 on the native ligand derivative xiamycin, and molnupiravir following the energy minimization guide https://avogadro.cc/docs/menus/extensions-menu/. The minimization of energy at the RdRp receptor (PDB ID: 7AAP) uses the Swiss PDB Viewer with GROMOS96 parameters following the

guidelines https://spdbv.unil.ch/Swiss-PdbViewerManualv3.7.pdf. Then, comparing the energy of the ligands and receptors before and after energy minimization.

Method validation

The validation of the molecular docking method was carried out by redocking the native ligand on the target protein using the AutoDock Tools 4.2 program. The redocking processes are carried out following the guidelines for using the Autodock program https://autodock.scripps.edu/wpcontent/uploads/sites/56/2021/10/AutoDock4.2.6_UserGuide.pdf. As explained in the manual that the RdRp receptor (PDB ID: 7AAP) has metal ions, it is necessary to add the AD4.1_bound.dat parameter in the grid settings. The data analyzed from the docking of native ligands is the root mean square deviation (RMSD), if RMSD <2 then the molecular anchoring method is declared valid and can be continued for anchoring test and comparison ligands.

Molecular Docking

Energy-minimized ligands and receptors were docked with the AutoDock Tools 4.2 program according to the Autodock program guide. The analysed data from the results of the docking of the test and comparison ligands is the docking score form of binding affinity and inhibition constants (Ki) from various anchoring poses. The lower the binding energy xiamycin derivatives against the RdRp receptor, the more potential this compound as an inhibitor of the SARS-CoV-2 RdRp enzyme compared to molnupiravir.

Identification and Visualization

The output of molecular docking is pose and docking score which can be seen from the DLG file. From this output, it can be seen which pose the ligand with the lowest energy binds to the receptor. Residues from the molecular interactions of ligands and receptors can be seen by visualization through Biovia on the ligand-protein interactions menu. Furthermore, to see the binding pocket can be visualized in 3D using the Pymol program. 2D visualization can be used to identify amino acid residues and metals from xiamycin derivatives compared to molnupiravir. 3D visualization can be used to see binding pocket ligands to RdRp.

RESULTS AND DISCUSSION

Selection of Comparative Pharmaceutical Agents

The results of the molecular structural analysis of the antioxidant properties of antiviral agents conducted by Yasri & Wiwanitki (2022) **(Table I)** show that new antiviral drugs for COVID-19 RNA-dependent RNA polymerase targets that have the best antioxidant properties are molnupiravir and favipiravir. Because the antioxidant scores were equal, a molecular structural similarity analysis was performed to decide whether to use molnupiravir or favipiravir as a comparison for molecular docking of xiamycin derivatives.

The calculation of structure similarity with ChemFPS structure fingerprint Molecular ACCess System (MACCS) and the dice similarity method. The test results (Table II) showed that the structural similarity score of molnupiravir to xiamycin was higher than that of favipiravir to xiamycin. Based on the results of this similarity calculation, molnupiravir was chosen as a comparison for molecular docking of xiamycin derivatives.

Table I. Antioxidant Properties of Antivirus

Antiviral drugs	Data on molecular redox capacity		Antioxidant characteristics and overall (unit)
	Donor electron molekul (unit)	Molecular electron donor (unit)	
Molnupiravir	4,5	0.4	4.1
Favipiravir	4,5	0.4	4.1
Lopinavir	4.4	0.4	4.0
Remdesivir	4.4	0.4	4.0
Tenofovir	4.1	0.4	3.7
Galidesivir	4.4	0,3	4.1
Ribavirin	5.0	0.4	4.6

Table II. Structural Similarity Test Results

Similarity	Method	Fingerprint	Score
Molnupiravir-Xiamycin	Dice	MACCS	0.54
Favipiravir-Xiamycin	Dice	MACCS	0.46

Ligand Screening

The choice the ligand is closely related to the optimal result of molecular docking. Therefore, ligand screening was carried out based on Lipinski's Rule of Five Ro5 parameters.

The xiamycin and molnupiravir derivatives selected as test ligands and comparison ligands in this study were screened using the parameter Ro5. The Ro5 parameters used were molecular weight (MW) <500, partition coefficient (logP) <5, hydrogen bond donor (HBD) <5, hydrogen bond acceptor (HBA) <10. If two or more Ro5 incompatibility occur, it is predicted that the compound cannot be used orally (Benet et al., 2016; Lipinski et al ., 2012).

It is known that the Ro5 parameters of the xiamycin and molnupiravir derivatives in the Pubchem database processed in **Table III** showed no more than two Ro5 discrepancies, so it was predicted that the xiamycin and molnupiravir derivatives could be taken orally. Therefore, it was continued by testing the spectrum of activity of molnupiravir and xiamycin through the PASSonline program.

Table III. Lipinski *Rule of Five Parameter*

Senyawa	MW (< 500)	Log P (<5)	HBD (<5)	HBA (<10)	Ket
Xiamycin A	363.4	5.1	3	3	□
Xiamycin B	379.4	4.4	4	4	□
Xiamycin C	379.4	3.8	4	4	□
Xiamycin D	393.5	4.2	3	4	□

Xiamycin E	391.5	4.4	2	4	□
Xiamycin Methyl Ester	377.5	5.5	2	3	□
19 Carbonyl Xiamycin	377.4	4	3	4	□
19 Metoxyl Xiamycin	393.5	4.4	3	4	□
Molnupiravir	329.31	-0.8	4	7	□

Description: qualified (□)

Ligand and Receptor Preparation

This RdRP receptor (PDB ID: 7AAP) has metal ions that play an active role in structural stability or reactions. The metal ions are Zn and Mg metals. Two zinc ions (Zn2+) are needed to maintain the stability of the RdRp structure (Kirchdoerfer & Ward, 2019). Two magnesium ions (Mg2+) play a role in RNA synthesis reactions as a trigger for the nucleophilic attack of the 3'-hydroxyl group of RNA on the phosphate of nucleotide triphosphate (NTP), while another Mg2+ ion facilitates the release of pyrophosphate (PPi) molecules (Carvalho et al., 2011). It is hoped that by including these metal ions in the receptor as a molecular docking target during receptor preparation **(Figure 1),** the ligand will also bind to metal ions so that the RdRp mechanism in virus replication will be disrupted.

The native ligands GE6 and POP present at the 7AAP receptor were prepared for validation of the molecular docking method. The native 7AAP receptor ligand, xiamycin, and molnupiravir derivatives were prepared with the addition of a hydrogen atom using the Biovia program. The prepared ligand was then minimized with Avogadro.

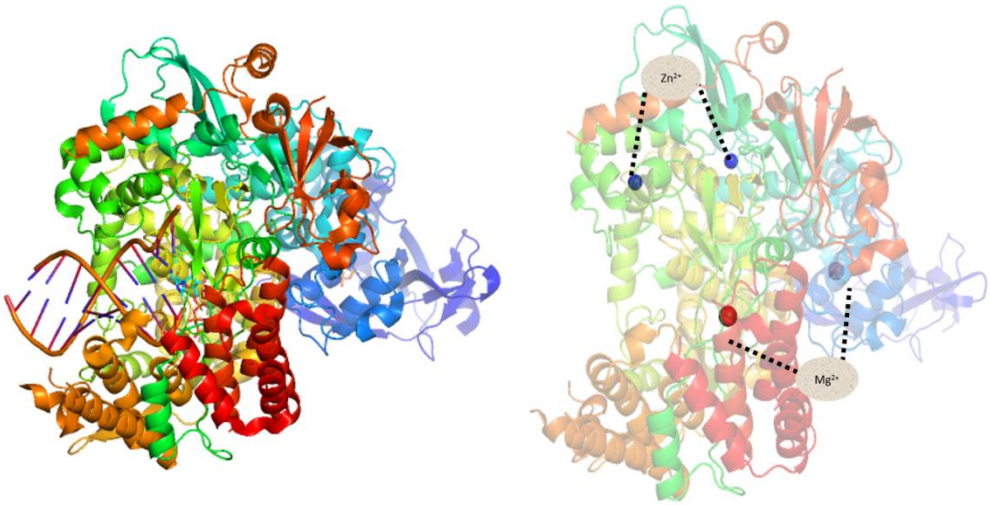


Figure 1. 7AAP Receptors Before and After Preparation

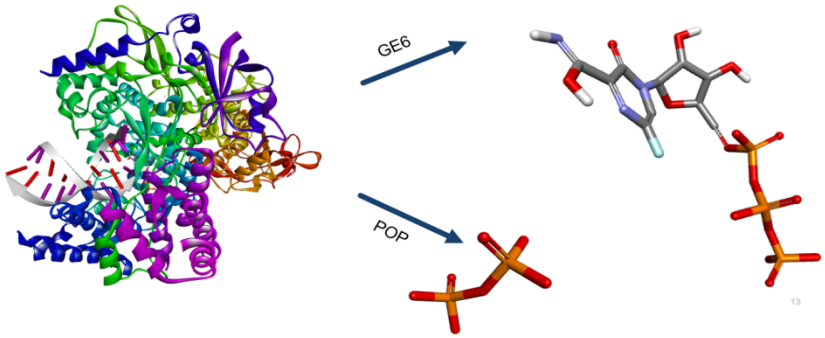


Figure 2. 7AAP Receptor Native Ligand Preparation

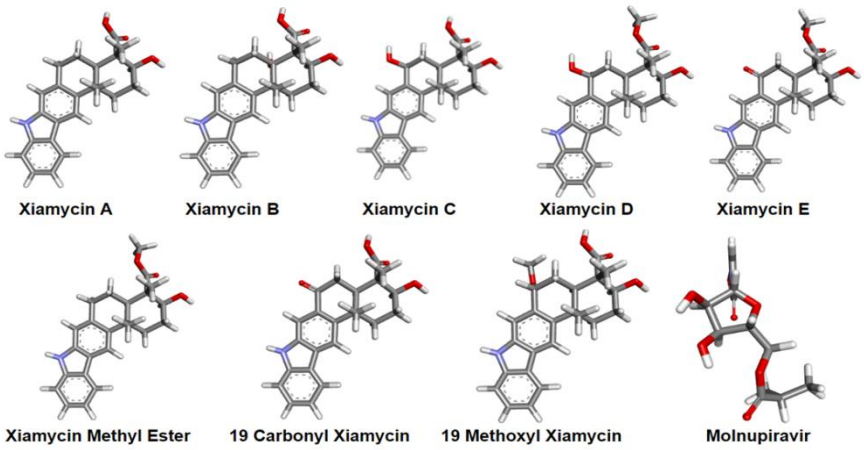


Figure 3. Test and Comparison Ligand Preparation Results

Method Validation

The validation of this molecular docking method was carried out by redocking the native ligand to the RdRp (7AAP) receptor. RMSD value <2 indicates that the docking method is valid, and RMSD 2-3 indicates a deviation of the native ligand from the reference position, but still within the orientation limit. However, an RMSD value > 3Å indicates a significant position deviation or improper docking (Ramírez & Caballero, 2018).

The validation of the native ligand pyrophosphate method using a grid box center on the ligand setting resulted in a root mean square deviation (RMSD) of 1.43 < 2. These results indicate that there is no significant position deviation from the native ligand after redocking **(Figure 4)**, so it can be used for bonding tests and comparison ligands.

Table IV. *Grid Box Redocking*

Native Ligand	Number Grid Point			Coordinate			Spacing Grid Point (A)	RMSD (Å)
	X	Y	Z	X	Y	Z		
GE6	40	40	40	99.517	96.001	112.613	0.375	4.25
POP	40	40	40	128.694	101.016	71.526	0.375	1.46

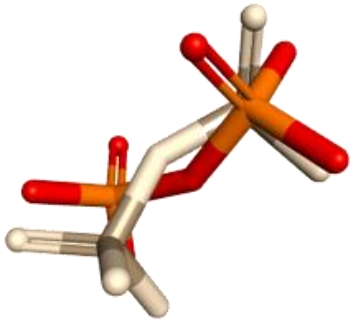


Figure 4. Superimpose Native Ligand POP

Molecular Docking

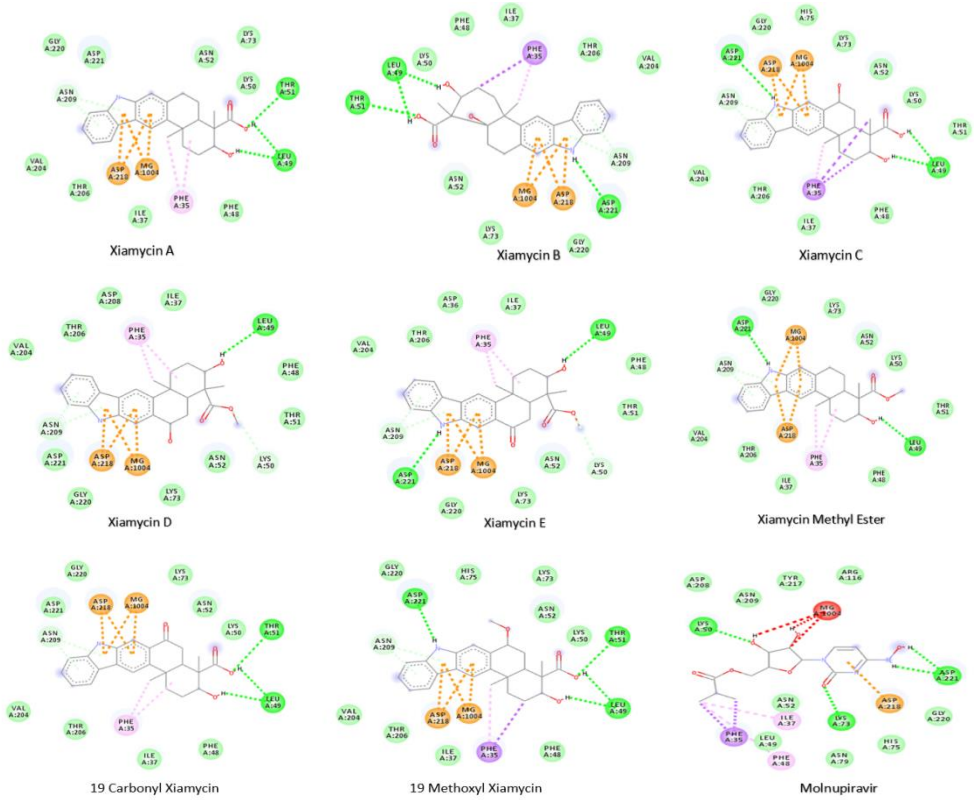
The molecular docking parameters analyzed were binding affinity, inhibition constant (Ki), and amino acid residues and metals. Good binding affinity can be seen from the negative Gibbs free energy (ΔG) value because the level of protein-ligand association is determined by the magnitude of the negative ΔG. The value of ΔG correlates with the stability of a given protein-ligand complex or alternatively the binding affinity of the ligand to a receptor (Du et al., 2016).

The value of G is directly proportional to the value of Ki. The Ki value is the inhibitor concentration needed to reduce half of the enzyme activity. The smaller the Ki value, the stronger ligand effect on the receptor (Balle , 2016). From the molecular docking results which can be seen in **Table V**, it is known that xiamycin E showed the best results because it has binding energy with an ΔG of -6.92 kcal/mol and an inhibition constant (Ki) of 8.41 uM.

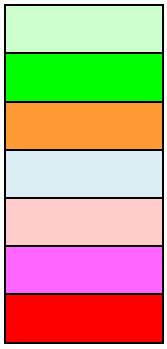
Table V. Affinity Energy Value and Inhibition Constant

Rank	Compound	Binding affinity (kcal/mol)	Inhibition Constant (uM)
1	Xiamycin E	-6.92	8.41
2	Xiamycin D	-6.70	12.27
3	19 Carbonyl Xiamycin	-6.62	13.98
4	Xiamycin Methyl Ester	-6.58	14.96
5	Xiamycin A	-6.57	15.32
6	Xiamycin C	-6.38	20.93
7	Xiamycin B	-6.27	25.38
8	19 Methoxyl Xiamycin	-6.27	25.51
9	Molnupiravir	-4.55	460.42

Visualization and Identification of Results



Description:



Van der Walls
Conventional Hydrogen Bond
Pi-Kation/Pi-Anion
Pi-Donor Hydrogen Bond/Carbon Hydrogen Bond
Alkyl/Pi-Alkyl
Pi-Sigma
Unfavorable Bump/Unfavorable Metal Donor

Figure 5. Molecular Docking 2D Visualization

Xiamycin E which has the lowest binding affinity and inhibition constant is known to have seven types of residues formed from different types of bonds. The residue ASP 218 was formed in the presence of pi-anion interactions, MG 1004 was formed in the presence of pi-cation interactions, ASP 221 and LEU 49 were formed in the presence of conventional hydrogen bonds. The residue of PHE 35 was formed in the presence of pi-alkyl interactions. The residue ASN 209 is formed by the interaction of pi-hydrogen donor bonds and LYS 50 with hydrocarbon bonds.

Table VI. Interaction Residue Classification

Rank	Compound	Hydrogen Bond	Electrostatic Interaction	Hydrofobic Interaction
1	Xiamycin E	ASP 221, LEU 49, **LYS 50, ASN 209**	**ASP 218**, MG 1004	PHE 35
2	Xiamycin D	LEU 49, LYS 50, ASN 209	**ASP 218**, MG 1004	PHE 35
3	19 Carbonyl Xiamycin	THR 51, LEU 49, **ASN 209**	**ASP 218**, MG 1004	PHE 35
4	Xiamycin Methyl Ester	ASP 221, LEU 49, **ASN 209**	**ASP 218**, MG 1004	PHE 35
5	Xiamycin A	THR 51, LEU 49, **ASN 209**	**ASP 218**, MG 1004	PHE 35
6	Xiamycin C	ASP 221, LEU 49, **ASN 209**	**ASP 218**, MG 1004	PHE 35
7	Xiamycin B	ASP 221, LEU 49, THR 51, **ASN 209**	**ASP 218**, MG 1004	PHE 35
8	19 Methoxyl Xiamycin	ASP 221, THR 51, LEU 49, **ASN 209**	**ASP 218**, MG 1004	PHE 35
9	Molnupiravir	ASP 221, LYS 50, LYS 73	**ASP 218**, MG 1004	PHE 35

The residue variations can be classified into three, namely: hydrogen bonding (ASP 221, LEU 49, ASN 209, and LYS 50), electrostatic interactions (ASP 218 and MG 1004), and hydrophobic interactions (PHE 35). In the aspect of bond strength, it is known that hydrogen bonds > electrostatic interactions > hydrophobic interactions (Biovia, 2019). Hydrogen bonding is the most important bond in the stability of the ligand binding to the receptor. Hydrogen bonding can also increase the binding affinity of the ligand (Williams & Ladbury, 2003; Zhou et al., 2012). These binding variations and interactions contribute to the affinity of the ligand and receptor on the binding pocket of xiamycin E to RdRp **(Figure 6).**

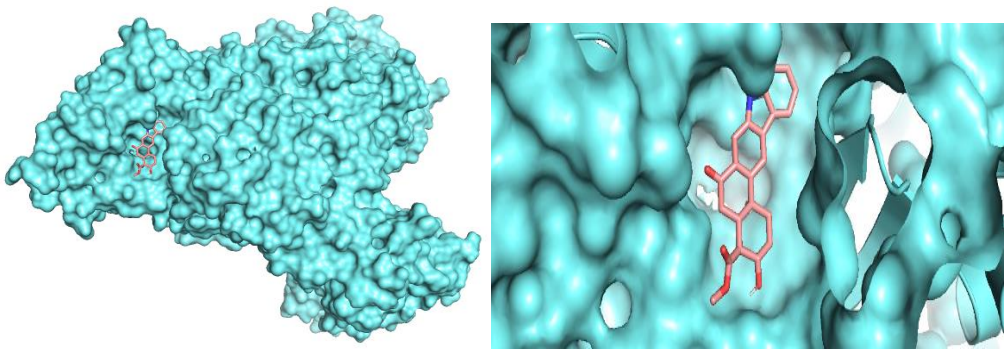


Figure 6. Xiamycin E Binding Pocket 3D Visualization against RdRp 7AAP

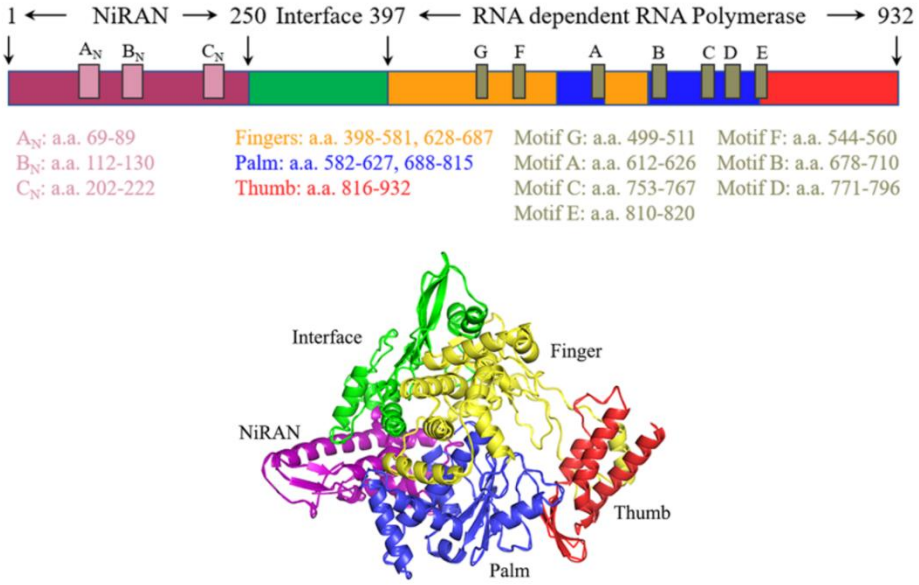


Figure 7. SARS-CoV-2 Nsp-12 domain (Zhang et al., 2020)

The docking coordinates used in the docking of the xiamycin and molnupiravir derivatives are the coordinates of the native ligand pyrophosphate. The crystallographic structure of RdRp 7AAP has a native pyrophosphate ligand located at the catalytic site of the nidovirus RdRp-associated nucleotidyltransferase (NiRAN) which is coordinated and stabilized by key amino acids such as ASN 209, ASP 218, LYS 50, LYS 73, and ARG 116 (Naydenova et al., 2021; Shannon et al., 2022).

NiRAN is one of the domains in Nsp12. Nsp12 consists of NiRAN, In-phased, and polymerase domains. The NiRAN domain contains 1-250 amino acid strands. The inter-phase domain contains the 251-397 amino acid strand. The polymerase domain consists of the finger (fingers), palm (palm), and thumb (thumb) domains. The domain fingers are built with amino acids 398–581 and 628–687, the palm domain is amino acids 582–627 and 688–815, and the thumb domain contains amino acids 816–919.

The results of the amino acid analysis showed that the key amino acids that appeared in xiamycin E were LYS 50, ASN 209, and ASP 218. The key amino acids that appeared in molnupiravir were LYS 50, LYS 73, and ASP 218. The amino acid residues LYS 50, LYS 73, and ASN 209 are formed by hydrogen bonding, then ASP 218 is formed by electrostatic interactions. Although xiamycin E and molnupiravir both produce three key residues, the binding affinity and Ki values of xiamycin E are lower than that of molnupiravir.

In addition, no unfavorable bond was found in the interaction of xiamycin E with RdRp as was the case with molnupiravir. This bond is not beneficial because it reduces the stability of the complex interactions due to repulsion between atoms and molecules (Dhorajiwala et al., 2019). Judging from the assessment of the docking parameters studied, xiamycin E was chosen as the best docking result and then the ADMET profile was assessed. The balance of clinical dose, efficacy, and toxicity of a drug candidate is not only correlated with its potency/specificity to its molecular target through the structure-activity relationship (SAR). Aspects of exposure/selectivity to disease target organs and normal organ structure-tissue exposure/selectivity relationship

(STR) also need to be comprehensively understood. Structure–tissue exposure/selectivity–activity relationship (STAR) studies can be used in the drug optimization process (Sun et al., 2022).

Molecular docking studies in this study prove the specificity of xiamycin to RdRp, but it is not known how selective it is against other molecular targets. The ADMET study in this study proved that the pharmacokinetic profile of xiamycin E was not optimal and had toxic potential.

In general, the results of this study indicate that xiamycin derivatives have the potential as inhibitors of SARS-CoV-2 virus replication and it is known that xiamycin E has the potential to be the best inhibitor based on molecular docking. ADMET prediction results indicate that the pharmacokinetic profile of xiamycin E is not ideal and has potential toxicity. Therefore, further studies related to STAR can be used in the process of optimizing xiamycin E as an inhibitor of SARS-CoV-2 virus replication in order to obtain optimal efficacy, ideal pharmacokinetic profile, and low potential toxicity.

CONCLUSION

Based on the results of this study, it is known that the xiamycin derivative has the potential to be developed as an RNA-dependent RNA polymerase inhibitor with energy affinity parameters (-6.92 - -6.27 kcal/mol) and inhibition constant (8.41 – 25.38 uM) which is better than molnupiravir. Xiaycin E had the best docking parameters with an affinity energy of -6.92 kcal/mol, an inhibition constant of 8.41 M, and three key amino acid residues of the NiRAN domain (ASN 209, LYS 50, and ASP 218). This finding is useful to identify the potency of xiamycin as RNA-dependent RNA polymerase inhibitor.

Further research is needed to identify xiamycin derivatives specificity as RNA-dependent RNA polymerase inhibitor. to optimize pharmacological potential, improve the pharmacokinetic profile, and reduce the toxicity of xiamycin E through STAR studies. This study is the first step for the development of bioactive mangrove endophytes in COVID-19 drug research.

ACKNOWLEDGMENT

Special thanks to Prof. Dr. Apt. Nurkhasanah, M. Si who has guided and given much advice in this research.

REFERENCES

Balle T, L. T. (2016). Molecular Recognition. Di dalam Strømgaard K, Krogsgaard-Larsen P, Madsen U (Eds.), Textbook of Drug Design and Discovery (5th ed.). CRC Press.

Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). BDDCS, the Rule of 5 and Drugability. *Advanced Drug Delivery Reviews*, *101*, 89–98. https://doi.org/10.1016/j.addr.2016.05.007

Biovia. (2019). *Comprehensive Modeling and Simulations for Life Sciences. Dassault Systemes*. https://crrd.kmu.edu.tw/images/studyonline/108.03.19-%0ADiscovery_Studio_%25E8%25A8%2593%25E7%25B7%25B4%25E8%25AA%25B2%25E7%25A8%258%0AB_20190319.pdf

Carvalho, A. T., Fernandes, P. A., & Ramos, M. J. (2011). The Catalytic Mechanism of RNA Polymerase II. *Journal of Chemical Theory and Computation*, *7*(4), 1177–1188.

Dhorajiwala, T. M., Halder, S. T., & Samant, L. (2019). Comparative in Silico Molecular Docking Analysis of l-threonine-3-Dehydrogenase, a Protein Target Against African Trypanosomiasis using Selected Phytochemicals. *Journal of Applied Biotechnology Reports*, *6*(3), 101–108.

Ding, L., Münch, J., Goerls, H., Maier, A., Fiebig, H. H., Lin, W. H., & Hertweck, C. (2010). Xiamycin, a Pentacyclic Indolosesquiterpene with Selective anti-HIV Activity from a Bacterial Mangrove Endophyte. *Bioorganic & Medicinal Chemistry Letters*, *20*(22), 6685–6687. https://doi.org/10.1016/j.bmcl.2010.09.01

0

Du, X., Li, Y., Xia, Y. L., Ai, S. M., Liang, J., Sang, P., Ji, X. L., & Liu, S. Q. (2016). Insights into protein–ligand interactions: Mechanisms, models, and methods. *International Journal of Molecular Sciences*, *17*(2), 1–34. https://doi.org/10.3390/ijms17020144

Kim, S. H., Ha, T. K., Oh, W. K., Shin, J., & Oh, D. C. (2016). Antiviral Indolosesquiterpenoid Xiamycins C-E from a Halophilic Actinomycete. *Journal of Natural Products*, *79*(1), 51–58. https://doi.org/10.1021/acs.jnatprod.5b00634

Kirchdoerfer, R. N., & Ward, A. B. (2019). Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nature Communications*, *10*(1), 1–9. https://doi.org/10.1038/s41467-019-10280-3

KKP. (2021). *Kondisi Mangrove di Indonesia*. Direktorat Pendayagunaan Pesisir Dan Pulau-Pulau Kecil. https://kkp.go.id/djprl/p4k/page/4284-kondisi-mangrove-di-indonesia

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviewsrug Delivery Reviews*, *64*, 4–17.

Naydenova, K., Muir, K. W., Wu, L. F., Zhang, Z., Coscia, F., Peet, M. J., CastroHartmann, P., Qian, P., Sader, K., Dent, K., Kimanius, D., Sutherland, J. D., & Löwe, J., Barford, D., & Russo, C. J. (2021). Structure of the SARS-CoV-2 RNA-dependent RNA polymerase in the presence of favipiravir-RTP. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.2021946118

Radji., M. (2005). Peranan Bioteknologi dan Mikroba Endofit dalam Pengembangan Obat Herbal. *Pharmaceutical Sciences and Research*, *2*(3), 113–126.

Ramírez, D., & Caballero, J. (2018). Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution without Considering Available Structural Data? *Molecules*, *23*(5), 1–17. https://doi.org/10.3390/molecules23051038

SATGAS COVID-19. (2022). *Peta Sebaran COVID-19*. https://covid19.go.id/peta sebaran-covid19

Shan, W., Zhou, Y., Liu, H., & Yu, X. (2018). Endophytic Actinomycetes from Tea Plants (Camellia sinensis): Isolation, Abundance, Antimicrobial, and PlantGrowth-Promoting Activities. *BioMed Research International*.

Shannon, A., Fattorini, V., Sama, B., Selisko, B., Feracci, M., Falcou, C., Gauffre, P., El Kazzi, P., Delpal, A., Decroly, E., Alvarez, K., Eydoux, C., Guillemot, J. C., Moussa, A., Good, S. S., La Colla, P., Lin, K., Sommadossi, J. P., Zhu, Y., … Canard, B. (2022). A dual mechanism of action of AT-527 against SARS-CoV-2 polymerase. *Nature Communications*, *13*(1), 1–9. https://doi.org/10.1038/s41467-022-28113-1

Sun, D., Gao, W., Hu, H., & Zhou, S. (2022). Why 90% of clinical drug development fails and how to improve it? *Acta Pharmaceutica Sinica B*, *12*(7), 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002

Williams, M. A., & Ladbury, J. E. (2003). Hydrogen bonds in protein-ligand Complexes. Protein-ligand Interactions: from Molecular Recognition to Drug design.

Worldometer. (2022). *COVID-19 Coronavirus Pandemic*. https://www.worldometers.info/coronavirus/

Yasri, S., & Wiwanitki, V. (2022). Molnupiravir, Favipiravir and Other Antiviral Drugs with Proposed Potentials for Management of COVID-19: a Concern on Antioxidant Aspect. *International Journal of Biochemistry and Molecular Biology*, *13*(1), 1–4.

Zhang, W. F., Stephen, P., Stephen, P., Thériault, J. F., Wang, R., & Lin, S. X. (2020). Novel Coronavirus Polymerase and Nucleotidyl-Transferase Structures: Potential to Target New Outbreaks. *Journal of Physical Chemistry Letters*, *11*(11), 4430–4435. https://doi.org/10.1021/acs.jpclett.0c00571

Zhou, W., Yan, H., & Hao, Q. (2012). Analysis of Surface Structures of Hydrogen Bonding in Protein–Ligand Interactions Using the Alpha Shape Model. *Chemical Physics Letters*, *545*, 125–131.