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Article

Identification of Antiviral Compounds against Hepatitis C Virus (HCV) targeting NS3 Protein by Pharmacophore Modeling, Molecular Docking, and ADMET Approach

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Ratih Rahayu[1], Linda Erlina[1,2*], Suratno Lulut Ratnoglik[1,3], Andi Yasmon[1,3], Fadilah[1,2], Rafika Indah Paramita[1,2]

[1]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Abstract. Hepatitis C Virus (HCV) is a world health problem. HCV infection is initiated by various structural and non-structural proteins. The HCV NS3 protein has an important function in viral replication. The N-terminal domain of NS3 acts as a protease to process most of the viral polypeptides. NS3 also acts as an RNA helicase and NTPase and triggers liver fibrosis which accelerates the development of liver disease. Thus, this study aims to provide information on potential new antiviral candidates against HCV that target the NS3 protein. This study was conducted in-silico with a ligand-based and structure-based pharmacophore model to the cavity of the active protein site generated after virtual screening and molecular docking. The results of this study showed that three compounds, namely stigmasterol, gamma-mangostin, and erycristagallin, were found as HCV antiviral candidates that target the NS3 protein with a lower binding affinity than the native ligand. The binding energy of each compound is -9.23 Kcal/mol, -8.58 Kcal/mol, and -8.17 Kcal/mol. Based on ADMET analysis, the three compounds have high absorption in the small intestine. The cytotoxicity analysis of stigmasterol compounds is not potentially mutagenic, and the LD50 value of stigmasterol is also lower than other compounds.

Corresponding Author :
Linda Erlina
Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
Email : linda.erlina22@ui.ac.id

1. Introduction

Hepatitis C Virus (HCV) is a world health problem and a risk factor that significantly plays a role in the development of liver disease. Caused liver diseases such as liver cirrhosis and hepatocellular carcinoma. Infection with HCV can even cause death. According to the World Health Organization (WHO) in 2022, new cases of chronic infection by HCV amounted to 58 million people and caused the death of 290,000 people [1].

The current therapy for HCV infection is a combination Direct-acting antivirals (DAA) regimen. DAA is a protease and polymerase inhibitor used in the latest treatment of HCV infection which provides excellent efficacy and safety [2]. However, there are still around 3-5% of patients who experience treatment failure. One of the factors causing treatment failure is resistance-associated substitution (RAS), RAS is generally detected in failed DAA regimens. Based on studies with analysis of drug target sequences of recovered viruses showed that RAS is known to occur in NS3 proteases after treatment failure. These RAS are genetically stable after virus passage and lead to significant phenotypic resistance in viruses with RAS [3-5].

The NS3 protein is a non-structural HCV protein that plays an important role in HCV replication, where its N-terminal domain acts as a protease that is responsible for processing most of the viral polypeptides [6-7]. Aside from being a viral protease, this protein also functions as an RNA helicase, nucleoside triphosphatase (NTPase), and stimulates liver fibrosis which accelerates the development of liver disease [8-9]. Thus, the protease NS3 represents a potential target for antiviral identification against HCV.

Compounds derived from plants have been widely used as therapeutic drugs for viral infections [10]. Various plant compounds of *Terminalia chebula* (*T. chebula*) have been identified in-silico for their ability to inhibit HCV protein NS3/4A. The results of this study predict that the tannins from T. chebula have the potential to inhibit HCV NS3/4A and modulate host immune activity [9]. Another study that identified *Aptenia cordifolia* root extract, using chromatography and spectroscopy techniques, resulted in the isolation and identification of eight compounds. The resulting compound fraction was tested for its activity as an anti-HCV. The results of this study showed that only the basic ethyl acetate fraction had high activity against HCV with an IC50 value of 2.4 μg/mL. Based on the virtual silico screening in this study, the tortuosamine compound showed the strongest binding to the active site of the NS3/4A helicase with a binding affinity of -7.1 kcal/mol [11]. This is the basis that plant compounds can be anti-HCV candidates that can target functional viral proteins.

Computer-aided drug design (CADD) is one approach that can be used in drug discovery from compounds derived from plants. CADD is a field that utilizes computational methods and tools to aid in the discovery, development, and analysis of drugs and related compounds [12]. CADD is an important part of the drug discovery project. This contributes to the identification and optimization of hit compounds, all the way to the later stages of the drug discovery pipeline. The goal of CADD is to improve the efficiency and success rate of drug discovery by providing insights into the interactions between drugs and their targets, predicting their properties, and optimizing their design [12-13].

There are two main approaches in CADD, namely Ligand-Based Drug Design (LBDD) and Structure-Based Drug Design (SBDD). LBDD is a method that involves analyzing the chemical and structural properties of known ligands and using this information to design new compounds with similar properties. It includes techniques such as quantitative structure-activity relationship (QSAR) modeling, pharmacophore modeling, and ligand-based virtual screening. SBDD is an approach that involves studying the three-dimensional structure of the target protein and designing drugs that can interact with it in a specific way. It includes techniques such as molecular docking, molecular dynamics simulations, and structure-based virtual screening [14].

Docking facilitates the development of novel medicinal drugs, the molecular prediction of ligand-target interactions, and the description of structure-activity relationships (SARs) [15]. Therefore, this study was conducted in silico screening with pharmacophore modeling, virtual screening, and

molecular docking of anti-HCV compounds that target the NS3 protein. This study aims to be able to provide information on potential new antiviral candidates against HCV.

2. Experimental Section

2.1. Materials and Methods

2.1.1. Ligand-Based Drug Design (LBDD)

Small molecule drug compounds for HCV therapy were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), selected compounds that have been approved, and some are still in clinical trials (Table 1). Canonical SMILES from all compounds were taken and used to make test sets, training sets, and decoys. The number of test sets and training sets was divided randomly until a good set was obtained. Decoy was created by inputting the compound canonical SMILES into the LIDeB Tools - LUDe web server (https://lideb-lude-v2.streamlit.app/). Pharmacophore modeling was carried out using Ligandscout 4.3 software, which produced 10 pharmacophore models. Pharmacophore model validation was carried out with a threshold on the ROC curve, namely AUC 100% ≥ 0.75 and EF1% ≥ 10.

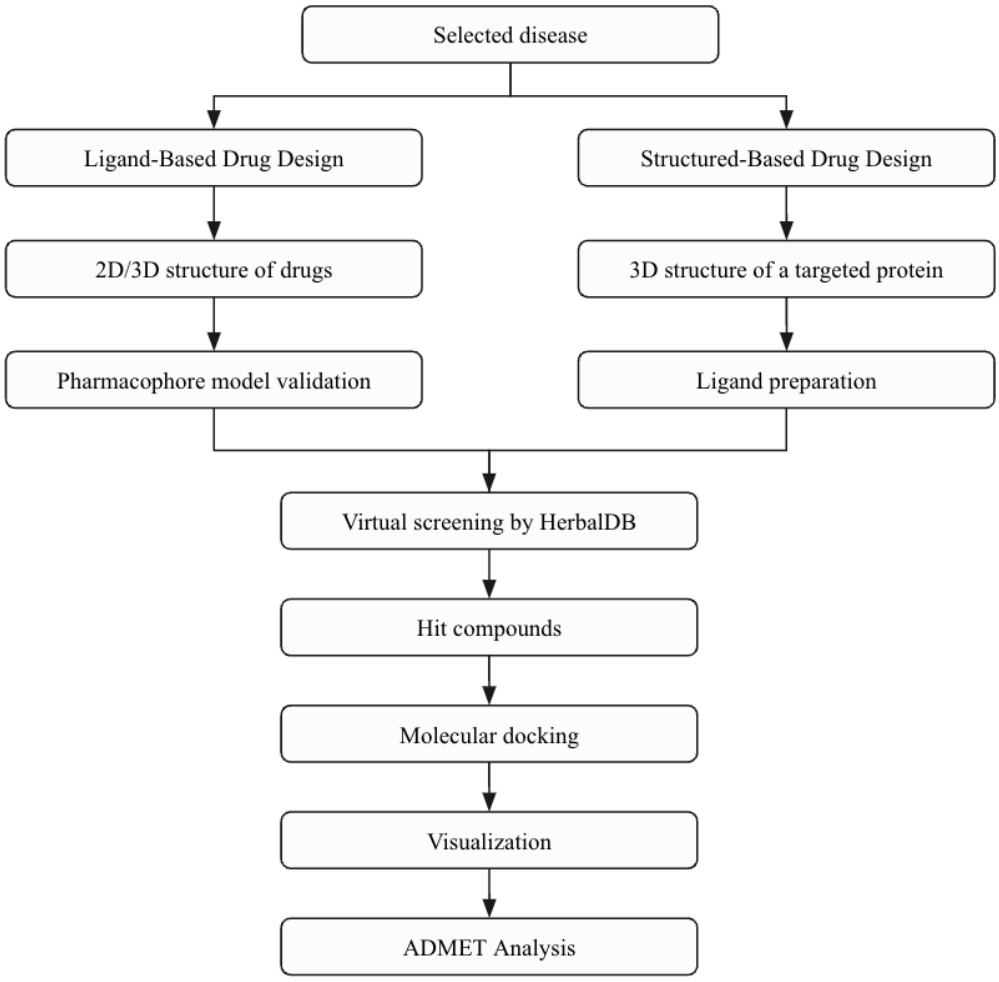

Figure 1. Pharmacophore modeling and molecular docking workflow.

Table 1. Antiviral drugs (small molecules) are used to make pharmacophore models

No	Antiviral drugs
1	Sofosbuvir
2	Ribavirin
3	Dasabuvir
4	Elbasvir
5	Grazoprevir
6	Boceprevir
7	Ledipasvir
8	Ombitasvir
9	Pibrentasvir
10	Simeprevir
11	Daclatasvir
12	Glecaprevir
13	Ritonavir
14	Asunaprevir
15	Voxilaprevir
16	Telaprevir
17	Paritaprevir
18	Velpatasvir
19	Ciluprevir
20	Danoprevir

2.1.2. Structure-Based Drug Design (SBDD)

The structure of the HCV NS3 protein (4OK5) obtained from PDB RCSB (https://www.rcsb.org/) was used in SBDD. The best pharmacophore model from LBDD results was also used. Virtual screening was performed on the HerbalDb compound library with NS3 proteins and a validated pharmacophore model. HerbalDb is a library of herbal compounds from Indonesia from the database of the Department of Pharmacy FKUI database (http://herbaldb.farmasi.ui.ac.id/v3). Virtual screening was carried out using Ligandscout 4.3 software and hit compounds were obtained.

2.1.3. Molecular Docking

The 3D structure of the hit compound obtained from the pharmacophore screening was taken from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The hit compound and the NS3 protein (4OK5) were carried out by molecular docking using the AutoDock tool software with the Lamarckian genetic algorithm (LGA) as a scoring function. The resulting binding compounds with a better binding affinity (kcal/mol) were extracted and visualized using Ligandscout 4.3.

2.1.4. ADMET Analysis

Evaluation of pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADME) including toxicity is one of the main criteria before developing compounds into drugs. The pharmacokinetic and toxicity profiles of the compounds were analyzed in silico using the ADMET pkCSM descriptor algorithm protocol. (https://biosig.lab.uq.edu.au/pkcsm/prediction). The ADME profile of the compound must meet the criteria of the Lipinski Rule of 5 consisting of a molecular weight < 500 daltons, hydrogen bond donor < 5, hydrogen bond acceptor < 10, and log P value < 5 [16-17].

3. Results and Discussion

3.1. Ligand-Based Pharmacophore Modeling

Pharmacophore modeling is a technology used in drug discovery and development to identify possible interactions between ligands and receptors. Pharmacophore modeling involves constructing models that represent the essential steric (shape) and electronic (charge) features of ligand-receptor interactions, which are critical for triggering biological responses [18]. Validation is important to do to get a good pharmacophore model. Where the pharmacophore model proves useful for drug design [19-20].

The ligand-based pharmacophore model in this study was validated using 20 HCV antiviral therapy drugs (Table 1.), resulting in 10 pharmacophore models for each validation. The results of the classification model such as the AUC (area under the curve) value and the EF (enrichment factor) value of the compound are predicted from the receiver operating characteristic (ROC) curve. ROC is a specificity graph that displays the results of a classification model that can provide an overview of the degree of separation, where AUC is used to describe a summary of model performance. If the model has a higher AUC value, then it has better predictability [21].

In this study, the threshold used for AUC 100% is above 0.75 and EF1% is above 10. The number of hits is another parameter to pay attention to, the less the better because it shows true positive and false positive values. The results of the validation of the pharmacophore model in this study, it was found that the EF1% was 25.5 with an AUC value of 100% which was 0.88 (Figure 1). This pharmacophore model has the ability to differentiate the actual active compound from the decoy compound. The number of hits obtained is 220 hits, with true positives of 11 and false positives of 209.

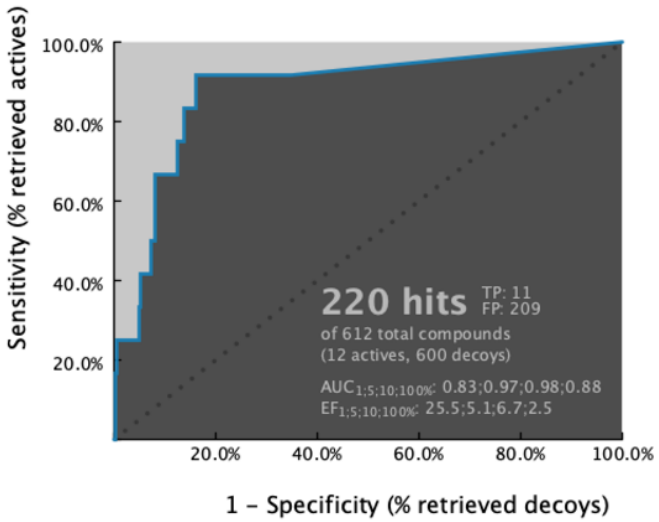

Figure 2. ROC curves of validated pharmacophore models. The pharmacophore model was validated using 20 sets of HCV antiviral therapy drugs and 600 decoy compounds (Ligandscout 4.3).

3.2. Structure-Based Pharmacophore Modeling

Virtual screening is one of the cornerstones of drug discovery today. Pharmacophore-based virtual screening, such as structure-based methods, is a highly efficient technique [22]. Drug design requires the 3D structure of the protein. The validated 3D structure of the HCV NS3 protein was taken from the protein databank (https://www.rcsb.org/). The ligand binding capacity of selected NS3 proteins was determined experimentally and validated through the x-ray diffraction method with an IC value of 50 3.1 x 10^4 nM and an analytical resolution of 2.15 Å. The 4OK5 protein has a native ligand with an ID of 2T2 [23].

The effectiveness of a compound is analyzed by binding to the active site of the NS3 protein. However, incompatible binding can result in unreliable protein inhibitory ability of the compounds. Therefore, it is important to determine the active sites of compounds and examine their interactions in order to obtain more biological activity compared to existing drugs. This can be done by testing the ability of compounds to bind to active proteins and inhibit substrate binding. LigandScout4.3 is computer software that enables the creation of pharmacophore models and virtual screening to define compounds whose interactions will be analyzed to determine their biological activity [21].

The virtual screening process can be accelerated by using a validated pharmacophore model used as a query. Validated pharmacophore models are used for screening large ligand databases or compound libraries to get a new candidate that has chemical features similar to the native model [24], [25]. In this study, the validated pharmacophore model was screened with a compound library containing 1,377 compounds. The hits obtained were 23 compounds with pharmacophore-fit scores from 55.25 to 58.01. All the hit compounds obtained have matching features similar to the validation pharmacophore model, namely having three hydrophobic bonds and two hydrogen bond acceptor donors. Compounds marked as hits were retrieved and stored for further evaluation. The hits compound obtained can be seen in Table 2.

Table 2. List of hit compounds

No	Compound	Pharmacophore-Fit Score
1	Orientanol C.mol	58.01
2	Mangostanin.mol	56.80
3	9,9'-Diapo-10,9'-retro-carotene-9,9'-dione.mol	56.73
4	Kuwanon T.mol	56.63
5	Erythrabyssin 11. mol	56.50
6	Erycristagallin.mol	56.44
7	5,7-Dihydroxy-4'-methoxy- 8-C- renyl-3'-(3-h...	56.42
8	Gartanin.mol	56.27
9	Dehydrocycloguanandin.mol	56.20
10	5,7-Dihydroxy-4'-methoxy-8,3'-di-C- renylfla...	56.15
11	Plucheoside A. mol	56.13
12	trans,trans-Farnesol.mol	56.13
13	Heteroartonin A. mol	56.01
14	Artocarpin.mol	56.01
15	Lute in. mol	55.80
16	Linoleic acid.mol	55.75
17	beta-Tocotrienol.mol	55.60
18	(Z,Z,Z)-3,6,9-Dodecatrien-1-01. mol	55.60
19	alpha-Tocotrienol.mol	55.59
20	alpha-Tocopherol.mol	55.38
21	gamma-Mangostin.mol	55.34
22	Sanggenol O. mol	55.33
23	Anacyclin.mol	55.25

3.3. Molecular Docking

3.3.1 Molecular Docking NS3 Protein with Compounds from Virtual Screening Results

Molecular docking is important in silico approach in the drug discovery process. This method is used to evaluate the binding ability of the hit compound to the target protein and its possible interactions [21]. Grid box native ligand validation was carried out to obtain a good grid box to be used in ligand-protein docking analysis. The grid box was validated with 3 sizes located in the central protein and ligand binding areas, namely 40x40x40, 50x50x50, and 60x60x60 (Table 3).

Table 3. Validation of grid box native ligand

No	Grid Center	Binding Energy	Koordinat (x,y,z)	RMSD	Inhibition constant
1	40x40x40	-8.01 kcal/mol	-19.170, -31.489, 31.798	0.865 Å	1.35 uM
2	50x50x50	-8.10 kcal/mol	-19.170, -31.489, 31.798	0.877 Å	1.15 uM
3	60x60x60	-8.02 kcal/mol	-19.170, -31.489, 31.798	0.839 Å	1.32 uM

Note: RMSD (Root Mean Square Deviation)

In this study, there are several parameters that must be considered in choosing a good grid box, namely the lowest binding energy value and RMSD below 2. RMSD is a measure of the average distance between the ligand and the protein in the simulation. If the RMSD value is small, then the distance between the ligand and the protein is also getting smaller. This indicates a good bond between the ligand and the protein, and vice versa [26]. Based on the validation results, it was found that the smallest binding energy value, namely -8.10 kcal/mol, is in the grid center of 50 x 50 x 50 with constant inhibition 1.15 nM and RMSD 0.877. The NS3 protein has an active site which is the native ligand attachment site, the position of the ligand is then matched to the hits compound. The test ligand was prepared and the receptor grid with a grid box measuring 50 x 50 x 50 with coordinates (x, y, z) -19,170, -31,489, 31,798 according to a good validation grid box.

In this study, Some of the hit compounds obtained from virtual screening results that have 3D structures on PubChem (https://pubchem.ncbi.nlm.nih.gov/) and some taken from the literature are used in the docking analysis. The total compounds analyzed by molecular docking were 22 compounds. The results of the docking analysis of the test compounds and NS3 protein can be seen in Table 4.

Table 4. Results of docking analysis of compounds and NS3 protein

No	Ligand	Binding Energy (Kcal/mol)	Inhibitor Constant (μM)
1	Native ligand	-8.10	1.15
2	Alphatocotrienol	-6.79	10.62
3	Anacyclin	-5.49	94.49
4	Artocarpin	-7.74	2.11
5	Betatocotrienol	-6.38	21.05
6	Dehydrocycloguanandin	-6.46	18.54

7	Erycristagallin	-8.17	1.03
8	Erythrabissin II	-6.96	7.96
9	Gamma Mangostin	-8.58	0.51
10	Gartanin	-7.73	2.16
11	Heteroartonin A	-6.54	16.16
12	Hisperidin	-6.04	37.30
13	Kaempferol	-5.91	46.49
14	Kuwanon T	-7.59	2.72
15	Linoleic acid	-4.61	420.01
16	Lutein	-5.87	49.89
17	Mangostanin	-7.61	2.62
18	Myricetin	-5.86	51.05
19	Naringenin	-5.94	43.99
20	Orientanol C	-7.96	1.46
21	Quercetin	-5.70	66.55
22	Sanggenol O	-8.01	1.35
23	Stigmasterol	-9.23	0.17

Based on the results of the docking analysis in the Table.4, three compounds were obtained that had a lower binding energy than the native ligand. The three compounds that have the lowest binding energy are Stigmasterol, Gamma mangostin, and Erycristagallin. The binding energies of each compound are -9.23 Kcal/mol, -8.58 Kcal/mol, and -8.17 Kcal/mol; with constant inhibitors of 0.17 μM, 0.51 μM and 1.03 μM respectively. In molecular docking, binding affinity refers to the strength of the interaction between the ligand and the receptor [27]. Bind affinity is affected by non-covalent intermolecular interactions such as hydrogen bonds, electrostatic interactions, hydrophobic, and Van der Waals forces between two molecules. In general, a lower binding affinity indicates a stronger interaction between the ligand and the receptor [28-29].

Stigmasterol or stigmasta-5,22-dien-3-ol is a wulzen anti-rigidity factor or known as stigmasterin. Stigmasterol is one of the many phytosterols found in plants. It is a naturally occurring bioactive substance found in plant cell membranes, and its chemical structure is similar to cholesterol in mammalian cells. Stigmasterol is widely distributed in plant foods such as olive oil, nuts, seeds, and legumes. Stigmasterol synthesizes many hormones, including estrogen, progesterone, corticoids, and androgens. Based on in-vitro and in-vivo studies, stigmasterol has various biological effects, including antioxidant, anti-cancer, anti-diabetic, respiratory disease, and lipid-lowering effects [30].

One of the health benefits and pharmacological properties of stigmasterol is that it has antiviral properties. The results of a study by Soekamto et al (2019), identified the antiviral effect of this compound isolated from the bark extract of *Melochia umbrella* against the flaviviridae family, namely dengue (DENV-2). Dengue antiviral test results revealed strong inhibitory activity with IC_{50} 9.11 μg/mL [31]. This compound has been identified as the main ingredient in several plant extracts such as *Calophyllum soulattri* Burm F., *Ophiopogon japonicus*, *Annona muricata* L., *Aegle marmelos* L., *and Icacina trichantha* [32-34].

Gamma-mangostin is one of the main xanthone compounds in mangosteen (*Garcinia mangostana*). Gamma-mangostin is known to have several pharmacological effects including anti-inflammatory, anticancer, and antidiabetic. Based on an in-silico study by Ansori et al (2022), it was revealed that gamma-mangostin exhibits antiviral capabilities with a binding affinity to a target protein

of -7.6 kcal/mol [35-36]. However, not many studies have experimentally tested the ability of gamma-mangostin compounds as antivirals.

Erycristagallin is a phytochemical compound found in *Erythrina variegate*. The pharmacological property of erycristagallin that has been identified in vitro is its ability as an antioxidant and antibacterial [37-38]. Based on a study by Syahdi et al (2012), who conducted a virtual screening of Indonesian herbal databases, it is predicted that erycristagallin can inhibit HIV-1 reverse transcriptase [39].

3.3.2 Interpretation of Protein-Ligands Interactions

Protein-ligand interactions can be seen from their amino acid interactions. Based on the results of the analysis of the ligand-protein interactions of the 3 compounds which have a lower binding energy than the native ligand, there are several amino acids that are always the sites of interaction between the tested ligand and the NS3 protein. The amino acids that are always present in every ligand-protein interaction are Tyr 502, Ala 275, Trp 501, Ala 297, Ala 497, Thr 298, and Gly 255. Gamma-mangostin compounds have the same amino acids as native ligands that interact with proteins (Figure 3).

However, there were differences in the interactions between the Tyr 502 and Thr 298 amino acids. The Tyr 502 amino acid in the native ligand interacted with hydrophobic bonds, while in Gamma-mangostin it interacted with hydrogen bonds. The same thing happened to the amino acid Thr 298. The Stigmasterol compound has similar interactions with the native ligand on all the amino acids involved in the interaction. Where the same interaction occurs, namely the interaction of hydrophobic bonds on the amino acids Tyr 502, Ala 275, Trp 501, Ala 297, Ala 497, and Thr 298. Another similar interaction is the interaction with hydrogen bonds on the amino acid Gly 255. The Erycristagallin compound only has the similarity of the hydrophobic bond interaction with the native ligand in the amino acids Tyr 502, Ala 275, Trp 501, Ala 297, Ala 497, and Thr 298. The consensus results of amino acid interactions between the 3 selected ligands and the native ligand in the complex with the NS3 protein can be seen in Table 5.

Table 5. The result of the interaction between the 3 selected ligands and the native ligand in a complex with the NS3 protein

No	Amino acid	Interaction with ligands				Number of interaction
		Native ligand	Stigmasterol	Gamma mangostin	Erycristagallin	
1	Leu 274	x		x		2
2	Tyr 502	x	x	v	x	4
3	Ala 275	x	x	x	x	4
4	Trp 501	x	x	x	x	4
5	Leu301	x		x		2
6	Ala 297	x	x	x	x	4
7	Ala 497	x	x	x	x	4
8	Thr 298	x	x	v	x	4
9	Thr 269	v		v		2
10	Gly 255	v	v	v		4
11	Lys 272		v			2
12	Val 232			x		1
13	Asp 269			v	v	1

Note: x is a hydrophobic bond, v is a hydrogen bond

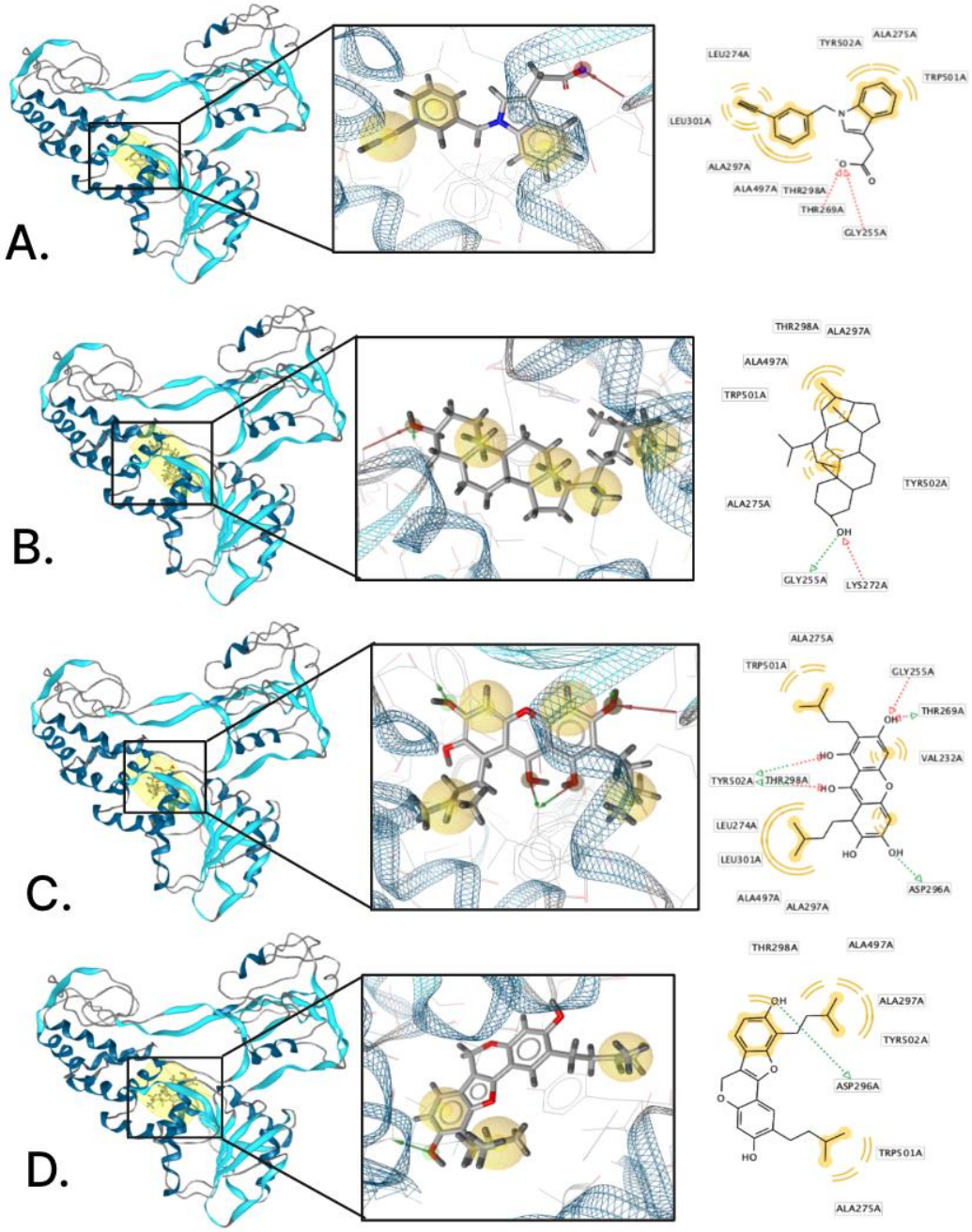

Figure 3. 2D and 3D visualization of NS3 protein interactions with ligands. A) native ligand, B) Stigmasterol, C) Gamma-mangostin, and D) Erycristagallin. (LigandScout 4.2.6.)

3.4. ADMET Analysis

The properties of ADMET refer to the absorption, distribution, metabolism, excretion, and toxicity of the drug. These properties play an important role in drug discovery and development, as high-quality drug candidates not only possess sufficient efficacy against therapeutic targets but also exhibit suitable ADMET properties at therapeutic doses [40-41]. Computational methods are often used to predict ADMET properties, as they can provide valuable insights into the fate of drug molecules in an organism [42].

In this study, we evaluated the ADMET properties of 3 selected compounds using the in silico Pkcsm (https://biosig.lab.uq.edu.au/) and AdmetSAR (http://lmmd.ecust.edu.cn/) to test pharmacokinetic properties such as lipophilicity, water solubility, and drug similarity, including to obtain the toxicity properties of compounds. The lipophilicity of the compounds was analyzed to determine the ability of the compounds to diffuse easily through the cell membrane, so injection preparations can be a better choice for rapid onset reaction. This is due to low gastrointestinal absorption. The ADMET properties of the four selected compounds are shown in Table 6.

Table 6. Results of ADMET analysis of selected ligands.

Properties	Parameters	Stigmasterol	Gamma-mangostin	Erycristagallin
Physico-chemical properties	MW (g/mol)	412,702	396, 439	390,479
	H-bond acceptors	1	6	4
	H-bond donors	1	4	2
Lipophilicity	Log Po/w	7,8	4,79	6,42
Water solubility	Log S (log mol/L)	-6.682	-3,579	-5.985
Pharmacokinetics	Intestinal absorption (%)	94,97	89,405	89,827
	AMES toxicity	-	+	+
	Hepatotoxicity	No	No	No
	Carcinogenicity	-	-	-
Toxicities	Max. tolerated dose (human) (log mg/kg/day)	-0.664	0,411	0,156
	Oral Rat Acute Toxicity (LD50) (mol/kg)	02.54	2.159	1.945

A compound being tested is a good compound when it meets the criteria of the Lipinski Rule of 5 consisting of a molecular weight < 500 daltons, hydrogen bond donor < 5, hydrogen bond acceptor < 10, and log P value < 5 [43]. Of the three selected ligand compounds, only gamma-mangostin meets the requirements of the Lipinski rule so it has good drug-likeness. Water solubility stigmasterol is the lowest than other compounds. The water solubility of stigmasterol, gamma mangostin, and erycristagallin were -6.682, -3.579, and -5.985, respectively. The water solubility of stigmasterol is the lowest among other compounds.

Water solubility is a measure of the ability of solubility of the substance in water. However, the pharmacokinetic intestinal absorption properties of the third compound are above 30%, so the intestinal absorption is high. Based on the results of the toxicity test where the compounds gamma mangostin and erycristagallin were positive for the AMES test, so they have the potential to be mutagenic, while the compound stigmasterol was negative for the AMES test. The LD50 value of stigmasterol is also lower than other compounds. However, the stigmasterol compound has a maximum recommended tolerated dose (MRTD) of -0.664 log mg/kg/day, which is a low dose.

4. Conclusion

In this in-silico approach, three compounds namely stigmasterol, gamma mangostin, and erycristagallin have been found as HCV antiviral candidates targeting the NS3 protein. These three selected compounds have a higher binding affinity than the native ligand. The binding energy of each compound is -9.23 Kcal/mol, -8.58 Kcal/mol, and -8.17 Kcal/mol. Based on the in-silico toxicity test, the

compounds gamma mangostin and erycristagallin were positive for the AMES test so they have the potential to be mutagenic, while the compounds stigmasterol were negative for the AMES test. The LD50 value of stigmasterol is also lower than other compounds. However, the stigmasterol compound has a low MRTD. Pharmacokinetic analysis of the three compounds has high absorption in the small intestine, namely absorption above 30%.

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