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Molecular Docking: Bioactive Compounds in Indramayu Mango (*Mangifera indica* L.) Peel Waste as NS5B Hepatitis C Virus (HCV) Inhibitor

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Abstract

Background*: Hepatitis C is caused by hepatitis C virus (HCV) infection. HCV infection is one of the biggest causes of chronic liver disease. About 60-80% of patients with acute hepatitis C will develop chronic hepatitis C.* ***Objective****: This study aimed to analyze the potential of mango peel compounds as HCV NS5B inhibitors.* ***Methods****: The methods in this study are ligand preparation, physicochemical and pharmacokinetic predictions, protein structure preparation, molecular docking, data analysis, and visualization.* ***Results****: The results showed that the test ligands had binding free energies close to the reference ligands, namely Mangiferin -7.862 kcal/mol and respectively D-(+)-Maltose -6.453 kcal/mol, Dibutyl – phthalate -6.326 kcal/mol, bis-β-D-fructofuranose 1,2':2,3'-dianhydride -6.249 kcal/mol, 16-Heptadecyne-1,2,4-triol -5.476 kcal/mol, 3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid -5,360 kcal/mol, Trigonelline -4.905 kcal/mol, Hexitol -4.552 kcal/mol, α-Glucoheptitol -4.403 kcal/mol. All the test ligands bind the NS5B active site with hydrogen bonds. Furthermore, the ligand-receptor complex has a dissociation constant value and hydrogen bond length.* ***Conclusion****: The results showed that Mangiferin was the most potential ligand in inhibiting NS5B HCV of all the test ligands used.*

Keywords*: bioactive compound, hepatitis c virus, mango, molecular docking, NS5B*

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INTRODUCTION

Hepatitis C is caused by hepatitis C virus (HCV) infection. HCV infection is one of the biggest causes of chronic liver disease. About 60-80% of patients with acute hepatitis C will develop chronic hepatitis C when the HCV overcomes the body's innate and adaptive immune systems. Many people with chronic hepatitis C are unaware of their condition, as most have been asymptomatic for a long time (Rabaan et al., 2020). The HCV was detected using a serological enzyme immunoassay that measures anti-hepatitis C antibodies to indicate recent or past infection. Chronic hepatitis C is one of the main causes of liver disease worldwide, such as fibrosis, cirrhosis, and hepatocellular carcinoma (Castro et al., 2015). In 2019, an estimated 290,000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (WHO, 2021).

HCV is a single-stranded RNA virus in the family *Flaviviridae*. HCV has one open reading frame, which is translated to produce 3000 amino acids and cleaved to produce three structural proteins (core proteins, E1, and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Bukh, 2016). The HCV life cycle begins when the virion binds to a hepatocyte receptor, then gets internalized and releases *genomic* RNA into the cytoplasm. The HCV replication process is catalyzed by NS5B and several other proteins. NS3 assists the replication process by playing an important role in the RNA binding process, NS4B initiates the replication complex, and NS5A is important in regulating viral replication processes (Li & Lo, 2015).

NS5B is an *RNA-dependent RNA polymerase* (RdRp) protein that plays a role in replicating positive-strand genomic RNA of HCV. NS5B has a polymerase structure consisting of fingers, palms, and thumbs subdomains. The active site of this polymerase is located in the *palm* region (Sabariegos et al., 2021; Wei et al., 2016). NS5B can transcribe viral RNA, which functions for protein translation and the formation of progeny genomes. NS5B is considered an attractive drug target because mammalian cells do not have RdRp polymerase so that it can be selectively inhibited (Boyce et al., 2014). However, drugs to treat hepatitis are still relatively expensive, less effective, and cause various side effects (Shakya, 2019).

Nature provides various human needs, such as food, shelter, and medicinal compounds that can be used as alternative medicine. In addition to having fewer side effects than conventional drugs, plant compounds also have the potential to overcome these problems (Permata & Khoirunnisa, 2020; Zahra et al., 2022). Indonesia is a tropical country with a high number of mango plant commodities. The results of research by Luthfia et al. (2021) showed that there were several compounds isolated from mango peel, some of which were Citroflex A-4, D-(+)-Maltose, 16-Heptadecyne-1,2,4-triol, Mangiferin, 3,4,5-trihydroxycyclohex -1-ene-1-carboxylic acid, Hexitol, Trigonelline, bis-β-D-fructofuranose 1,2':2,3'-dianhydride, α-Glucoheptitol, and Dibuty phthalate. Mangiferin is a xanthone with antiviral, anticancer, antidiabetic, antiaging, antioxidative, and hepatoprotective activities (Imran et al., 2017). This study aimed to analyze the potential of mango peel compounds as HCV NS5B inhibitors.

MATERIALS AND METHODS

Materials

The materials used in this study were the NS5B structure of the Hepatitis C virus, which was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https: //www.rcsb.org/) with PDB ID code "3UPI". The test ligands were from bioactive compounds found in the Indramayu mango (*Mangifera indica* L.) peel waste (Table 1). All test ligands were downloaded from PubChem.

Tools

The equipment used in this study was a *hp* 250 G5 Notebook PC with Windows 10 Professional 64-bit operating system, Intel ® Core ™ i3-6006U processor specifications, and 4.00 GB RAM. The software used to conduct the research was YASARA structure (version 19.9.17) (Krieger & Vriend, 2014), Discovery Studio by Dassault Systems BIOVIA (Biovia, 2017) and PyMOL by Schrödinger.

Methods

Ligand preparation

The ligands structures were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) in 3D and saved in (.pdb) format. Then, the ligand file was prepared using YASARA for energy minimization (*Options > Chose experiment > Energy minimization*) (Luthfia et al., 2021; Venkatachalam & Ettrich, 2021; Gholam et al., 2022). Hydrogens atom were added to all ligands (Gholam, 2022; Venkatachalam & Ettrich, 2021). The test ligands used in this study were Hexitol, Citroflex A-4, D-(+)-Maltose, 16-Heptadecyne-1,2,4-triol, Mangiferin, 3,4,5-trihydroxy cyclo hex-1-ene-1-carboxylic acid, Hexitol, Trigonelline, bis-β-D-fructofuranose 1,2':2,3'-dianhydride, α-Glucoheptitol, Dibutyl phthalate (Table 1). The reference ligand used was Dasabuvir (Shakya, 2019).

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Physicochemical and pharmacokinetic prediction

Predictions of physicochemical and pharmacokinetics were performed on the reference and test ligands. The ligands structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in (.sdf) format. The Physicochemical predictions were carried out using the Lipinski rule of five on the SCFBio ITT Delhi website (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The Lipinski rule of five parameters consists of molecular weight, hydrogen donor and hydrogen acceptor, the logarithm of octanol/water partition coefficient (log P), and molar refractivity (Lipinski et al., 1997). The pharmacokinetics prediction was performed by submitting the reference ligand and the test ligand SMILES structure to the pkCSM Biosig Lab website (http://biosig.unimelb.edu.au/pkcsm/prediction). A good ADMET pharmacokinetics profile is characterized by a compound that can be absorbed in the intestine, can be metabolized and excreted completely, and is not toxic (Fida et al., 2021; Luthfia et al., 2021; Pires et al., 2015).

Protein structure preparation

The three-dimensional crystallographic structure of NS5B polymerase bound to the 4, 5-dihydrofurano ligand was downloaded from the protein data bank (PDB ID: 3UPI) (Velázquez et al., 2012). The resolution of the crystallographic structure is 2.00 Å. Only chain B is used in this study, so the unused chains and residues are deleted (Shakya, 2019). Preparation was done using the YASARA structure. The NS5B polymerase receptor is energy-optimized. The optimized structure has the minimum energy for bonding with the test ligand. The crystallographic ligand in chain B has not been removed to determine the grid box area in the active site. It is also necessary to remove water molecules and add hydrogen molecules (Luthfia et al., 2021; Shakya, 2019; Venkatachalam & Ettrich, 2021).

Molecular docking

Validation is required before docking to determine the appropriate grid box. Validation was carried out by redocking the crystallographic ligands on the NS5B receptor. Validation was done using dock_run.mcr. macro from YASARA with runs=25 and Amber14 forcefield (Ali et al., 2020; Gholam & Firdausy, 2022). The grid box used is a cube-shaped 3Å grid box (X = 16.81, Y = 16.81, Z = 16.81). The parameter used is RMSD, with a valid score of less than 2 Å (Ali et al., 2020; Faridah et al., 2019).

The docking was done using the same command file as the validation process; it uses YASARA's dock_run.mcr (Krieger & Vriend, 2014), macro with runs=25, and Amber14 forcefield. YASARA implements AutoDock Vina (Ali et al., 2020; Trott & Olson, 2010; Venkatachalam & Ettrich, 2021). Amber14 forcefield is required to be able to calculate the ligands. Each docking run was ranked based on the strongest binding free energy (kcal/mol). The final result of the docking process shows the approximate binding free energy, dissociation constant (Kd), contact amino acid residues, and the binding conformational coordinates. The docking results are selected based on the lowest binding free energy. The (.yob) file was used to view the position of each ligand and contact amino acid residues through two-dimensional or three-dimensional visualization after being converted into (.pdb) format. (Ali et al., 2020; Venkatachalam & Ettrich, 2021).

Data analysis and visualization

The design for displaying the data in this study follows the research of Shakya (2019) by considering the binding free energy, conventional hydrogen bonds, hydrogen bond distances, and number of hydrogen bonds and visualizing both 2D and 3D conformations. The receptor's complex conformations, ligand interactions, and contact amino acid residues can be seen using the BIOVIA Discovery Studio in two dimensions (2D) (Komarudin et al., 2021). The three-dimensional (3D) visualization was done using PyMOL software (Zaelani et al., 2021).

Table 1. Selected bioactive compounds from Indramayu mango peel waste (Luthfia et al., 2021)

CID	Compound
10222764	Citroflex A-4
439186	D-(+)-Maltose
3015189	16-Heptadecyne-1,2,4-triol
5281647	Mangiferin
1094	3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid
453	Hexitol
5570	Trigonelline
440332	bis-β-D-fructofuranose 1,2':2,3'-dianhydride
101748	α-Glucoheptitol
3026	Dibutyl phthalate

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Table 2. Lipinski prediction results

Compound	Parameter Lipinski rule of five					Application of Lipinski rule of five
	Molecular weight (<500 Da)	Hydrogen donor (<5)	Hydrogen acceptor (<10)	LogP (<5)	Molar refractivity (40-130)	
Dasabuvir (reference ligand)	493	1	5	3.141550	130.175186	Passed
Citroflex A-4	402	0	8	4.542399	107.939987	Passed
D-(+)-Maltose	342	8	11	0.779680	72.029884	Passed
16-Heptadecyne-1,2,4-triol	284	3	3	4.053739	92.266380	Passed
Mangiferin	422	7	11	1.100710	89.980591	Passed
3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid	174	4	5	0.250540	37.080692	Passed
Hexitol	182	6	6	0.326940	39.968788	Passed
Trigonelline	137	0	2	0.550850	32.389000	Passed
bis-β-D-fructofuranose 1,2':2,3'-dianhydride	324	6	10	1.014480	68.844788	Passed
α-Glucoheptitol	212	6	7	0.514430	46.122288	Passed
Dibutyl phthalate	278	0	4	3.439459	77.445992	Passed

RESULTS AND DISCUSSION

This study used bioactive compounds in Indramayu mango peel waste. Research by Luthfia et al. (2021) using LC-MS found several active compounds in Indramayu mango peel waste. Luthfia et al. (2021) also analysed the potential of the compounds that have the potential as an antiviral for SARS-CoV-2 with the target protein of *Angiotensin-converting enzyme* 2 (ACE2) *in silico*. The research proved that Mangiferin had the highest activity inhibiting ACE2. This study analysed the interaction of bioactive compounds in Indramayu mango peel waste as hepatitis C antiviral.

Physicochemical and pharmacokinetic prediction

Before molecular docking was performed, the pharmacokinetics and the physicochemical properties of the bioactive compounds in Indramayu mango peel waste were predicted. The physicochemical properties of the reference and test ligands were predicted using Lipinski rule of five. The parameters in Lipinski's rule of five were; molecular weight, number of hydrogen donors and acceptors, the partition coefficient value of octanol to water (log P), and molar refractivity. Compounds with good physicochemical properties had molecular weights <500 Da, number of hydrogen donors and acceptors <5 and <10, log P values <5, and molar refractivity value of 40-130 (Lipinski et al., 1997; *Lipinski Rule of Five*, n.d.). Compounds that did not violate a maximum of two parameters are considered a good drug candidates. The physicochemical predictions showed that the reference and test ligands passed the physicochemical predictions based on the Lipinski rule of five (Table 2).

The molecular weight of a compound plays a role in determining the permeability of the compound. Compounds with a molecular weight of less than 500 Da can easily penetrate cell membranes. The number of hydrogen donors and acceptors acts as a secondary determinant of fractional absorption because it affects the molecular interactions that will be formed with water molecules. The number of hydrogen acceptors and donors in a compound that is too high indicates poor permeability, which will affect the absorption and distribution process of the compound (Lipinski et al., 1997).

The log P value expresses a compound's lipophilic nature and solubility in the water. A good compound has low lipophilic properties, indicating that the compound is soluble in water. Meanwhile, molar refractivity determines the permeability of a compound. Molar refractivity values of 40-130 indicate that the body can absorb these compounds well (Lipinski et al., 1997; *Lipinski Rule of Five*, n.d.).

The test ligands' pharmacokinetic properties were predicted in absorption, distribution, metabolism, excretion, and toxicity (ADMET) using pkCSM Biosig Lab website (http://biosig.unimelb.edu.au/pkcsm/prediction). The absorption profile was determined by intestinal absorption and Caco2 permeability parameters. The steady-state volume of distribution (VDss) and the blood-brain barrier (BBB) parameters determined the distribution profile. The value of CYP2D6 inhibitors

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and CYP3A4 inhibitors determined a metabolic profile. Meanwhile, the excretion profile can be determined based on the total clearance parameter, and the hepatotoxicity parameter can specify the toxicity profile (Pires et al., 2015).

A compound is considered to have good absorption if its absorption value in the intestine is 70-100%. Meanwhile, Caco2 permeability shows the ability of the compound to permeate the intestinal epithelium, which acts as a model for selecting candidates for oral administration. Caco2 permeability values with Ppap logs > 0.90 x 10^{-6} cm/s are considered to have high permeability (Singh, 2016). VDss shows the value of a total dose of a drug distributed thoroughly with the same concentration in blood plasma. Compounds with a high volume of distribution have a log VDss value > 0.45 and low if the log VDss value is < -0.15. The BBB parameter states the ability of a compound to penetrate the brain barrier, help reduce side effects and toxicity, and increase drug efficacy. A good compound has a log BBB value of > 0.3, and a bad one if the log BBB value is < -1 (Fida et al., 2021; Pires et al., 2015).

Metabolic profile is chemically changing drugs to form metabolites (detoxification). The liver carries this detoxification process using the cytochrome P450 (CYP) isoenzyme, consisting of CYP2D6 and CYP3A4. CYP2D6 plays a role in detoxifying drugs and xenobiotic substances in the body, while CYP3A4 plays of up to 50% in drug detoxification and is present in the small intestine and kidneys. The interaction of CYP inhibitors with the substrate can increase plasma levels (bioavailability), causing increased substrate activity and unwanted side effects (Fida et al., 2021; Pires et al., 2015).

Table 3. Predicted results of ADMET on test ligands

Compound	Absorption		Distribution		Metabolism		Excretion	Toxicity
	Intestinal absorption (%)	Caco2 permeability (log Ppap in 10^{-6} cm/s)	VDss (log L/kg)	BBB (log BB)	CYP2D6 inhibitor	CYP3A4 inhibitor	Total clearance	Hepatotoxicity
Dasabuvir (reference ligand)	85.507	-0.1111	-0.957	-1.03	No	Yes	0.735	Yes
Citroflex A-4	65.533	1.01	-0.071	-1.34	No	No	2.059	No
D-(+)-Maltose	6.412	-0.12	0.203	-1.024	No	No	1.545	No
16-Heptadecyne-1,2,4-triol	93.219	1.564	-0.369	-0.056	No	No	1.976	No
Mangiferin	46.135	-0.926	1.364	-1.573	No	No	0.347	No
3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid	46.681	-0.23	-0.618	-0.683	No	No	0.688	No
Hexitol	25.401	-0.441	-0.325	-1.309	No	No	0.919	No
Trigonelline	96.44	1.124	-0.758	-0.234	No	No	0.378	No
bis-β-D-fructofuranose 1,2':2,3'-dianhydride	32.563	0.448	0.281	-0.905	No	No	1.29	No
α-Glucoheptitol	21.174	-0.121	-0.304	-1.451	No	No	0.976	No
Dibutyl phthalate	95.044	1.622	-0.007	-0.054	No	No	0.93	No

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Table 4. Crystallographic ligand redocking (validation)

Ligand	Binding free energy (kcal/mol)	RMSD (Å)	Contact amino acid residue
4,5-dihydrofurano	-11.26	0.00	PHE B:193, PRO B:197, ARG B:200, ASN B:316, ASP B:318, ASP B:319, CYS B:366, SER B:367, SER B:368, LEU B:384, GLY B:410, ASN B:411, MET B:414, TYR B:415, GLN B:446, ILE B:447, TYR B:448, GLY B:449, SER B:556

The total clearance parameter, a combination of hepatic and renal clearance determines the excretion profile. This relates to bioavailability and determines the dose to reach a steady state. It is also important to know the toxicity profile, one of which is hepatotoxicity. Hepatotoxicity is the primary manifestation of drug toxicity involving hepatocytes as an approach used in drug development (Fida et al., 2021; Pires et al., 2015). The pharmacokinetic prediction results showed that dasabuvir had a reasonably good pharmacokinetics profile but was hepatotoxic. Meanwhile, all tested ligands had a good pharmacokinetics and were not hepatotoxic (Table 3).

The physicochemical properties of the reference and test ligands were predicted using the Lipinski rule of five (Table 2). The physicochemical prediction results showed that the reference and test ligands met the Lipinski rule of five qualifications. The eleven ligands were followed by pharmacokinetics predictions of absorption, distribution, metabolism, excretion, and toxicity (ADMET). The pharmacokinetic prediction of dasabuvir (as a reference ligand) showed a relatively good pharmacokinetic profile but was hepatotoxic. Meanwhile, all tested ligands had a good pharmacokinetics and were not hepatotoxic (Table 3).

Molecular docking

The target enzyme is NS5B from the hepatitis C virus (HCV), which has a resolution of 2.00 Å (PDB ID: 3UPI) (Velázquez et al., 2012). Luthfia et al. (2021) explain that the quality of good protein structures ranges from 1.5 to 2.5 Å in molecular docking studies. NS5B is an RNA polymerase enzyme that plays an essential role in HCV replication (Shakya, 2019). The NS5B polymerase target receptor prepared using the YASARA structure has a structure composed of A and B chains. The A chain and water molecule were deleted. The B chain and the crystallographic ligand 4,5-dihydrofurano bound in the B chain are used to determine the grid box size. Ten test ligands from Indramayu mango peel waste and one reference ligand were prepared using the YASARA structure. The three-dimensional structure is downloaded from the PubChem page in (.sdf) file format. The ligand energy was minimized and saved in the form of (_ligand.sdf) to be docked to the NS5B HCV protein receptor.

Before docking, validation was carried out to find the grid box size, with an RMSD value <2 Å to limit the movement area of the test ligands. This study used a grid box size of 3 Å (X = 16.81, Y = 16.81, Z = 16.81). The macro play option from the YASARA structure is used for the docking, using the dock.run macro (Gholam & Firdausy, 2022) with settings of runs=25 and Amber14 force field. Validation was carried out by redocking the crystallographic ligand,5-dihydrofurano, into the NS5B protein receptor. The results of the grid box validation can be seen in Table 4. This validation stage is intended to measure the validity of a molecular docking method. The parameter used is Root Mean Square Deviation (RMSD). RMSD is a parameter that shows the difference between the crystallographic ligand poses before and after redocking (Damayanti et al., 2021). Based on the validation results (Table 4), the resulting RMSD has an RMSD score of 0.00 Å. This result can be considered valid because the redocking process is able to mimic the initial pose of the crystallographic ligand when it is downloaded from the PDB (Damayanti et al., 2021). A grid box with a size of 3 Å is chosen to perform the molecular docking of the test ligand against the target protein. The results of the validation can be seen in Table 4.

The eleven ligands were docked on a 3 Å grid box (X = 16.81, Y = 16.81, Z = 16.81). The eleven ligands include Dasabuvir as a reference ligand and bioactive compounds from Indramayu mango peel waste consisting of Citroflex A-4, D-(+)-Maltose, 16-Heptadecyne-1,2,4-triol, Mangiferin, 3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, Hexitol,

Trigonelline, bis-β-D-fructofuranose 1,2':2,3'-dianhydride, α-Glucoheptitol, Dibutyl phthalate as the test ligand. Eleven ligands were docked to inhibit the NS5B polymerase target receptor. Molecular docking was done using YASARA structure with play macro dock_run settings runs=25 and Amber14 force field (Ali et al., 2020; Gholam et al., 2022). The docking results using the YASARA structure are binding free energy (kcal/mol), the resultant file containing receptor-ligand complex and contact amino acid residues. The overall results that follow the parameters in the study by Shakya (2019) are presented in Table 5. In addition, this study also calculates the dissociation constant. The dissociation constant in YASARA structure docking results can be seen in Table 5, unchanged as in Srivastava et al. (2018) research. Visualization was carried out to show the various amino acid residues involved and three-dimensional visualization on the surface.

Molecular docking can be used to investigate the possibility of binding interactions between the test ligand and the protein at the binding site.(Srivastava et al., 2018). The results showed that amino acid residues that form hydrogen bonds in the ligand-receptor complex were ASN B:291, ASN B:316, ASP B:318, ASP B:319, CYS B:366, SER B:367, SER B:368, SER B:407, ASN B:411, TYR B:415, GLN B:446, TYR B:448, GLY B:449 (Table 5). The total number of these amino acid residues is thirteen. It is known that all thirteen amino acids that form hydrogen bonds occur at the binding site. This is known because Shakya (2019) has provided a series of amino acid residues that have the potential as binding sites on NS5B polymerase. This study's overall contact with amino acid residues also occurred in Chain B.

Table 5. Molecular docking results and interaction data

Compound	Binding free energy (kcal/mol)	Dissociation constant (pM)	Hydrogen bonds	Hydrogen bonds distance (Å)	Number of hydrogen bonds
Dasabuvir (reference ligand)	-9.175	188207.031	ASN B:291	1.90	5
			ASP B:318	1.60, 2.10, 2.50	
			GLN B:446	2.36	
Mangiferin	-7.862	1726146.25	ASP B:318	2.14	8
			ASP B:319	2.47, 2.98	
			CYS B:366	2.62, 4.69, 4.72	
			SER B:367	2.19	
			TYR B:415	1.99	
Hexitol	-4.552	460616768	SER B:407	2.10	5
			ASN B:411	2.39, 2.84	
			GLN B:446	1.92, 2.23	
3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid	-5.360	117779008	CYS B:366	2.49	2
			SER B:368	2.53	
Trigonelline	-4.905	253855936	ASN B:316	2.53	3
			CYS B:366	2.95, 4.97	
α-Glucoheptitol	-4.403	592322496	GLN B:446	2.58	1
D-(+)-Maltose	-6.453	18616082	CYS B:366	2.69, 2.99	5
			GLN B:446	2.19	
			TYR B:448	2.28	
			GLY B:449	2.71	
bis-β-D-fructofuranose 1,2':2,3'-dianhydride	-6.249	26267714	CYS B:366	2.35	6
			ASN B:411	2.66, 2.77, 3.01	
			TYR B:415	2.13	
			TYR B:448	2.35	
16-Heptadecyne-1,2,4-triol	-5.476	96836432	SER B: 407	2.16	3
			ASN B:411	2.48, 2.70	
Dibutyl phthalate	-6.326	23066438	TYR B:415	1.96	1

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This study showed that the reference ligand Dasabuvir (CID_56640146) had the lowest binding free energy than the test ligand. Dasabuvir has a score of -9.175 kcal/mol. Dasabuvir forms hydrogen bonds in the amino acids residue ASN B:291, ASP B:318, and GLN B:446 with five hydrogen bonds (Fig 1). However, a test ligand has a binding score close to the reference ligand, Mangiferin (CID_5281647). Mangiferin has a binding free energy score of -7.862 kcal/mol. Mangiferin forms hydrogen bonds in the amino acids residue ASP B:318, ASP B:319, CYS B:366, SER B:367, and TYR B:415 with a total of eight hydrogen bonds (Fig 10). Mangiferin is the test ligand with the lowest docking score, and it should be noted in this study that all test ligands had a docking score. Consecutively D-(+)-Maltose (CID_439186) with a -6.453 kcal/mol binding score. Hydrogen bonds are formed at the amino acid residues CYS B:366, GLN B:446, TYR B:448, and GLY B:449 with a total of five bonds (Fig 6). Dibutyl phthalate (CID_3026) has a binding score of -6.326 kcal/mol with contact amino acid residues that form one hydrogen bond at TYR B:415 (Fig 9). bis-β-D-fructofuranose 1,2':2,3'-dianhydride (CID_ 440332) has a score of -6.249 kcal/mol. The amino acid residues forming hydrogen bonds, are CYS B:366, ASN B:411, TYR B:415, TYR B:448, with six hydrogen bonds formed (Fig 7). 16-Heptadecyne-1,2,4-triol (CID_3015189) with a docking score of -5.476 kcal/mol and residues that form hydrogen bonds are SER B:407, ASN B:411 with a total of three hydrogen bonds (Fig 8). 3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid (CID_1094) has a score of -5.360 kcal/mol. Hydrogen bonds are formed at residues CYS B:366, SER B:368 with two hydrogen bonds (Fig 3). Trigonelline (CID_5570) has a -4.905 kcal/mol score with amino acid residues that form hydrogen bonds at ASN B:316, and CYS B:366 and have a total of three hydrogen bonds (Fig 4). Hexitol (CID_453) has a -4.552 kcal/mol score with amino acid residues formed at SER B:407, ASN B:411, and GLN B:446 and has a total of five hydrogen bonds (Fig 2). α-Glucoheptitol (CID_101748) had the lowest binding score of all ligands with a -4.403 kcal/mol score. Also, this ligand only formed one hydrogen bond at residue GLN B:446 (Fig 5). Mangiferin has been shown to have scores close to the reference ligand Dasabuvir, but Mangiferin has more hydrogen bonds than Dasabuvir, which is eight bonds. The findings of this study provide a potential test ligand candidate for Mangiferin in its role of inhibiting NS5B. All data from the test ligands and reference ligands from the docking results are presented in Table 5.

Shakya (2019) explains that the minimum binding free energy, the greater the bond affinity in molecular docking studies. In addition, one of the main concerns of in silico studies is the presence of hydrogen bonds because the presence of bonds can determine the binding strength of the receptor-ligand (Shakya, 2019). This study found that the average presence of hydrogen bonds was caused by the interacting -OH (hydroxyl) group, which is also known to help stabilize the receptor-ligand complex (Weni et al., 2020; Gholam et al., 2022). This study also showed that the more hydrogen bonds, the stronger the bonds formed between the receptor-ligands complex. This is following the research of Uzzaman et al. (2019), which explains that a strong hydrogen bond is a significant contributing factor in increasing the affinity of drugs with receptors. Some literature states that strong hydrogen bonds have a distance of less than 2.3 Å (Uzzaman et al., 2021).

Visualization was carried out to analyze the receptor-ligand complex that forms a bond at each amino acid residue on the receptor. Visualization was done using BIOVIA Discovery Studio to visualize in two dimensions and PyMOL in three dimensions (surface). Visualization of the receptor-ligand complex can be seen in Figures 1 to 10. Visualization on the surface shows magenta colour in the ligand area. This is because the magenta indicates the visualization of the binding site area.

CONCLUSION

The results showed that of all the test ligands used, Mangiferin was predicted as the most potential ligand in inhibiting NS5B HCV. Each test ligand has a molecular interaction in the form of hydrogen bonds that bind the active site of NS5B HCV. The physicochemical and pharmacokinetic predictions of the test ligands also showed that the ligands used were in good criteria according to the specified parameters. This research needs to be proven *in vitro* and *in vivo*.

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