A molecular docking study of dehydroevodiamine as an inhibitor of epstein-barr virus protease

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Abstract. Epstein-Barr Virus (EBV) is a type of γ-herpes virus which cause kissing disease. The virus induces cancer and causes latent infection. EBV protease is one of the constituent capsid proteins that play an important role in assembling virions on nucleus and spreading them. Therefore, this enzyme potentially became one of inhibition target which have impact on the termination EBV life cycle. During this time, drugs to inhibit this enzyme had not been studied. This study aimed to examine dehydroevodiamine as a potential inhibitor EBV protease by molecular docking method. The docking was done through both blind and specific docking techniques and the Kᵢ values were calculated using docking approach when RMSD is 0 Å. Molecule visualization was done using PyMol and dehydroevodiamine profile identification was done on Ro5. The results showed that dehydroevodiamine has binding affinity of $-9.8$ kcal/mol and $-7.3$ kcal/mol; predicted Kᵢ (STP) of $1.426729\times10^{-8}$ and $1.431479\times10^{-6}$ for blind and specific docking, respectively. Dehydroevodiamine profiles does not violate Ro5. These values indicated the potential of dehydroevodiamine as an oral drug candidate for kissing disease. This finding opens possibility to do further work on wet-lab-levels.

1. Introduction

Technology bring many benefits in science. One of them is supporting drug design process, especially in drug discovery step. Drug design is very important because many disease vectors exist around human. The purpose of drug design is to find chemical compounds that can enter certain cavities on target proteins both geometrically and chemically [1]. Drug design process using computer is also known as Computer-Aided Drug Design (CADD). In CADD, there are terms Ligand-Based Drug Design (LBDD) and Structure-Based Drug Design (SBDD). LBDD is a process designing drugs based on knowledge of molecules bound by biological targets, while SBDD is based on knowledge of 3D structure from biological targets.

In drug design, drug discovery step is often done using wet lab approach. However, this approach is not economic and effective. That approach needs to involve trials and errors. The estimated total incurred and capitalized R&D costs per new drug to be $1395 million and $2558 million, respectively in 2013. Furthermore, the increased out-of-pocket cost per approved drug to $1861 million and capitalized cost to $2870 million [2]. On the other hand, drug design is multidisciplinary and time consuming. Therefore, in silico approach can be considered to find potential medicines for diseases in drug discovery step. The method used for drug discovery is molecular docking. Molecular docking has become an increasingly important tool for drug discovery [3]. Molecular docking is a computational method for placing ligands which are often small molecules into receptor binding sites.[4] Molecular
docking aims to predict position, orientation, and conformation of ligand to the binding site of target macromolecule.[5] In general, molecular docking underlies the discovery position that best suits with pattern of flexible ligands and proteins as rigid receptors. Molecular docking method is used to find compounds which have potentially became inhibitor for receptor, especially receptor that initiate diseases. One disease which does not have clear treatment is disease caused by Epstein-Barr Virus (EBV).

EBV was first observed at Burkitt Lymphoma (BL) cells in 1964. EBV was the first human tumour virus to be discovered.[6] EBV is present in all populations and infects over 90% of individuals within the first 10 years of life.[7] EBV vaccine to prevent primary infection or disease, or therapeutic vaccines to treat EBV malignancies have not been licensed.[8] EBV is dangerous because it is latent and can induce other diseases, especially cancer. Primary EBV infection occurs through saliva contact with patients EBV. Therefore, disease caused by EBV is commonly referred to as kissing disease. EBV can also spread due to sexual contact because it can be found in genital fluid secretions. EBV is associated with infectious mononucleosis (IM) disease. The IM is characterized by fever, sore throat, cervical and general lymphadenopathy, hepatosplenomegaly, somatic complaints of fatigue and malaise.[9] Many neurologic manifestations of EBV infection have been documented, including encephalitis, aseptic meningitis, transverse myelitis, and Guillain-Barré syndrome.[10] Other diseases that may arise due to EBV include epithelial malignancies such as gastric carcinoma, mesenchymal malignancies such as follicular dendritic cell sarcoma and lymphoid malignancies such as pyothorax-associated lymphoma, Burkitt lymphoma, Hodgkin lymphoma and non-Hodgkin lymphoma.

Diseases often arise due to disorders of biomolecules, such as DNA and protein. EBV capsid proteins which form protease enzyme play an important role in the process of assembling virions at nucleus and spreading them from one cell to another. This enzyme catalyzes division of assembled proteins after formation procapsids. These cleavage sites are found in the bonds of -Ala-Ser-, -Ala-Ala- and Ala-Thr. Based on structure, EBV protease has an active site which shaped a very shallow pocket located on top of strands β5 and β6 of the middle β-barrel. The 3D structure of EBV protease was obtained from experiment using X-ray crystallography method with a resolution of 2.3 Å after that enzyme was inhibited with diisopropyl-fluorophosphate (DFP).[11] This enzyme has potentially became a biological target to be inhibited. EBV protease inhibition aims to fully inhibit enzyme work, thereby impacting in the termination of EBV life cycle.

Drugs as inhibitors most often are small molecules and produce therapeutic benefits for patients. EBV protease does not have a standard inhibitor as a drug. Kissing disease had been treated with drugs that did not have selectivity and specific toxicity to EBV. Therefore, this study tries to examine the ability of dehydroevodiamine as a potential inhibitor of EBV protease which is able to work with selectivity and specific toxicity. Dehydroevodiamine was chosen because it belongs to the type of alkaloid derivative. Alkaloids are often used as samples of ligands because they have good biological activity. Alkaloids had been the subject of many research related to their biological activities, such as anti-tumour[12], antiinvasive and expectorant[13], anti-alzheimer and antioxidant[14], analgesic activity[15] and antiviral[16]. This compound also has N atoms which is expected to donate electrons to the active site enzyme. The reason underlies that dehydroevodiamine has potentially became used as an inhibitor EBV protease, all at once as a drug for kissing disease. The purpose of this study was to examine dehydroevodiamine as an inhibitor of EBV protease with molecular docking method and its possibility to be implemented as an oral kissing disease drug. This study also will open possibility to do further work on wet-lab-levels.

2. Experiment

2.1. Research object

The research objects are 3D structure EBV protease and dehydroevodiamine. The device used are hardware consisting of a set computer with specifications: Processor Intel(R) Core(TM)2 Quad CPU
Q8300 @2.50GHz (4 CPU), 4GB RAM and 500GB hard disk and software consisting of Windows 7 64-bit Ultimate (6.1, Build 7601), Edu PyMOL v.1.7.4.5., PyRx 0.9.5 and LIGPLOT+. 

2.2. Procedure

2.2.1. Molecular docking and visualization result of molecular docking

The structure of dehydroevodiamine ligand is downloaded at https://pubchem.ncbi.nlm.nih.gov in 3D with (*.sdf) format. The structure is minimized using Open Babel which is integrated in PyRx 0.9.5 software. Insert new item is selected and dehydroevodiamine ligand is opened. Minimization is carried out by right clicking and choosing to minimize selected. Ligand is changed to pdbqt format, then save is selected.

The 3D structure of EBV protease is downloaded at https://www.rcsb.org in (*.pdb) format. This enzyme is opened in the Edu PyMOL software. In the names panel, letter "A" (action) selected, then the polar hydrogen atom is added by selecting hydrogens and clicking add. Enzyme sterilization is carried out by removing ligands and water. The procedure is done by selecting show sequence "S". Ligand and water sequences are selected, then right clicked and selected remove. Sterile enzyme are stored by clicking save molecule.

Molecular docking process between EBV protease and dehydroevodiamine is carried out using Vina Wizard program integrated in PyRx 0.9.5 software. The start button is clicked, add ligand and add macromolecule panels will be appeared. Ligand preparations and enzyme sterile are added. The molecular docking process is continued by clicking on forward. The binding site is determined through grid box position. In blind docking technique, the grid box used is Center X: 5,934, Center Y: 9,336, Center Z: 28,411 and Dimensions (Å) X: 67,785, Dimensions (Å) Y: 57,655, Dimensions (Å) Z: 94,953. In specific docking technique, grid box is adjusted to position catalytic triad at the active site enzyme (His48-Ser116-His139). These residues of triad catalytic are chosen with selected molecules menu in the navigator. The "+" button next to 1O6E sterile enzyme are clicked. The toggle selection spheres is also clicked, then catalytic triad is selected. Full screen is selected, and grid box is organized to limit catalytic triad area. Forward is chosen to continue molecular docking process. The results of molecular docking are enzyme-ligand complex, binding affinity value enzyme-ligand and Root Means Square Deviation (RMSD).

The binding affinity value has a relationship with Inhibition Constant (Ki). Ki values are predicted under Standard Temperature and Pressure (STP) conditions. The calculation of predicted Ki is at Equation 1.

\[
\text{Equilibrium} \quad : E + I \leftrightarrow EI \\
\text{Binding} \quad : E + I \rightarrow EI \left( K_b \right) \\
\text{Dissociation} \quad : EI \rightarrow E + I \left( K_d \right)
\]

Therefore,

\[
K_b = \frac{1}{K_d} \\
\ln K_b = - \ln K_d
\]

Ki = dissociation constant enzyme-inhibitor complex (−Kd)

\[
\Delta G_{\text{inhibition}} = \Delta G \\
\ln K_i = \frac{\Delta G}{RT} \\
K_i = e^{\frac{\Delta G}{RT}}
\]

Visualization of enzyme-ligand complex is carried out using Edu PyMOL (3D) and LIGPLOT+ (2D) software. In Edu PyMOL software, the results of docking and sterile enzymes are opened in PDB.
file format at the same time. In names panel section, letter "A" is clicked, then preset-ligand site is selected. The desired form of visualization is clicked. Visualization with LIGPLOT+ software is carried out by selecting menu file and open PDB file. Complex is entered by clicking browse. In the select ligand to plot section, ligands in the complex are selected.

2.2.2. Rule of five (Ro5)
Lipinski rule also known as Rule of Five (Ro5) consists of (1) molecular mass <500 Dalton, (2) lipophilicity level (log P) <5, (3) molar refraction must be in the range of 40-130, (4) hydrogen bond donors <5 and (5) hydrogen bond acceptors <10. Analysis Profile analysis of drug candidates with Ro5 is performed using SCF Bio IITD webservice. File in PDB format is uploaded and submitted.

3. Results and Discussion

3.1. Molecular docking and visualization result of molecular docking
EBV protease has a 3D structure composed of A chain and B chain. These chains are presented in Table 1. These chains form the structure of EBV protease which can be observed in Figure 1. The active site of this enzyme is composed of catalytic triad His48-Ser116-His139, so this enzyme can be categorized as serine protease. Histidine has an imidazole group, making it possible for proton transfer process. Serine can act as a covalent binding on the acyl group. ISP (propan-2-yl dihydrogen phosphate) ligand has been co-crystallized with EBV protease and it bound at its active site suggesting that ISP ligand acts as an inhibitor. ISP is a general inhibitor for serine protease. The enzyme binds ISP ligand at its active site through A chain and B chain. The distance between ISP binding to EBV protease in A chain and B chain can be observed in Figure 2(A) and 2(B).

| Table 1. A chain and B chain sequences of EBV protease |
|---------------------------------------------------------|
| **A Chain**                                             |
| MVQAPSVYVCGFVERPDAPPKDACLHLDPLTVKSQPLKKPLPLTVEHPGVPVGSFGLYQSSAGLFSASITSGDFLSLLDSIYHDCAIAQRSLPREPKVEALHAWLPSLSSLHPDIPQTTADGGKLSFFDHVSICALGRRGTTAVYGTDLA WVLKHSDFLEPSIAAQIENDANAAKRESGCPEHDPLTQLKIAIDAGFLNRTVRQDRG VANIPAESYLKA |
| **B Chain**                                             |
| MVQAPSVYVCGFVERPDAPPKDACLHLDPLTVKSQPLKKPLPLTVEHPGVPVGSFGLYQSSAGLFSASITSGDFLSLLDSIYHDCAIAQRSLPREPKVEALHAWLPSLSSLHPDIPQTTADGGKLSFFDHVSICALGRRGTTAVYGTDLA WVLKHSDFLEPSIAAQIENDANAAKRESGCPEHDPLTQLKIAIDAGFLNRTVRQDRG VANIPAESYLKA |

![Figure 1. Visualization of EBV protease structure using Edu PyMOL (A) cartoon and (B) surface](image-url)
ISP ligand binds to the enzyme using noncovalent and covalent interactions. Covalent interaction indicates that ISP inhibits protease EBV irreversibly. ISP has corrosive properties to metal and tissue. Thus, ISP might not be used as inhibitors which could be consumed by human. When ISP enters the body, enzymes that contain serine in its active site will become permanently inactive. This is very dangerous. One of the enzymes in the body that contain serine in the active site is acetylcholinesterase. Acetylcholinesterase works on the nervous system in human body. Paralysis in some organs can occur if this enzyme is inhibited. Therefore, ISP in this study was substituted using dehydroevodiamine. Dehydroevodiamine is classified as a secondary metabolite compound alkaloid derivative. Dehydroevodiamine have heterocyclic nitrogen atoms which are expected to donate electrons to bind in the active site of enzyme and contribute to the formation of hydrogen bonds. Many hydrogen bonds will make the binding affinity value more negative.

![Figure 2. The position of ISP binding in protease EBV at (A) A chain and (B) B chain](image)

Binding of drugs at active site enzyme will produce a competitive inhibition mechanism, whereas binding which does not occur at the active site enzyme will produce a noncompetitive inhibition mechanism. Implementation of blind docking technique will generally obtain drug candidates that work with noncompetitive inhibition mechanisms. This is due to the active site enzyme located on the inside, so it takes a much higher energy to arrive at that place. This technique will bind ligand in any binding site of enzyme that produces the most negative binding affinity value. Conversely, implementation of specific docking technique will obtain drug candidates which work with competitive inhibition mechanism.

Based on calculation by PyRx 0.9.5 software, each ligand has binding affinity value varies in the number of certain modes. The difference in these modes lies in the position, orientation and conformation when ligand is bound to enzyme. The scoring taken is the most negative of the calculated modes. A negative scoring value refers to an increasingly stable interaction between enzyme and ligand. Another parameter that can be observed is RMSD. The RMSD value is calculated relatively in terms of the best mode by only utilizing movable heavy atoms. RMSD affects the orientation of ligand position when interacting with target enzyme. The prioritized RMSD from these results of molecular docking is valued at 0 Å which means identical. RMSD value less than 2 Å means good.

The results blind docking and specific docking of EBV protease and dehydroevodiamine, respectively, obtained binding affinity values were -9.8 kcal/mol and -7.3 kcal/mol, when RMSD value 0 Å. Binding affinity values are obtained in kcal/mol. This value indicates total bonding energy from molecular docking process, so it can be referred as Gibbs free energy ($\Delta G_{\text{binding}}$). $\Delta G_{\text{binding}}$ expresses the spontaneity of chemical reactions.\[17\] $\Delta G_{\text{binding}}$ is a thermodynamic benchmark that
shows stability of ligand conformation when bound to a biological target. The ligand conformation to bind with receptor will be more stable if binding affinity value is getting negative. \( \Delta G_{\text{binding}} \) has a relationship with \( K_i \). In this study, the value of binding affinity is converted to \( K_i \). \( K_i \) values are prediction for reference when laboratory experiments are performed. The predicted \( K_i \) values from calculation based on blind docking and specific docking techniques are \( 1.42679 \times 10^{-8} \) and \( 1.431479 \times 10^{-6} \), respectively. The smaller binding affinity value, the smaller predicted \( K_i \) value. Binding affinity and predicted \( K_i \) values show if dehydroevodiamine has low energy when bind with EBV protease. It means that dehydroevodiamine can bind to biological target easily. Thus, dehydroevodiamine was proposed as drug candidate for kissing disease.

Visualization blind docking with Edu PyMOL software showed residues EBV protease that interact directly with dehydroevodiamine to form hydrogen bonds are Gln35 and Lys40. The distance hydrogen bond between dehydroevodiamine-GLN35 is 3.2 Å and dehydroevodiamine-LYS40 is 2.3 Å. Visualization is strengthened using LIGPLOT+ software to see hydrophobic interactions. Based on this visualization result, a hydrogen bond is formed between dehydroevodiamine and Lys40 residue. This hydrogen bond distance is 3.07 Å. Some residues that carry out hydrophobic interactions with dehydroevodiamine are Phe136, Pro41, Phe137, Gln35, Leu38, Leu42, Leu60, Gly59, Val57 and Pro37. The results of blind docking visualization using Edu PyMOL software can be observed in Figure 3(A) and 3(B), while visualization with LIGPLOT+ software can be observed in Figure 3(C).

![Figure 3. Visualization blind docking dehydroevodiamine to EBV protease (A) cartoon (PyMOL), (B) surface (PyMOL) and (C) 2D diagram (LIGPLOT+)](image)

Visualization specific docking with Edu PyMOL software showed residue that interact directly with dehydroevodiamine is SER116 with hydrogen bond distance 2.6 Å. The dehydroevodiamine is...
precisely attached to the binding site ISP ligand. Visualization specific docking with Edu PyMOL software is strengthened using LIGPLOT software to see hydrophobic interactions. Based on the results, hydrogen bonds are formed between dehydroevodiamine with Ser116 residue. This hydrogen bond distance is 3.01 Å. Other residues that contribute in hydrophobic interactions for stability enzyme binding with ligand are Leu145, Glu47, Cys143, Gly146, Arg148, Leu25, Arg147 and His48. The results of specific docking visualization using Edu PyMOL software can be observed in Figure 4(A) and 4(B), while visualization with LIGPLOT software can be observed in Figure 4(C).

3.2. Ligand profile based on rule of five (Ro5)

Medicines taken orally will pass through the body with possibilities that can prevent it from reaching target. Factors which influence the body’s treatment of drugs, namely absorption, distribution, metabolism and excretion (ADME) are better known as pharmacokinetics. Pharmacokinetics of drugs need to be considered when designing a drug, so the drug can effectively reach target.

The Lipinski Rule also known as Rule of Five (Ro5) provides guidance in the process of finding a drug which is good for oral consumption. Ro5 is based on a distribution of calculated properties among several thousand drugs [18]. Compounds that have high molecular weight, are generally large. The greater size of compound will more difficult to penetrate cell membrane (lipid bilayer). Lipophilicity is also known as the logarithmic value of partition coefficient (P). Partition coefficient is ratio of solubility compounds in a water-fat system. The main factors which contributes partition of solutes in a two-phase system, in this case water-fat, are molecular volume and intermolecular interaction of solution-solute. Log P related with hydrophobic characteristic of compounds. The higher log P value indicates that the compound is increasingly hydrophobic. Compound used as drug candidate may not be too hydrophobic, because it will block the rate of movement to arrive at lipid bilayer. It will affect the distribution of drug in body in a long time. As a result, the selectivity of the bond between drug candidate and target enzyme is reduced. Log P value that is too negative makes it difficult for drug candidate to enter lipid bilayer. Molar refraction has a relationship which is directly proportional with polarisability. The higher molar refraction value make compound more polar and will be penetrated difficulty to the lipid bilayer. Conversely, the lower molar refraction value make compound more nonpolar and will be difficulty to reach lipid bilayer. The value of H-donor and H-acceptor supported by molecular weight play important role in determining permeability of drug to pass lipid bilayer. Lipinski rules states if molecules profile fall outside its limits of the rules, the molecule will be difficult to absorb orally. Lipinski rules refers to candidate drug's properties as a suitable or unsuitable molecule as an oral drug. In practice, minimum of two Lipinski Rules must be achieved. Violations exceeding two rules are predicted to have a high failure rate for drugs to reach the target receptor and this is a potential problem in the drug discovery process. In general, drugs that do not suit with Lipinski Rules are recommended to be made as intravenous injection types.

The dehydroevodiamine profile in this study was adjusted to Ro5, because it would be used as an oral drug candidate. Dehydroevodiamine profile test with Ro5 parameters was carried out by Supercomputing Facility for Bioinformatics & Computational Biology, IIT Delhi Webserver. Ro5 data for dehydroevodiamine are presented in Table 2. Based on Table 2, dehydroevodiamine can be used as a potential oral drug candidate for kissing disease, because its profile does not violate the Lipinski Rules.

| No. | Rule of 5       | Specification  | Dehydroevodiamine Profile |
|-----|-----------------|----------------|---------------------------|
| 1.  | Molecular weight| <500 Dalton    | 301 Dalton                |
| 2.  | Log P           | <5             | 2,167700                  |
| 3.  | H-bond donors   | <5             | 0                         |
| 4.  | H-bond acceptors| <10            | 4                         |
| 5.  | Molar refraction| (40-130)       | 89,9544750                |
4. Conclusion
Dehydroevodiamine compound has potential to be an EBV protease inhibitor with carried out hydrophobic interactions and hydrogen bonds. This compound is also potentially used as an oral kissing disease drug.

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