Cathafiline from *Cassytha filiformis* and BR-Xanthone A from *Garcinia mangostana* as potential bone morphogenetic protein receptor type I (BMPR-I) inhibitor for iron-refractory iron deficiency anemia *in silico*

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Abstract. Oral iron supplementation has become a standard therapy for anemia around the world but some anemic patients are not responsive to it, as well known as Iron Refractory Iron Deficiency Anemia (IRIDA). One of the causes of IRIDA is BMPR-I activation. LDN-193189 is a synthetic small molecule that is developed for inhibition of BMPR-I. In the human liver, this receptor consists of 4 isoforms of Activin Like Kinase (ALK) 1, 2, 3, and 6. However, the synthetic molecule is not specifically bind to BMPR-I. This study aimed to identify Indonesian phytochemicals that can inhibit BMPR-I as IRIDA therapy with molecular docking approach. This bioinformatics study used 517 phytochemicals, which were registered in HerbalDB, had molecular structure and met the criteria for Lipinski’s rule of five. Three dimensional structure of LDN-193189 as a standard compound was found in complex with BMPR-I subtype ALK2 and obtained from Protein Data Bank (ID: 3Q4U). The AutoDock Vina 1.1.2. software was used to perform molecular docking between LDN-193189-ALK2 and phytochemicals-ALK-2. Binding complexes of ALK2 and LDN-193189/phytochemicals were visualized using PyMol 1.3 and Chimera 1.12 programs. The potential candidate of BMPR-I inhibitor was analysed based on docking score, binding site and conformation of phytochemicals toward ALK2. It revealed that BR-Xanthone A had lower docking score than LDN-193189 (-11.40 kcal/mol vs 11.30 kcal/mol). Phytochemicals which have hydrogen bonds to ATP binding site of ALK2 and similar conformation with LDN-193189 were Cathafiline and BR-Xanthone A. Both of those phytochemicals met the criteria of Lipinski’s rule of five. Cathafiline and BR-Xanthone A were potential as inhibitor BMPR-I for treatment of IRIDA.

1. Introduction

Iron deficiency anemia is an imbalance of iron intake, iron storage, and iron loss in the body that cause insufficiency of iron reserves to produce erythrocytes [1]. The prevalence of anemia in Indonesia is still relatively high at 28.1% in children aged 12-59 months and 37.1% in pregnant women. About 50% of those cases are iron deficiency anemia [2,3].

First-line therapy for iron deficiency anemia is oral iron supplementation. However, there are some patients who do not respond to oral administration. Level of serum hepcidin in unresponsive patients is higher than responsive patients of oral supplementation [4]. Low oral responsiveness can be caused by
chronic inflammation or polymorphism in genes involved in iron regulation such as Divalent Metal Transporter (DMT) -1 or TMPRSS6 (Transmembrane Protease, Serine 6) [5]. Polymorphism in TMPRSS6 gene causes hepcidin production increase, therefore iron export from lumen into the circulation decrease. This condition is called Iron Refractory Iron Deficiency Anemia (IRIDA), which patients will be unresponsive of oral iron therapy [6].

Other therapies targeting the hepcidin axis have been developed at this time [7]. Hepcidin is the main regulator of iron which can prevent the release of iron from the intestinal lumen into the circulation [8]. Hepcidin axis consist of BMP (Bone Morphogenetic Protein)/ SMAD (Sma and Mothers Against Decapentaplegic) pathway and IL-6 (Interleukin-6)/ STAT (Signal Transduction and Activator of Transcription) pathway. One molecular therapy that targets the hepcidin axis is LDN-193189, a small molecule inhibitor of BMPR-I (Bone Morphogenetic Protein Type I), specifically the ALK (Activin Like Kinase) 2 and ALK3 subtypes [7]. LDN-193189 works by ATP-mimetic method, which is to compete with ATP to occupy the ATP binding site of ALK2 [9,10]. ALK2 has some ATP binding site in the ATP binding pocket which are Val214, Gly215, Lys216, Gly217, Arg218, Tyr219, Gly220, Val222, Ala233, Lys235, Thr283, His284, His286, Ser290, Lys340, Asr34 Leu343, and Asp354 residue [11]. High concentration of LDN-193189 can inhibit other kinases that also require ATP in their activities therefore it has low specificity towards ALK2 and ALK3 [12]. Thus, LDN-193189 is not developed anymore. Even so, BMPR-I inhibitors are proven to be effective therapy for IRIDA, so alternative therapy other than LDN-193189 should be further developed [13].

Indonesia has 9,600 plant species that have pharmacological activities [14]. So far, no research has been conducted to identify phytochemical compounds of Indonesian herbal plants that have BMPR-I inhibitor activity to suppress the production of hepcidin in IRIDA. The method that is widely used in drug development today is the molecular docking method, which is a computational method for predicting the bond between ligands and macromolecules efficiently [14]. So this study aimed to identify Indonesian phytochemical compounds that have the potential as BMPR-I inhibitors of IRIDA by molecular docking approach.

2. Experimental
The samples of this bioinformatics study were obtained from the HerbalDB database and their three-dimensional structures were obtained from PubChem. The standard compound used in this research was LDN-193189. 3D structure LDN-193189 binding to ALK2 subtype of BMPR-I was obtained from Protein Data Bank with code: 3Q4U. AutoDock Vina 1.1.2 was used to analyze the binding energy of molecular docking between phytochemicals toward ALK2. The docking results was visualized using PyMol 1.3 and Chimera 1.12.

Before running the molecular docking, the structure of ALK2 was prepared first. ALK2 structure was separated from its ligand (LDN-193189) using AutoDock Tools version 1.5.6. Water molecules were then removed from ALK2 and polar hydrogen molecules were added. The initial visualization of ALK2 and LDN-193189 binding was performed using Chimera 1.12 to get the information of LDN-193189 binding site on ALK2 and to determine the grid box that would be used in docking process then. The established grid box was at X center = +22.65 size=22 Å, Y center = -19.05 size=30 Å, and Z center = +6.56 size=24 Å. Secondly, validation of LDN-193189 toward ALK2 was performed using AutoDock Vina 1.1.2. The validation aimed to get the standard molecular docking score between ALK2 and LDN-193189 for phytochemicals screening. The validation was performed at least three times to get valid score. Then phytochemicals were molecularly docked with ALK2 to get the binding energy scores. The analysis was performed by comparing docking scores, binding locations, binding types, and conformation of phytochemical toward ALK2.

3. Results and Discussion
Initial visualization was performed firstly to get the information of LDN-193189’s binding site on ALK2. The result of initial visualization was LDN-193189 interacted with ALK2 on several non-polar amino acids such as Val214, Val222, Ala233, Leu263, Tyr285, Leu343, and Ala353. In addition, there
were also interactions between LDN-193189 with several amino acids such as Lys235, His286, Thr283, Glu287, Gly289, Asp293, Lys340, and Asn341 to strengthen LDN-193189's position towards ALK2. There was a hydrogen bond between the N atom on LDN-193189 and the H atom on His286 with a distance of 2.212Å. LDN-193189 was also predicted having water-mediated hydrogen bond at Glu248. However, this study used Chimera 1.12 software to perform visualization therefore water-mediated hydrogen bond could not be visualized. Those interactions helped us to determine the grid box needed in docking process then.

Secondly, interaction of LDN-193189 and ALK2 was validated to get the standard binding energy score. The obtained binding energy score from the interaction between LDN-193189 with ALK2 is -11.30 kcal/mol (Table 1).

| Ligand  | Docking score (kcal/mol) | Docking score mean (kcal/mol) | Deviation standard |
|---------|--------------------------|-------------------------------|-------------------|
| LDN-193189 | -11.30 -11.30 -11.30 -11.30 -11.30 | -11.30 | 0 |

Validation results showed that LDN-193189 interaction toward ALK2 was found at Val214, Val222, Ala233, Lys235, Leu263, Thr283, Tyr285, His286, Glu287, Gly289, Lys340, Asn341, Leu343, and Ala353 as shown in figure 1. Water-mediated hydrogen bond could not be visualized because water molecules had been removed during the preparation. The absence of water-mediated hydrogen bond caused a slight shift in the position of LDN-193189 when compared to the prediction in initial visualization as shown in figure 2, so the hydrogen bond with His286 could not be seen. However, there were still interactions between LDN-193189 and His286, which was the interaction of N atom on His286 with H atom on LDN-193189 with a distance of 2.497Å and the interaction of N-N atoms with a distance of 3.49Å.

Figure 1. The binding of LDN-193189 toward ALK2. Visualization was using Chimera 1.12.

Figure 2. Visualization of the validation result (light blue) compared with the prediction (purple). Visualization was using Chimera 1.12.
Molecular docking was performed on 517 Indonesian phytochemicals in the HerbalDB database which had PubChem ID to identify their potential as BMPR-I inhibitor. Molecular docking was performed three times to get valid docking scores. It revealed that there was only one compound having docking score lower than the standard, which was BR-Xanthone A, with a docking score of -11.40 kcal/mol. It means that BR-Xanthone A binds ALK2 easier than the standard do. BR-Xanthone A had hydrogen bonds with Thr283 and Asp293 as shown in figure 3. In addition to hydrogen bonds, BR-Xanthone A also had hydrophobic interactions with non-polar amino acids such as Val214, Val222, Ala233, Leu263, Tyr285, Leu343, and Ala353. There were also interactions of BR-Xanthone A with other amino acids, such as His286, Glu287, Gly289, Asp293, and Lys340 (Table 2). BR-Xanthone A also had similar conformation with the standard as shown in figure 4 although it had different binding site of hydrogen bonds. Figure 5 shows all interactions of BR-Xanthone A toward ALK2.

Figure 3. The hydrogen bonds of BR-Xanthone A toward ALK2. Visualization was using PyMol 1.3.

Figure 4. Visualization of the BR-Xanthone A (light blue) compared with the standard (purple). Visualization was using Chimera 1.12.

Figure 5. Interactions of BR-Xanthone A toward ALK2. Visualization was using Chimera 1.12.
This study assessed the phytochemical’s potential not only by the docking score, but also the binding types, the number of ATP binding sites occupied, as well as the conformation or phytochemical position toward ALK2 compared with the standard. Therefore, this study also analysed seven other phytochemicals with the lowest docking scores to compare their potential with BR-Xanthone A and standard. Another phytochemical having higher potential among others was Cathafiline. It had docking score -10.60 kcal/mol and similar binding sites compared with the standard. It also had hydrogen bonds on ATP binding sites more than the standard had. Cathafiline had hydrogen bonds on Lys235, His284, and His286, hydrophobic interactions with Val214, Val222, Ala233, Leu263, Leu281, Tyr285, Leu343, and Ala353, and several other bonds (Table 2). All interactions of cathafiline toward ALK2 can be seen in figure 6, while its hydrogen bonds are shown in figure 7. Cathafiline also had similar conformation compared with LDN-1933189 as shown in figure 8.

![Figure 6. Interactions of Cathafiline toward ALK2. Visualization was using Chimera 1.12.](image)

![Figure 7. The hydrogen bonds of Cathafiline toward ALK2. Visualization was using PyMol 1.3.](image)

![Figure 8. Visualization of the Cathafiline (light blue) compared with the standard (purple). Visualization was using Chimera 1.12.](image)
Table 2. Interactions of phytochemicals toward ALK2 compared with LDN-193189a

| Ligand                  | Docking score (kcal/mol) | Hydrogen bond location | Other interactions                                      | ATP binding sites occupied | Conformation |
|-------------------------|--------------------------|------------------------|--------------------------------------------------------|----------------------------|--------------|
| LDN-193189              | -11.30                   | His286                 | Val214, Val222, Ala233, Lys235, Leu263, Thr283, Tyr285, His286, Glu287, Gly289, Lys340, Asn341, Leu343, Ala353, Asp354 | Val214, Val222, Ala233, Lys235, Thr283, His286, Lys340, Asn341, Leu343, Asp354 | -            |
| BR-Xanthone A           | -11.40                   | Thr283, Asp293         | Val214, Val222, Ala233, Leu263, Tyr285, Leu343, Ala353, His286, Glu287, Gly289, Asp293, Lys340 | Val214, Val222, Ala233, Leu343, His286, Lys340, Thr283 | Similar      |
| Cathafiline             | -10.60                   | Lys235, His284, His286 | Val214, Val222, Ala233, Lys235, Leu263, Leu281, Thr283, His284, Tyr285, His286, Gly289, Asn341, Leu343, Ala353, Asp354 | Val214, Val222, Ala233, Lys235, Thr283, His284, His286, Asn341, Leu343, Asp354 | Similar      |

a Bold residues were the same binding sites as standard’s

Lipinski’s rule of five is a theory used to determine the bioavailability of a drug compound. If a compound meets these criteria, the compound can be developed as a drug, because it will be well absorbed by the body. There are four things that must be fulfilled by a compound to be absorbed by the body, which are: the donor hydrogen bond < 5, the acceptor hydrogen bond < 10, molecular weight < 500 Daltons, and the logarithmic coefficient of the octanol-water ratio in the compound (logP) < 5 [15]. BR-Xanthone A and Cathafiline have met the criteria of Lipinski. BR-Xanthone A has a molecular weight of 396,439 Da, lower than LDN-193189, indicating that the absorption rate is better than LDN-193189. Cathafiline has a molecular weight of 369,373 Da, lower than the standard. It also has lipophilicity (LogP) less than the standard, therefore Cathafiline is more hydrophilic and has lower toxicity (Table 3).

Table 3. Lipinski’s criteria of LDN-193189 and phytochemicals

| Active compound    | Molecular Formula | Molecular weight (<500) (Da) | H-Bond Donor (<5) | H-Bond Acceptor (<10) | Lipophilicity (<5) |
|--------------------|-------------------|-----------------------------|-------------------|-----------------------|--------------------|
| LDN-193189         | C_{25}H_{22}N_{6} | 406,493                     | 1                 | 5                     | 3,3                |
| BR-Xanthone A      | C_{24}H_{24}O_{6} | 396,439                     | 2                 | 6                     | 4,8                |
| Cathafiline        | C_{20}H_{19}NO_{6} | 369,373                     | 1                 | 6                     | 2,8                |
BR-Xanthones A is a compound found in the skin of *Garcinia mangostana* (mangosteen) [16]. This compound belongs to the family of xanthonoid type, pyranoxanthone class. This compound is considered to have antioxidant activity [17]. Cathafiline is a compound of *Cassytha filiformis* [18]. This plant has been used as a therapy for gonorrhea, cancer, kidney disease, as a diuretic, and other diseases. Parts of plants used to obtain pharmacological activities are leaves. This plant has pharmacological activity as an antioxidant, antitrypanosomal, antplatelet, and vasorelaxant [19].

We realized that this study has some limitations. AutoDock Vina used in this study needs removal of water molecule when doing the receptor preparation. Therefore, water-mediated hydrogen bonds cannot be performed. In addition, Chimera 1.12 dan PyMol 1.3 used in this study cannot identify other bonds than hydrogen bonds. This study is just a prior study, therefore next development study is needed.

4. Conclusion
Cathafiline and BR-Xanthone A were potential as BMPR-I inhibitor for treatment of IRIDA *in silico* with the docking score of -10.60 kcal/mol and -11.40 kcal/mol respectively. Both compound had higher amount of hydrogen bonds than the standard and similar conformation as the standard. Those compound can be found in *Cassytha filiformis* and *Garcinia mangostana* respectively.

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