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Sterol Constituents of Sea Fan (*Gorgonia mariae*) as Potential Candidates of M^{Pro} Protein SARS-CoV-2 Inhibitor: *in silico* Study

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Abstract

Ethnobotanically, sea fan was a group of gorgonian coral that have used the Maluku people as medicinal ingredients with secondary metabolites containing sterols, terpenoids, and alkaloids that have anti-inflammatory, anti-viral, anti-cancer, analgesic, gastroprotective, anti-bacterial, anti-cancer, and anti-fouling agents. However, the effectiveness of sterols as anti-viral SARS-CoV-2 has not been reported, so research was needed. The initial stage of targeting (SwissTargetPrediction), binding affinity (Autodock Tools 4.2), and amino acid interactions (Discovery Studio 2016 Client®). The route of administration, pharmacokinetic properties, and acute oral toxicity (LD_{50}) were predicted by Lipinski's rule of five, pre-ADMET, and ProTox-II. The results of target class obtained probability of 10.6% (4,24-dimethyl cholesta-7,22-dien-3ß-ol and 4,24-dimethyl-22-dehydro-cholestanol) and 11, 8% (dinosterol). Binding affinity (ΔG, kcal/mol and Ki, nM) potentially 4,24-dimethyl cholesta-7,22-dien-3ß-ol (-9.90; 55.13) > dinosterol (-9.77; 68.66) > 4,24-dimethyl-22-dehydro-cholestanol (-9.48; 113.33), respectively with the crucial amino acid, Asp187. The test compound has a log P value > 5, so solubility must be considered. Pre-ADMET showed an excellent disposition as a drug and was not mutagenic and carcinogenic. However, the distribution of plasma proteins and the dose of LD_{50} need to be considered. Thus, sea fan sterols have potential as M^{Pro} protein inhibitors.

Keywords: Maluku, sea fan, M^{Pro} protein, in silico, pre-ADMET

INTRODUCTION

Three types of coronavirus cause deadly pneumonia, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle-Eastern respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 (Walls et al., 2020). SAR-CoV was first identified in Guangdong, China, in 2002, which infected 8098 people with 774 deaths. In 2012, MERS-CoV appeared in the Arabian Peninsula, which infected 2494 people and 858 cases of death (Huang et al., 2020). Meanwhile, in December 2019, a new type of coronavirus emerged, namely SARS-CoV-2 in Wuhan, Hubei, China which is currently happening, which has infected 90000 people and 3000 are deaths in 60 countries (Zhu et al., 2020). The latest WHO release until August 25, 2021, shows the number of confirmed positive COVID-19 in Indonesia reached 4,026,837 people with death cases 129,293 people and recovery 3,639,867 people (WHO, 2021).

The world is currently experiencing coronavirus disease-19 (COVID-19) pandemic, a dangerous infectious disease that SARS-CoV-2 causes. This virus can infect because it has a bond between the viral protein (spike protein) and the angiotensin-converting enzyme-2 (ACE-2) receptor in humans (Wan, Shang, Graham, Baric, & Li, 2020). In addition, it was also reported through M^{Pro} protein (Frengki et al., 2020).

M^{Pro} protein is the main protease of COVID-19 (Frengki et al., 2020), which releases functional polypeptides from polyproteins through a proteolytic process. These polyproteins are required for the process of replication and transcription. In addition, M^{Pro} is also used by coronaviruses (SARS-CoV and SARS-CoV-2) to interact directly with ACE-2 to enter target cells (Fakih & Dewi, 2020). By inhibiting the M^{Pro} protein, it will disrupt the replication and

transcription of non-structural proteins of the virus, resulting in the death of the virus (Purwaniati & Asnawi, 2020).

There was no effective therapy to overcome this viral infection, but various efforts continue to be made. The use of remdesivir has been reported by Wang et al., (2020), which effectively treats COVID-19. However, compounds (drugs) as anti-COVID-19 viruses in humans are still controversial in terms of effectiveness and side effects, so the discovery and development of drugs from natural ingredients is very much needed. The results of research conducted by Frengki et al., (2020) show that catechin compounds and their derivatives derived from natural ingredients have great potential as anti-virus SARS-CoV-2 through an *in silico* approach, where the binding affinity values (ΔG and Ki) were higher than commercial drugs that have been used.

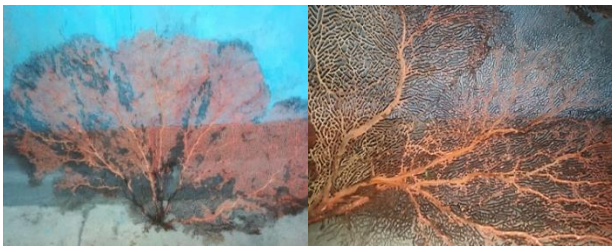


Figure 1. *G. mariae* (Kelutur, Saptarini, Mustarichie, & Kurnia, 2021b)

Coral gorgonians have been reported to have anti-inflammatory, anti-viral, anti-cancer, analgesic, gastroprotective, anti-bacterial, and anti-fouling activities with the secondary metabolites of sterols as the primary compounds (Kelutur, Saptarini, Mustarichie, & Kurnia, 2021a). One type of gorgonian, sea fan (*G. mariae*), can be seen in Figure 1, has been used by the people of Maluku for generations as a medicinal ingredient. However, it has not been scientifically proven and explored, so it has promising prospects for research. In this study, an initial study was conducted using the *in silico* method to determine the effectiveness of sea fan sterols as an anti-virus SARS-CoV-2 before moving on to the next stage. This method provides an economical and effective strategy in discovering new drugs by utilizing computers (La Kilo, Aman, Sabihi, & La Kilo, 2019). In addition, predicting the potential and properties of the material before proceeding to another stage (synthesis) (Male, Sutapa, & Ranglalin, 2015).

In addition, the target class, route of oral administration, pharmacokinetic properties, and acute oral toxicity were predicted to know the effectiveness and safety when consumed as anti-viral drug candidates.

METHODOLOGY

Materials and Instrumentals

Receptor. The M^{Pro} protein SARS-CoV-2 downloaded from https://www.rcsb.org/ with the code of Protein Data Bank (PDB) 6LU7 can be seen in Figure 2 (Jin et al., 2020).

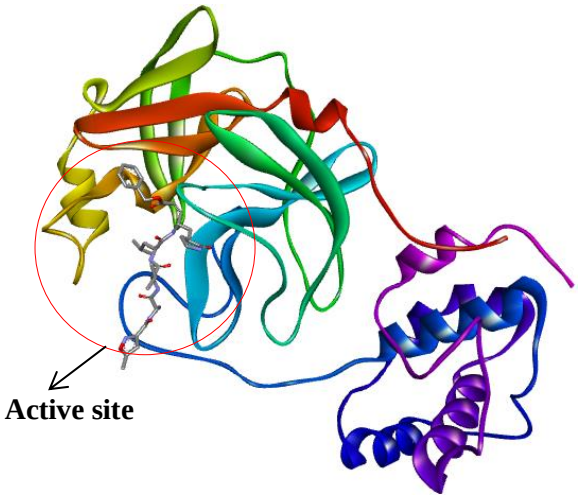


Figure 2. The active site of the M^{Pro} protein SARS-CoV-2

Table 1. Chemical structure of the test ligands

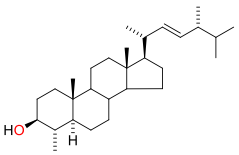
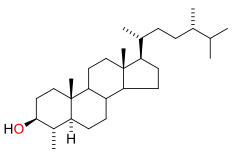
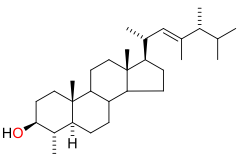
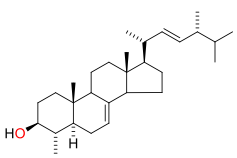
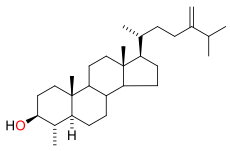
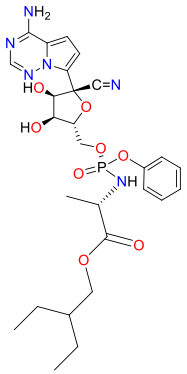
Compounds	Chemical Structure
4,24-dimethyl-22-dehydro-cholestanol	
4,24-dimethyl-cholestanol	
Dinosterol	
4,24-dimethyl cholesta-7,22-dien-3β-ol	

Table 1. Chemical structure of the test ligands (*continuous*)

Compounds	Chemical Structure
4-methyl-24-methylene cholestanol	
Remdesivir	

Test ligands. The sterol secondary metabolites of sea fan consist of 4,24-dimethyl-22-dehydro-cholestanol; 4,24-dimethyl-cholestanol; dinosterol; 4,24-dimethyl cholesta-7,22-dien-3β-ol; and 4-methyl-24-methylene cholestanol (Kokke, Bohlin, Fenical, & Djerassi, 1982) and comparison (remdesivir) downloaded from https://pubchem.ncbi.nlm.nih.gov/ with a 2D structure in SDF format. The chemical structure of the test ligands can be seen in Table 1.

Software. Operating system windows 10 home single language 64-bit (10.0, build 18362) intel (R) Core (TM) i5-10210u CPU @ 1.60 GHz (8 CPUs), ~2.1 GHz, Chem3D Pro 12.0, ChemDraw Ultra 12.0, Autodock Tools 4.2 (The Scripps Research Institute, 2020), and the Biovia Discovery Studio 2016 Client® (Dassault Systèmes BIOVIA, 2020).

Methods

Preparation of receptor.

Protein was separated from water molecules and native ligand using Discovery Studio 2016 Client® and stored in .pdb format (Wibowo, Sri Widyarti, Sabarudin, Soeatmadji, & Sumitro, 2019). Then it was re-prepared using Autodock Tools 4.2 by adding Kollman charge and hydrogen polar only and saved in pdbqt format (Kolina, Sumiwi, & Levita, 2018).

Preparation of ligands.

The native ligand was obtained when separated from protein using Discovery Studio 2016 Client® via the scripts menu, selection then selection of protein chains and saved in .pdb format (Wibowo et al., 2019). While the test ligands were manually drawn using ChemDraw Ultra 12.0, otherwise available in Pubchem. Then minimize energy (MM2 method) using Chem3D Pro 12.0 and save in *.pdb format (Narayanaswamy, Wai, & Esa, 2017). After that, it was re-prepared using Autodock Tools 4.2 by adding Gasteiger, all hydrogen, and non-polar merge compute loads and saved in pdbqt format (Kelutur & Mustarichie, 2020).

Parameter settings of grid and docking.

Determination of the grid box on the active site of the M^{Pro} protein in the form of the parameter box's location and the grid box's size (distance, Å) using the Autodock Tools 4.2 program (Roy, Kumar, & Acharya, 2014). The results obtained are the center values of x, y, and z and the distance of the grid points used as a reference for the process of docking the test ligand to the protein. While the docking parameters were a Genetic Algorithm for the number of GA runs, others were left as default (Grolmusz, 2008).

Molecular docking process.

Docking between native ligands and proteins using the Autodock Tools 4.2 program (run-autogrid and run-autodock), then editing the cmd directory (autogrid4 or autodock4 –p dock.dpf –l dock.dlg &) and click launch, which indicates the molecular docking process has started.

Method validation.

The software parameters used must be valid first, where the Root Mean Standard Deviation (RMSD) value of the re-docking results is close to the crystallographic results in the selected active side area (Pratama, 2016).

Target class determination.

All test ligands were identified as target class by Swiss Target Prediction (http://www.swisstarget prediction.ch/). The ligands were converted into "SMILES" type files. Then the Homo sapiens protein was selected. Its test aims to evaluate which compounds can interact with proteins that play a role in proteases.

Docking of the test ligands to the M^{Pro} protein.

The molecular docking process for the test ligands was carried out in the same way as the validation process, which used grid parameters and the docking of the validation results. The results observed in the form of binding affinity include free energy of binding (ΔG) and inhibition constant (Ki) as well as amino acid residues (Kim & Skolnick, 2008).

Analysis of docking result and visualization.

The output of molecular docking is in the form of a notepad file, then the ligand with the lowest bond energy value (best pose) is selected based on clustering. Visualization of the position and orientation of ligands when interacting with proteins and amino acids and bonds formed using the Discovery Studio 2016 Client® (Kelutur, Mustarichie, & Umar, 2020; Wibowo, Sri Widyarti, Sabarudin, Soeatmadji, & Sumitro, 2019).

Lipinski's rule of five.

Prediction of the route of oral administration using http://www.scfbio-iitd.res.in/software/drug-design/lipinski.jsp. The potential test ligand for the SARS-CoV-2 protease inhibitor was inputted with the file type ".pdb," then the pH was set to 7.4 for the human body. Thus, we can know Lipinski's rule of five rules.

Pre-ADMET.

Prediction of pharmacokinetic properties through the website https://preadmet.bmdrc.kr/. Test ligands that have potential as medicinal ingredients are drawn and uploaded in the Mol file format (*.mol) so that they can automatically determine parameters such as the permeability of Human colon adenocarcinoma (Caco-2) cells, Human Intestinal Absorption (HIA), and Plasma Protein Binding (PPB) as well as mutagenic and carcinogenic (Kelutur, Mustarichie, & Umar, 2020).

Acute oral toxicity (LD_{50}).

The LD_{50} prediction is based on the Globally Harmonized System using ProTox-II (https://tox-new.charite.de/protox_II/). The test ligand was formatted into type files "SMILES" then the prediction parameters for the acute oral toxicity model were clicked.

RESULTS AND DISCUSSION

Minimize energy

Protein was minimized by adding a Kollman charge which aims to provide a charge to amino acid residues in the form of electrostatic potential energy based on quantum mechanical calculations (Kolina et al., 2018). In addition, a polar only hydrogen atom was added. It means M^{Pro} protein was water-soluble.

Gasteiger charge was added to the ligand to adjust to the molecular docking environment so that the calculation process could be carried out correctly (Forli et al., 2016). Then a hydrogen atom was added (protonation process), which aims to adjust to the pH conditions in the cell cytoplasm (pH ~7) (Drie, 2005). In addition, to complete the amino acid residues, the hydrogen atoms were lost due to structural damage due to X-ray radiation during the crystallographic process. The considered hydrogen atom was polar because it has an essential role in hydrogen bonding. Non-polar hydrogen atoms were not considered in the ligand-receptor interaction in molecular docking, so they need to be combined with the binding atom (Yanuar, 2012). Therefore, the non-polar merge was chosen (Kolina et al., 2018).

Minimize energy aims to minimize the steric effect of a 3D compound to be stable so that it has the exact resemblance or closeness to bonds of compound and receptor (protein) in the human body (Sliwoski, Kothiwale, Meiler, & Lowe, 2014). The minimize energy process was carried out so that the molecular docking results remain on the target or active site when binding. The active site was selected based on amino acid residues that affect function or activity (Cuzzolin, Sturlese, Malvacio, Ciancetta, & Moro, 2015).

Method validation

The method validation results can be evaluated based on the RMSD value and the binding location, where the docking parameters that were run are appropriate or not, and describe how much influence the native ligand conformation has before and after being validated. The system used for the docking process must be in a flexible ligand condition to allow the ligand to adjust its structure to achieve a stable conformation when it binds to the active site of the receptor or protein (Muttaqin, Pratama, & Kurniawan, 2019).

Determination of the grid box and Lamarckian Genetic Algorithm was very important because it becomes a reference parameter during the docking process between the test ligand and the receptor (protein). In this study, the coordinates of the grid box docking coordinates for the M^{Pro} protein were x = -9,768; y = 11.436; and z = 68.904 with a grid point of 40×54×40 and a grid point distance of 0.375 Å. The setting of the grid box aims to determine the active site area of the macromolecule (Roy et al., 2014).

While algorithm method, namely Lamarckian Genetic Algorithm for the number of GA runs was 100 times (one docking process). It aims to find the best position or conformation when the ligand and receptor bind (Grolmusz, 2008).

The docking parameter in this study was declared valid because it has an RMSD value of 3.011 Å. It shows that the position of the native ligand as a result of re-docking after being superimposed was getting closer to the co-crystal results (Jain & Nicholls, 2008). In addition, the effect of the closer receptor (protein) binding distance was directly proportional to the

smaller the RMSD value (Puspaningtyas, 2013). Thus, M^{Pro} protein can be used for molecular docking processes.

Target class

Based on the results of the target class prediction, it was found that the sterol compounds of the sea fan have a probability value of 10.6–11.8% to interact with protease protein, it can be seen in Table 2.

Table 2. Identification of the target class of the test ligands

No.	Compounds	Probability (%)
		Protease protein
1.	4,24-dimethyl cholesta-7,22-dien-3ß-ol	10.6
2.	4,24-dimethyl-22-dehydro-cholestanol	10.6
3.	4,24-dimethyl-cholestanol	-
4.	4-methyl-24-methylene cholestanol	-
5.	Dinosterol	11.8

Table 2 shows that the compounds 4,24-dimethyl-cholestanol and 4-methyl-24-methylene cholestanol were not found for protease protein targets. Meanwhile, 4,24-dimethyl cholesta-7,22-dien-3ß-ol; 4,24-dimethyl-22-dehydro-cholestanol; and dinosterol have incidence probability of 10.6% (0.106) and 11.8% (0.118), which is a probability between 0 and 1. This identificate the possibility that sterol compounds can interact with protease protein.

Docking interaction of test ligands to protein and visualization

The results obtained for the molecular docking process was the interaction of the test ligands with amino acid residues, which indicates binding to the active site of the M^{Pro} protein. In addition, the binding affinity values were obtained in the form of free energy of binding, ΔG and the inhibition constant, Ki as shown in Table 3.

Binding affinity was an important factor that must be considered when interacting between ligands and proteins. If the binding affinity was low, the compound requires less energy to bind or interact with proteins. In other words, it can be said that low binding affinity values have a more significant potential to interact with target proteins (Pangastuti, Amjn, & Indriwati, 2016).

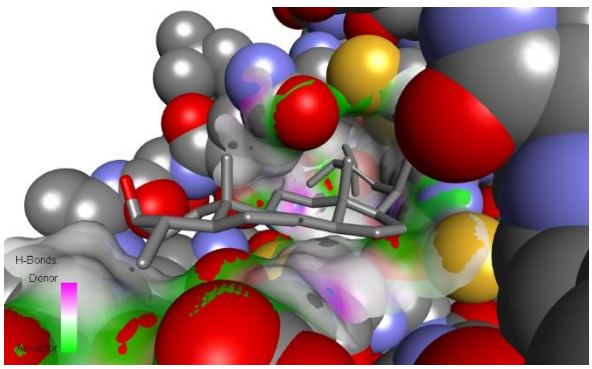
Table 3. Results of docking test ligands to the M^{Pro} protein SARS-CoV-2

Compounds	Amino acid residue interactions			ΔG (kcal/mol)	Ki (nM)
	Hydrogen bonds	Binding distances (Å)	Van der Waals bonds (hydrophobic)		
Sea fan sterols					
4,24-dimethyl cholesta-7,22-dien-3ß-ol	-	-	Cys145, His164, Glu166, Arg188, Asp187, Tyr54, Gln189, Leu167, and Pro168	-9.90	55.13
Dinosterol	Thr26 Thr25	2.98 3.21	Gln189, Asp187, Arg188, Tyr54, Leu27, His164, Thr24, Gly143, Asn142, and Glu166	-9.77	68.66
4,24-dimethyl-22-dehydro-cholestanol	Thr26 Thr25	2.98 3.21	Pro52, Arg188, Gln189, Glu166, Tyr54, Asp187, His164, Leu27, Thr24, Gly143, and Asn142	-9.48	113.33
Remdesivir	Glu166 His164	2.84 2.96	Gly143, His163, Leu141, Ser144, Phe140, Asp187, Thr190, Ala191, and Arg188	-7.43	3610

Free energy analysis aims to determine the spontaneity of a reaction and its stability when the ligand-receptor interaction was indicated by a low (minus) ΔG value. In addition, the stability of the interaction was proportional to the compound's potential when it forms strong chemical bonds (Adelina, 2014). The smaller number or more negative energy of ΔG means the stronger binding of a ligand to a receptor (Mulyati & Panjaitan, 2021; Umamaheswari, Madeswaran, & Asokkumar, 2013). According to the equation, ΔG was closely related to Ki, $\Delta G = -RT \ln Ki$. Thus, the value of ΔG indicates the ability of the compound to inhibit macromolecules (Ki) (Kartasasmita, Herowati, Harmastuti, & Gusdinar, 2009).

The molecular docking results in Table 3 show that the sterol compounds from the sea fan have a better binding affinity than the comparator (drug) with a potential ΔG value 4,24-dimethyl cholesta-7,22-dien-3ß-ol (-9.90 kcal/mol) > dinosterol (-9.77 kcal/mol) > 4,24-dimethyl-22-dehydro-cholestanol (-9.48 kcal/mol) > remdesivir (-7.43 kcal/mol), respectively.

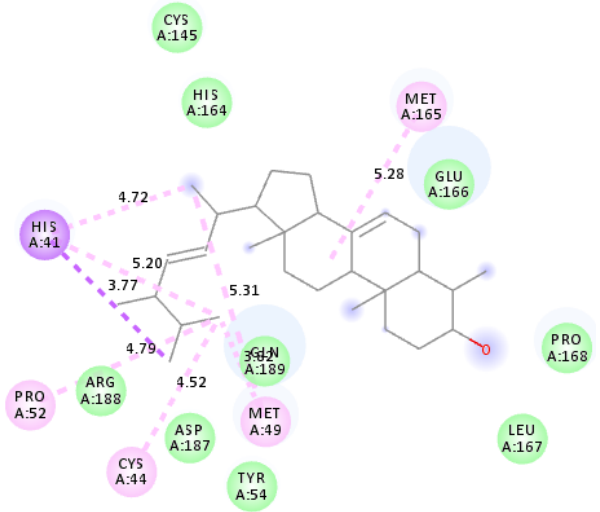
While Ki indicates the ability of a compound to inhibit the receptor (protein). The smaller the Ki value, the stronger the resistance (Umamaheswari et al., 2013). The Ki values obtained in Table 3 from the most potent were 4,24-dimethyl cholesta-7,22-dien-3ß-ol (55.13 nM) > dinosterol (68.66 nM) > 4,24-dimethyl-22-dehydro-cholestanol (113.33 nM) > remdesivir (3610 nM).



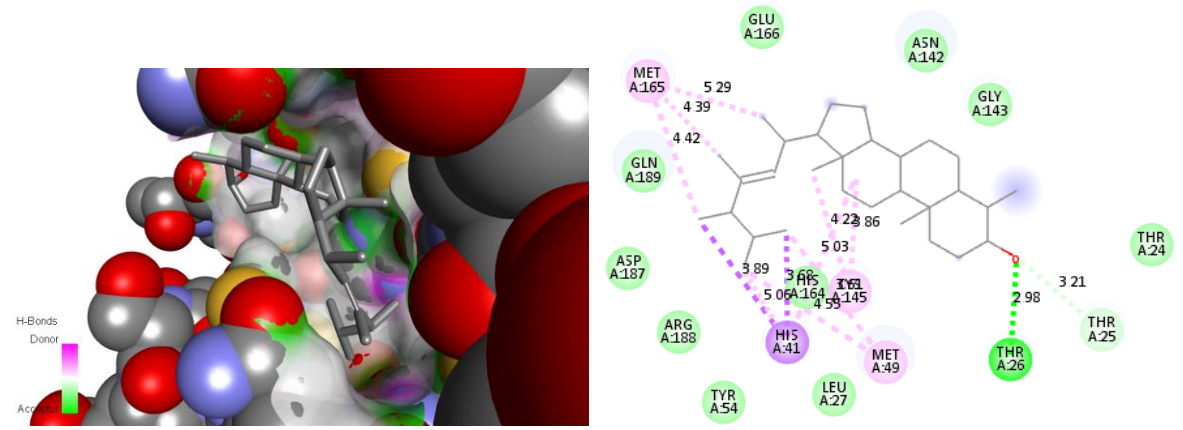
The interaction between the test ligands and M^{Pro} SARS-CoV-2 protein (Figure 3) formed hydrogen bond (HB) and Van der Waals (VdW) interactions with varying amino acid residues and bond distances. In this study, 4,24-dimethyl cholesta-7,22-dien-3ß-ol had no HB interaction but only VdW (Cys145, His164, Glu166, Arg188, Asp187, Tyr54, Gln189, Leu167, and Pro168).

Compounds of dinosterol and 4,24-dimethyl-22-dehydro-cholestanol form HB with Thr26 (2.98 Å) and Thr25 (3.21 Å). This interaction occurs because tyrosine (Thr) has a hydroxyl group on the side chain to form OH···O. While on VdW, there are Gln189, Asp187, Arg188, Tyr54, Leu27, His164, Thr24, Gly143, Asn142, and Glu166 for dinosterol and Pro52, Arg188, Gln189, Glu166, Tyr54, Asp187, His164, Leu142, Thr24, Asn142 for 4,24-dimethyl-22-dehydro-cholestanol. Remdesivir obtained residue on HB in the form of Glu166 (2.84 Å) to form OH···O and His164 (2.96 Å) is NH···O. For VdW, namely Gly143, His163, Leu141, Ser144, Phe140, Asp187, Thr190, Ala191, and Arg188.

The difference of amino acids in HB is due to the presence of side chains, which contain hydroxyl (Ser and Thr), amide (Asn and Gln), charge (Lys, Arg, Asp, and Glu), and aromatic (His, Tyr, Trp) (Scheiner, Kar, & Pattanayak, 2002). The visualization results show crucial amino acid residues when the sterol compounds from *G. mariae* interact with M^{Pro} as an anti-virus inhibitor were Asp187.



(**a**)



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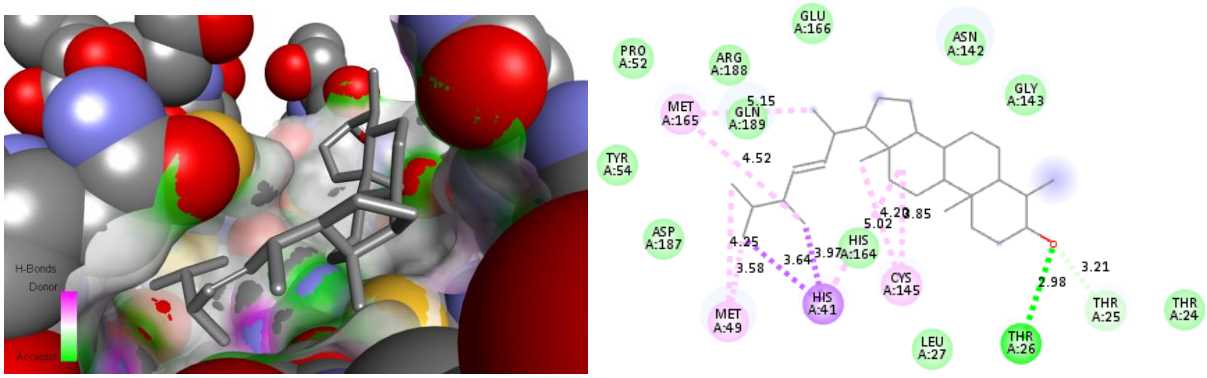

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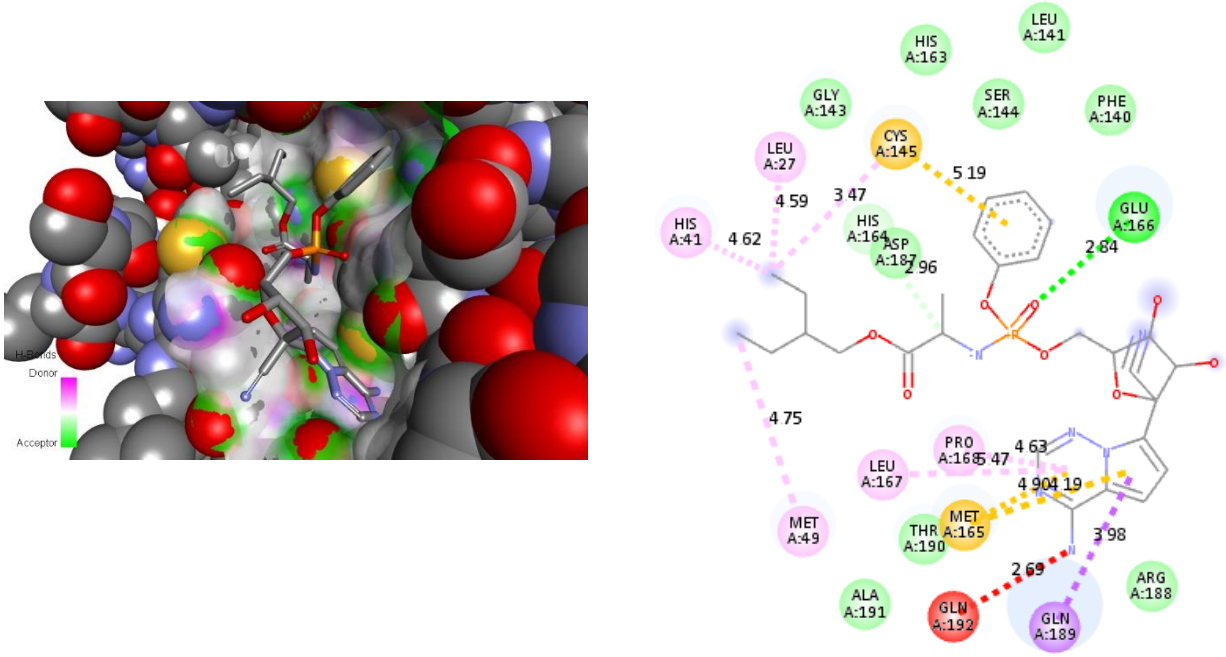

(**d**)

Figure 3. The active pocket and amino acid residues formed when 4,24-dimethyl-choleste-7,22-dien-3ß-ol (a), dinosterol (b), 4,24-dimethyl-22-dehydro-cholestanol (c), and remdesivir (d) binds to the protein of M^{Pro} SAR-COV-2

Prediction of solubility and permeability

Criteria for a good drug was if it meets the rules of Lipinski's Rule of Five. Its rule determines the physicochemical properties as hydrophobic/hydrophilic when absorption and permeability occur in the lipid bilayer in the human body (Nursamsiar, Toding, & Awaluddin, 2016; Syahputra, Ambarsari, & Sumaryada, 2014).

Lipinski's Rule of Five was used to predict a candidate drug orally, which must meet five requirements, namely molecular weight (MW) 500 g/mol, partition coefficient (Log P) 5, hydrogen bond donor 5, hydrogen bond acceptor 10, as well as the molar refractivity between 40–130 (Lipinski, 2004) as shown in Table 4.

Table 4. Prediction results of Lipinski's rule of five

Compounds	MW (g/mol)	Log P	Hydrogen Donor	Hydrogen Acceptor	Molar refractivity	Information
4,24-dimethyl cholesta-7,22-dien-3ß-ol	412	7.7	1	1	128	×
Dinosterol	428	8.1	1	1	133	×
4,24-dimethyl-22-dehydro-cholestanol	414	7.7	1	1	128	×
Remdesivir	602	2.3	5	13	147	×

The prediction results in Table 4 show that all the test ligands have a log P value > 5. It means that they are not suitable to be given, or modifications were needed for oral use. A log P value of more than 5 have the potential to have a toxic effect because it has low solubility in water, so it was difficult to be excreted, accumulates, easily binds to hydrophobic targets compared to the intended target, and was difficult to metabolize (Gao, Gesenberg, & Zheng, 2017; Hongmao, 2016).

Electron donor substituents were essential to note in the P log (Rachmania, Supandi, & Larasati, 2015). The logarithm of the partition coefficient (log P) of a compound was influenced by the length of the carbon chain, the number of alkyl substituents (-CH_3) and lone pairs (-OH) (Czyrski & Kupczyk, 2013). It was known that sterols belong to the lipid group with aliphatic or aromatic carbon chains, so they tend to be more non-polar or lipophilic (Kelutur, Mustarichie, & Umar, 2020).

The number of hydrogen bond donors and acceptors explains the high hydrogen capacity so that the absorption process that occurs also requires a high level of energy (Syahputra, Ambarsari, & Sumaryada, 2014). All the test ligands met the number of hydrogen bond donors and acceptors. Likewise, molar refractivity explains the interaction of compounds (Sawale, Kalyankar, George, & Deosarkar, 2016).

Determination of pharmacokinetic properties

The development of a drug substance must pay attention to aspects of absorption, distribution, metabolism, excretion, and toxicity before clinical trials are carried out. The predicted parameters include pharmacokinetic properties and toxicity. Pharmacokinetic parameters were absorption (Human Intestinal Absorption, HIA and permeability of Human Colon Adenocarcinoma cells, Caco-2) and distribution (Plasma Protein Binding, PPB). At the same time, the toxicity predicts mutagenic and carcinogenic properties (Kelutur & Mustarichie, 2020).

HIA shows the percentage of drugs absorbed from the ratio of cumulative excretion in urine, bile, and feces. Caco-2 was widely used as a model in vitro testing when predicting drug absorption in humans through intestinal epithelial barrier cells (O'Hagan & Kell, 2015). While PPB was related to the ability of drug disposition when giving effect (Megantara, Levita, Iwo, & Ibrahim, 2018) and toxicity predicts the test compounds as a drug candidate in providing biological activity to the body (Muttaqin et al., 2019). Pre-ADMET prediction results can be seen in Table 5.

Table 5. Prediction results of pre-ADMET

Compounds	Absorption		Distribution	Toxicity	
	HIA (%)	Caco-2 (nm sec^{-1})	PPB (%)	Mutagenic	Carcinogenic
4,24-dimethyl cholesta-7,22-dien-3ß-ol	100	51.8	100	No	No
4,24-dimethyl-22-dehydro-cholestanol	100	51.8	100	No	No
Dinosterol	100	52.1	100	No	No
Remdesivir	53.5	3.3	81.3	No	Yes

Table 5 shows an excellent %HIA value for the test ligand, which was 70–100%, so it can be said to have good absorption when passing through the intestine. The Caco-2 cells showing moderate permeability (4–70 nm sec-1) and %PPB showing strong binding (> 90%), but it needs to be modified because the stronger plasma protein binding, the pharmacological effect given was not good because it does not diffuse outward from the systemic circulation (Aslam, Tan, & Prayitno, 2003). Meanwhile, the toxicity prediction shows that the test ligand was safe to use because it was not mutagenic and carcinogenic.

LD_{50} Prediction

The toxic dose was given with an LD_{50} value (mg/kg). The higher the LD_{50}, the better activity. Based on the results of prediction of acute oral toxicity, it was known that the sterol compounds from the sea fan belong to class IV ($300 < LD_{50} \leq 2000$) with the possibility of dangerous toxicity if ingested, so it needs special attention and consideration for the route of administration (Table 6).

Table 6. Prediction of acute oral toxicity in test ligands

Compounds	LD_{50} acute (mg/kg)	Class	Similarity (%)	Accuracy (%)
4,24-dimethyl cholesta-7,22-dien-3ß-ol	500	IV	100	100
4,24-dimethyl-22-dehydro-cholestanol	500	IV	100	100
Dinosterol	500	IV	100	100
Remdesivir	1000	IV	40.9	54.3

CONCLUSION

The results of the research that have been carried out can be concluded that the sterol compounds from sea fans have the probability to interact with protease proteins based on the Swiss Target Prediction prediction, namely only 4,24-dimethyl cholesta-7,22-dien-3ß-ol; dinosterol; and 4,24-dimethyl-22-dehydro-cholestanol. Then based on molecular docking, it showed that the test ligands had a higher binding affinity value than the comparison (drug) with important amino acid residues of Asp187. It could be said to be the potential candidate of inhibitor for the M^{Pro} protein SARS-CoV-2. However, it needs to be considered and a serious concern if it was given orally. It was because of a lipophilicity value of more than 5. In addition, it does not provide a pharmacological effect if distributed to plasma proteins because it does not diffuse out of the systemic circulation and the dose of acute oral toxicity (LD_{50}) was dangerous if swallowed.

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