ORIGINAL ARTICLE

The interaction of alpha-mangostin and its derivatives against main protease enzyme in COVID-19 using *in silico* methods

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ABSTRACT

More than 111 million people worldwide have been affected by the COVID-19 outbreak caused by SARS-CoV-2. The main therapeutic target of COVID-19 is main protease (Mpro). It plays a key role as an enzyme in the SARS-CoV-2 replication and transcription. In this case, the alpha-mangostin potentially has antiviral activity against Mpro by inhibiting this enzyme. Nevertheless, the alpha-mangostin has low solubility and a lack of information about alpha-mangostin activity against the SARS-CoV-2. The aim of this study is to describe the molecular interactions and identify the pharmacokinetics profile between alpha-mangostin and its derivatives. *in silico* study was conducted by pharmacokinetics and toxicity prediction, molecular docking simulation, and Lipinski's rule of five. FKS9 has a Gibbs free energy value of-10.5 kcal/mol with an inhibition constant of 36.45 μM and an interaction with amino acid His41 residue. Its human intestinal absorption and Caco-2 values were 95.13% and 47.71% while the plasma protein binding and blood-brain barrier values were 96.66% and 6.99%. FKS9 also has no mutagenic and carcinogenic potential. FKS9 as an alpha-mangostin derivative had the best interaction with the Mpro enzyme and its pharmacokinetic profiles was identified.

Key words: Alpha-mangostin, COVID-19, *in silico,* main protease

INTRODUCTION

According to the World Health Organization (WHO), more than 111 million confirmed COVID-19 cases have been reported in 216 countries with 2.46 million confirmed deaths.[1] This pandemic is a major and recurrent global public health concern. This outbreak is an emerging infection and rapidly spreading globally.[2] The COVID-19 was caused by the SARS-CoV-2, which is an RNA virus that can spread widely to cause respiratory diseases.[3]

Currently, the primary care for COVID-19 patients is symptomatic therapy. Based on the clinical trials conducted by WHO from around the world, hydroxychloroquine, lopinavir, remdesivir, and interferon have proven ineffective in COVID-19 treatment.[4] These drugs have a tendency to develop acute toxicity and show poor therapeutic results in overcoming COVID-19.[5] As a result, further research related to the discovery of the COVID-19 drug is required to find active compounds that effectively reduce the spread of COVID-19.

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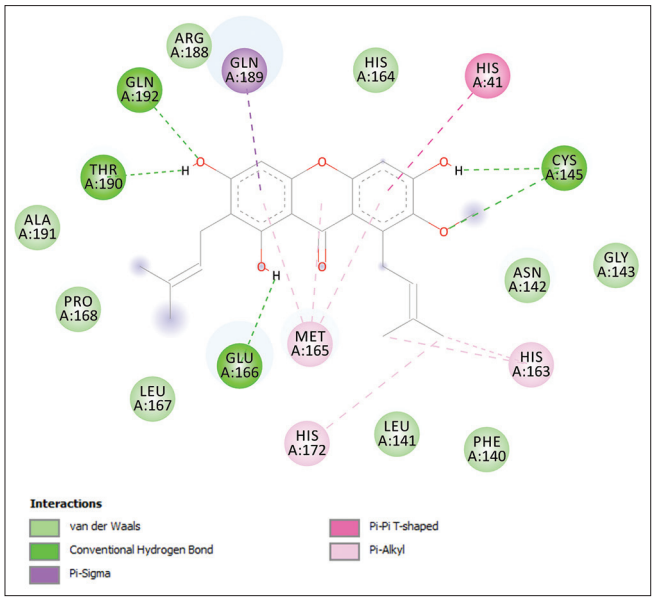


Figure 1: Interaction between alpha-mangostin and main protease

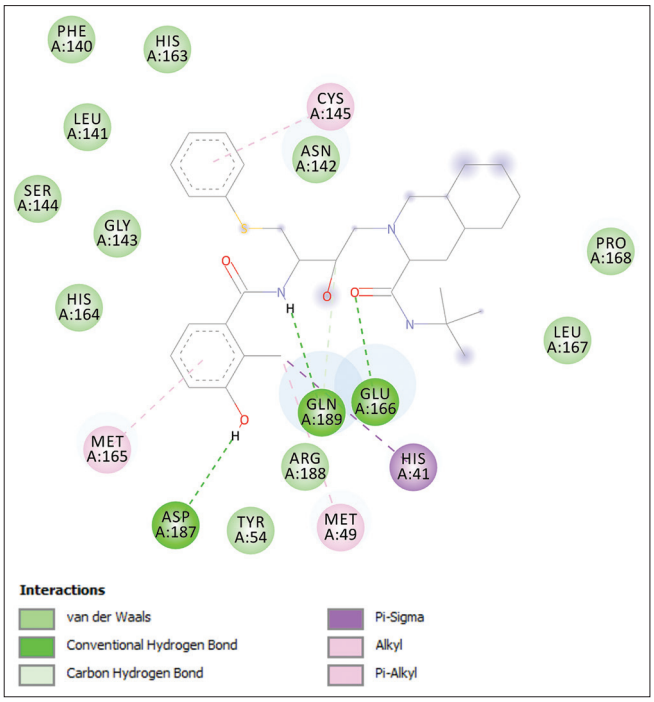


Figure 2: Interaction between nelfinavir and main protease

The search for active compounds through natural ingredients is accomplished in the search for parent compounds for COVID-19 therapy. Alpha-mangostin, originated from mangosteen pericarp has the potential to be an alternative agent for COVID-19 therapy. This is supported by previous studies which show that alpha-angostin has activity on the protease enzymes of the HIV.[6-9] HIV protease has a genomic similarity level of 67.5% compared to SARS-CoV-2's main protease (Mpro).[10] Therefore, alpha-mangostin is also thought to be able to have the same activity against the Mpro.

Mpro is referred to as an ideal drug target because of its specific presence that is only owned by viruses.[11] This specific existence is able to suppress the side effects that will be accepted by humans because the compounds will only affect the virus.

Behind these potentials, there are several limitations of alpha-mangostin, such as a low pharmacokinetic profile and a lack of information regarding alpha-mangostin activity against SARS-CoV-2.[12,13] Therefore, structural modification of alpha-mangostin is required to obtain alpha-mangostin derivatives with better pharmacokinetic profiles and pharmacological activities.

MATERIALS AND METHODS

Hardware and software

Hardware: A personal computer with Intel® Core ™ i5-6600 CPU, CPU 3.90 GHz, and 8 GB RAM. Software: The ChemOffice 2010 and ChemDraw Ultra 12.0 programs (PerkinElmer Inc., downloaded at http://www.cambridgesoft.com/) for drawing two-dimensional structures and expressing three-dimensional (3D) structures of the ligands. The AutoDock 4.2.6 and AutoDockTools 1.5.6 programs (The Scripps Research Institute, USA) to conduct molecular docking simulations. Pre-ADMET 2.0 program to predict absoprtion, distribution, and toxicity profile. The BIOVIA Discovery Studio 2017 R2 Client (Dassault Systems, downloaded from http://www.accelrys.com/) to visualize 3D structures.

Structure acquisition

The 3D structure of Mpro was downloaded from Protein Data Bank (PDB) (http://www.rscb.org/) with ID code 6 LU7. Mpro was complexed with N3 inhibitor molecule. Separation was performed using BIOVIA Discovery Studio 2017 R2 Client. The 3D structure of the ligands (alpha-mangostin and its derivatives) was drawn and optimized utilizing ChemOffice 2010 and ChemDraw Ultra 12.0 (PerkinElmer Inc.). Nelfinavir was chosen as comparison compound and the structure was downloaded from Pubchem (http://pubchem.ncbi.nlm.nih.gov/).

Pharmacokinetics and toxicity prediction

The pharmacokinetics (absorption and distribution) and toxicity prediction include human intestinal absorption (HIA), Caco2, plasma protein binding (PPB), blood–brain barrier (BBB), Mutagenicity, and Carcinogenicity.

Molecular docking simulation

Ligands and enzyme are prepared using AutodockTools 1.5.6. Polar hydrogen and Kollman charges are added to protein and saved as PDBQT. Gasteiger charges were calculated. The box size is set at 26 × 52 × 32 at the coordinate

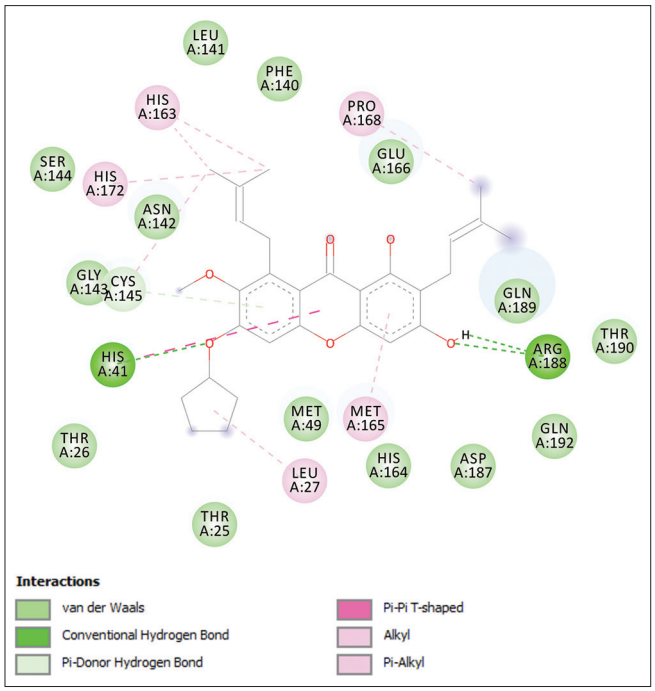


Figure 3: Interaction between FKS9 and main protease

x = −9,732; y = 11.403; and z = 68.925 with a distance of 0.375 Å. The genetic algorithm is set at 100x runs and other parameters are set by default. Autodock 4.2.6 is used to simulate the molecular docking process. The binding affinities of the compounds were studied using Discovery Studio Visualizer.

Lipinski's rule of five

According to Lipinski's rule of five (RO5), a reasonable compound for use as an orally active candidate must have no more than one violation of the following criteria: ≤5 hydrogen bond donors, ≤10 hydrogen bond acceptors, molecular weight ≤500, and logP ≤5.[14]

RESULTS

The alpha-mangostin structure modification was focused on C1 and C6 atoms which are more reactive than others to improve physicochemical properties, bioavailability, and pharmacological activity of alpha-mangostin. Alpha-mangostin modified compounds shown in the following [Table 1].

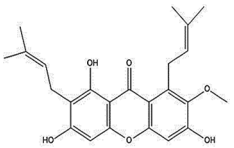
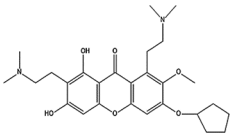
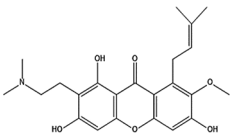
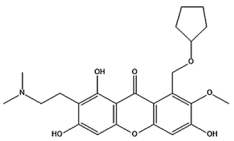
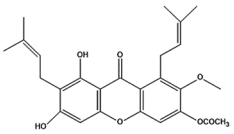
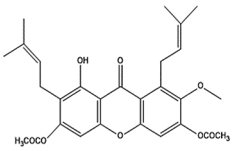
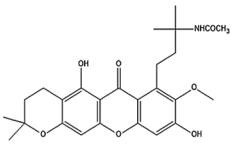
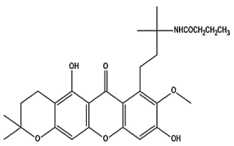
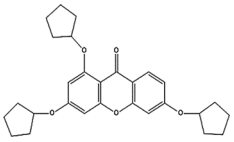
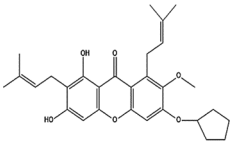
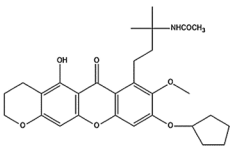
Pharmacokinetics and toxicity prediction

Table 2 shows the pharmacokinetic profile of these ligands. It shows the absorption profile of the compound represented by the HIA and Caco-2 values, while the distribution profile is shown by the PPB and BBB values, and the toxicity is represented by mutagenicity and carcinogenicity.

Molecular docking simulation

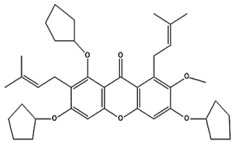
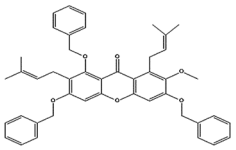
The molecular docking research control was used to validate the molecular docking parameters. The N3

Table 1: Alpha-mangostin and its derivative structures

Compound	Structure
Alpha-mangostin	
FKS1	
FKS2	
FKS3	
FKS4	
FKS5	
FKS6	
FKS7	
FKS8	
FKS9	
FKS10	

Contd...

Table 1: Contd...

Compound	Structure
FKS11	
FKS12	

compound from the 6 LU7 protein complex is reattached to the Mpro. The measured value is in the form of root mean square deviation (RMSD) which shows the deviation of the binding pose occurring in the test ligand compared to the reference binding pose. The lower the RMSD value, the better the model docked to the target structure.[15] The simulation results show that the RMSD value of molecular docking validation is 2.15 Å which indicates that the docking method used is qualified because the value obtained is ≤3 Å.[16] Molecular docking simulation of the alpha-mangostin derivatives that passed the pharmacokinetic profiles selection compared with nelfinavir are shown in [Table 3].

Lipinski's rule of five

The Lipinski's RO5 also considered regarding the active compound to be administered orally. This is due to the fact that 90% of the active compounds administered by the oral route have passed phase II clinical trials. The RO5 relates to the acceptance of solubility and permeability of compounds in the gastrointestinal tract and this is the initial stage in determining the oral bioavailability of active substances.[17] [Table 4] shows the Lipinski's RO5 parameters of alpha-mangostin and its derivatives.

DISCUSSION

The structural modification of alpha-mangostin is focused on modifying the substituted dihydroxy group on the aromatic ring (C1 and C6 atoms) to increase its affinity for the catalytic site, Cys145 and His41.[18]

Pharmacokinetics and toxicity prediction

The HIA value shows the degree of absorption of the active substance in the human intestine. There are three categories, namely HIA 0%–20% (low), 20%–70% (moderate), and 70%–100% (high).[19] The HIA value of alpha-mangostin and its derivatives is in the range of 90%–99% which indicates that it can be properly absorbed by the intestine.

In addition, Caco-2 cell modeling is recommended as a good *in vitro* model for predicting the absorption of orally administered active substances. The quality of absorption of Caco-2 cells was categorized into three groups, namely <4 (low), 4–70 (moderate), >70 (high).[20] The Caco-2 value of alpha-mangostin and its derivatives shows that the ability of these compounds to penetrate the cell membrane is in the medium category.

The degree of binding of the drug to plasma proteins affects the pharmacokinetic and pharmacodynamic properties of the drug. PPB values <90% indicate the drug is strongly bound to protein, whereas PPB values below 90% indicate the drug is weakly bound to plasma proteins so that the drug can be easily partitioned for distribution to cells. PPB values of alpha-mangostin, FKS4, FKS5, FKS7, FKS8, FKS9, FKS11, and FKS12 have PPB values above 90% which indicates that these compounds are strongly bound to plasma proteins so that only a small portion of the drug is in its free form which can then reach the Mpro. Meanwhile, the PPB of FKS1, FKS2, FKS6, FKS7, and FKS10 values were below 90% so that it could be partitioned much more easily in the blood and drugs would be much easier to distribute to cells.

The BBB value shows the drug concentration in the brain and blood. There are three categories, namely value >2.0 indicates that the compound has the ability to penetrate the central nervous system (CNS) so that it is thought to affect CNS activity. A BBB value between 2.0 and 1.0 indicates a moderate degree of absorption in the brain, and a BBB value below 1 indicates a low absorption in the brain.[21] The design of medicinal compounds for anti-COVID-19 is not targeted at the CNS, but at the Mpro enzyme, which is likely to be in the nasopharyngeal and lung cells. Therefore, drug penetration against the CNS needs to be avoided so that drugs do not have CNS side effects.[22] FKS1, FKS3, FKS5, FKS6, FKS7, and FKS10 have low penetration of SSP while other compounds are in moderate penetration. This shows that the alpha-mangistin and its derivatives are thought to have a mild CNS effect.

Meanwhile, the overall level of toxicity, nelfinavir, alpha-mangostin, and its derivatives have no potential for mutagens or carcinogens. However, FKS11 and FKS8 have the potential to be mutagenic or can cause mutation effects on the surrounding cells so that it is excluded from the anti-COVID-19 drug candidate.

Molecular docking simulation

The alpha-mangostin derivatives that passed the pharmacokinetics profile selection (FKS9) [Table 2] were subjected to molecular docking simulations. The results of molecular docking of the FKS9 compound [Table 4] were compared with nelfinavir, one of the COVID-19 drugs.[9]

There are four parameters considered to determine the affinity of the compounds, including Δ G, inhibition constant, hydrogen bond, and Van der Waals interactions. Based on Table 4, it can be seen that FKS9 (−10.15 kcal/mol)

Table 2: Pharmacokinetics and toxicity prediction of ligands

Compound	Absorption		Distribution		Toxicity	
	HIA (%)	Caco2	PPB	BBB	Mutagenecity	Carcinogenecity
Nelfinavir	93.91	48.32	82.89	4.04	Nonmutagenic	Negative
Alpha-mangostin	91.81	20.69	96.62	3.94	Nonmutagenic	Negative
FKS1	94.47	41.64	18.03	0.06	Nonmutagenic	Negative
FKS2	91.22	20.169	83.07	1.04	Nonmutagenic	Negative
FKS3	91.04	10.41	77.43	0.42	Nonmutagenic	Negative
FKS4	95.01	26.04	92.45	1.55	Nonmutagenic	Negative
FKS5	97.93	34.38	90.93	0.05	Nonmutagenic	Negative
FKS6	91.65	19.63	87.79	0.22	Nonmutagenic	Negative
FKS7	65.88	25.66	91.51	0.09	Nonmutagenic	Negative
FKS8	97.37	47.89	98.08	1.26	Mutagenic	Negative
FKS9	95.13	47.71	96.66	6.99	Nonmutagenic	Negative
FKS10	94.88	31.01	86.71	0.05	Nonmutagenic	Negative
FKS11	97.82	43.66	95.11	9.61	Mutagenic	Negative
FKS12	98.42	52.63	97.73	2.04	Nonmutagenic	Negative

HIA: Human intestinal absorption, PPB: Plasma protein binding, BBB: Blood-brain barrier

Table 3: Molecular docking parameters of nelfinavir, alpha-mangostin, and its derivative structure

Molecule	ΔG (kcal/mol)	Inhibition constant (μM)	Amino acids interaction	
			Hydrogen bond	Van der waals bond
Nelfinavir	−9.74	72.09	Gln189, Gly143, Asp187	Thr26, Asn142, His163, His172, Phe140, Leu141, Glu166, Arg188, Tyr154, Pro52, His164, Thr25
Alpha-mangostin	−8.58	511.49	Glu166, Thr190, Gln192, Cys145	His164, Asn142, Gly143, Phe140, Leu141, Leu167, Pro168, Ala191, Arg188
FKS9	−10.15	36.45	His41, Arg188	Gly143, Asn142, Ser144, Leu141, Phe140, Glu166, Gln189, Thr190, Gln192, Asp187, Met49, His164, Thr25, Thr26

Table 4: Physicochemical properties of alpha-mangostin and its derivative structures

Compound	Molecular weight	LogP	Hydrogen bond		Violations
			Donor	Acceptor	
Alpha-mangostin	418.530	3.71	3	6	Achieved
FKS9	478.585	6.04	2	6	Achieved

has a higher Δ G value than nelfinavir (−9.74 kcal/mol) and alpha-mangostin (−8.58 kcal/mol). This shows that the interactions that occur between FKS9 and Mpro are at a higher level compared to alpha-mangostin and also the comparison compound (nelfinavir). The more negative the ΔG value, the more stable the bonds are. As a result, the ligand-protein affinity is getting better which leads to better activity.[23]

The next parameter is the inhibition constant (Ki). The smaller the Ki, the smaller the doses required to demonstrate pharmacological abilities. The FKS9 has a Ki value of 36.45 μM. This was much smaller than that of nelfinavir (72.09 μM) and alpha-mangostin (511.49 μM).

The analysis of molecular docking results is also important to review the ability of the test ligand to interact with the ligand-binding domain (LBD) of the target protein. Cys145 and His41 are catalytic amino acid residues in LBD so that antagonistic compounds that are able to inhibit LBD by binding with these residues through hydrogen bond interactions cause virus replication to not occur.[18,24] In this case, The interactions of alpha-mangostin [Figure 1], nelfinavir [Figure 2], and FKS9 [Figure 3] were compared to see compounds with better interactions. FKS9 [Figure 3] is able to interact with Mpro catalytic residue, His41, via hydrogen bond, which indicates that FKS9 has a good potential as an Mpro antagonist in inhibiting SARS-CoV-2 replication compared to alpha-mangostin and nelfinavir.

Lipinski's rule of five

Based on Table 3, the FKS9 achieves the RO5 with only one violation, namely the logP value that exceeds the requirements (logP <5). Thus, FKS9 can be further investigated *in vitro* and it is predicted that it can be administered orally with a determinable oral bioavailability.

CONCLUSION

In conclusion, FKS9 as an alpha-mangostin derivative had

the best interaction with the Mpro enzyme as indicated by Δ G and Ki values of −10.5 kcal/mol and 36.45 μM and also an interaction with His41 residue. The pharmacokinetic profile of FKS9 has been known as shown by the HIA and Caco-2 values of 95.13% and 47.71%, these two values indicate that FKS9 can be well absorbed in the intestine and has the ability to penetrate the membrane. The PPB and BBB values of 96.66% and 6.99% indicating the distribution profile of FKS9 in terms of binding to plasma proteins and the ability to penetrate the BBB. FKS9 also has no mutagenic and carcinogenic potential.

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Conflicts of interest

There are no conflicts of interest.

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