In silico design of fragment-based drug targeting host processing α-glucosidase I for dengue fever

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Abstract. Dengue is a major health problem in the tropical and sub-tropical regions. The development of antiviral that targeting dengue’s host enzyme can be more effective and efficient treatment than the viral enzyme. Host enzyme processing α-glucosidase I has an important role in the maturation process of dengue virus envelope glycoprotein. The inhibition of processing α-glucosidase I can become a promising target for dengue fever treatment. The antiviral approach using in silico fragment-based drug design can generate drug candidates with high binding affinity. In this research, 198,621 compounds were obtained from ZINC15 Biogenic Database. These compounds were screened to find the favorable fragments according to Rules of Three and pharmacological properties. The screening fragments were docked into the active site of processing α-glucosidase I. The potential fragment candidates from the molecular docking simulation were linked with castanospermine (CAST) to generate ligands with a better binding affinity. The Analysis of ligand - enzyme interaction showed ligands with code LRS 22, 28, and 47 have the better binding free energy than the standard ligand. Ligand LRS 28 (N-2-4-methyl-5-((1S,3S,6S,7R,8R,8aR)-1,6,7,8-tetrahydroxyoctahydroindolizin-3-yl) penty1 indolin-1-yl) propionamide) itself among the other ligands has the lowest binding free energy. Pharmacological properties prediction also showed the ligands LRS 22, 28, and 47 can be promising as the dengue fever drug candidates.

1. Introduction

Dengue fever is a viral disease in humans caused by dengue virus (DENV)[1]. DENV belongs to the Flaviviridae family and because of that has a close relationship with other humans lethal viral pathogens such as Yellow Fever and West Nile virus. This virus has five serotypes, that are DENV-1, DENV-2, DENV-3, DENV-4, and DENV-5 [2,3]. Dengue virus is commonly found in tropical areas, especially in the regions of Asia and Africa. But recently, dengue infection has been reported in the Caribbean area, South America, and Europe [4,5]. DENV infection has spread around the world so that it has become one of the major health problems worldwide.

Each year, there are at least 40 to 100 million people are infected by DENV and more than half of the world’s population at risk of infecting by this virus. Infections caused by the DENV can cause high fever and flu-like symptoms. These infections, in some people, may also develop into a more acute phase called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [1,6,7]. DHF and DSS are responsible for causing death in major dengue cases, around 2.5 % from 500,000 clinical
cases [6]. Therefore, an effective treatment is needed to repress the number of death caused by a dengue infection.

Antiviral studies based on virus factor have been widely reported. Thiadiazoles that has been modified by Li et al (2009) could use to inhibit envelope protein in the binding site of β-OG, Sousa et al (2015) reported that the compound agathisflavone and myricetin have noncompetitive inhibitor activity on NS2B-NS3 protease dengue virus serotype 2 and Tambunan et al (2015) through its research reported that 5-(3,4-dichlorophenyl)-N-[2-(p-tolyl) benzotriazol-5-yl]furan-2-carboxamide can inhibit n-octyl-β-d-glucoside (β-OG) binding pocket on envelope protein of DENV-2 [8–10]. However, antiviral compounds that target the virus proteins usually only specific to a particular virus and sometimes fail due to the emergence of some resistant viruses. Antiviral targeting host protein that involved in the life cycle of the virus can be used as a therapy for various types of virus and the treatment of resistant viruses. There are a number of cellular proteins in the host cell that have been known to cause effects as anti-dengue when inhibited. Endoplasmic reticulum (ER)-resident α-glucosidase is one of the host proteins that can be used as a potential target [11–13].

Enzyme α-glucosidase is an enzyme that essential in the maturation process of the nascent glycoprotein. It involves in N-linked glycan part trimming process whereas their role is cutting glucose part on N-linked glycans that attached to immature glycoproteins. Envelope protein from some viruses have been known as glycoprotein and there is N-linked glycan attached to it. Therefore, inhibiting α-glucosidase in N-linked glycan part trimming process could interrupt the maturation of nascent envelope virus and hence it also disturb the function of viral envelope [12,14].

The α-glucosidase protein consists of processing α-glucosidase I and α-glucosidase II. Inhibiting processing α-glucosidase I by castanospermine (CAST) may cause an antiviral effect in the dengue infection [14–16]. Antiviral development with fragment-based drug approach could generate potential drug candidate. Fragment-based drug method aroused a larger and complex lead compound with higher binding affinity by joining two or more fragment [17].

In this study, we designed α-glucosidase I inhibitor using in silico fragments-based drug approach. Molecular docking is used to study the binding interaction between inhibitors and processing α-glucosidase I [18]. We used MOE 2014.09 software to perform molecular docking [19]. The objective of this study was to generate a lead compound using the silico fragment-based drug which showed inhibition activity toward processing α-glucosidase I and has good pharmacological properties. We expected the outcome from this study could provide an insight into a novel antiviral treatment for dengue infection.

2. Methods

2.1 Preparation of Processing α-glucosidase I

In this study, we used the three-dimensional structure of processing α-glucosidase I protein with PDB ID: 4J5T [16] that available in Protein Data Bank (http://www.rcsb.org/pdb). The other molecule that attaches in the enzyme, such as water (H\textsubscript{2}O), were removed. LigX function in MOE 2014.09 was utilized to minimize and optimize the processing α-glucosidase I structure.

2.2 Fragment Preparation

Fragment database was downloaded from ZINC15 [20]. We used an abiogenic database that contains natural product and metabolite compound as fragment database. The rule of three (RO3) [21] was applied using Osiris Data Warrior software (http://www.openmolecules.org/datawarrior/) on the database to reduce the amount of the molecules and construct a database with the potential fragment. The CAST compound was drawn and convert to .mol type file structure using ACD/ChemSktech 12.01 (www.acdlabs.com). The energy minimized was applied to the fragment database and CAST using MOE 2014.09 and then the partial charge was also applied using the same software in an MMFF94x force field.
2.3 Molecular Docking of CAST and Fragment Database
Molecular docking simulation for the CAST and fragment database against processing α-glucosidase I was performed using MOE 214.09 software. We used Triangle Matcher as placements methods, London dG as scoring function and 30 times of placement retain as parameters. The other parameters were set according to MOE 2014.09 default parameters. The active site from Baker’s (2013) processing α-glucosidase I model was chosen as docking area [16].

2.4 Hits Selection and Fragment linking
Potential fragments and CAST were linked using linker database in MOE 2014.09 to generated new ligands. The Lipinski Rules of Five (RO5) was applied in linking process. These new ligands were optimized using MOE 2014.09 and then were docked with processing α-glucosidase I. We choose several ligands that have low binding energies and good ligand-enzyme interaction to be continued into the next phase.

2.5 Analysis of Ligand Interactions and Drug Scan
The docked ligands with low binding energies were analyzed their interaction with the active site of processing α-glucosidase I. In this study, we also used some software such as Osiris DataWarrior and T.E.S.T (Toxicity Estimation Software Tool) (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test) in drug scan to predicted the drug-likeness and the toxicity.

3. Result and Discussion

3.1 Preparation of processing α-glucosidase I
In this study, we used processing α-glucosidase I structure that has been reported by Barker and Rose (2013) with PDB ID: 4J5T in Protein Data Bank (PDB). This structure has similarity with processing α-glucosidase I structure in eukaryotes more than the other model structure of processing α-glucosidase I. They also reported that processing α-glucosidase I has catalytic residues that retained in Asp568 and Glu771 [16]. Therefore, the interaction of the ligand with one of this residue is important for a potential inhibitor candidates of processing α-glucosidase I. We used LigX in MOE 2014.09 to minimization and optimation to prepared the processing α-glucosidase I in Amber10:EHT force field. The minimization of processing α-glucosidase I structure was done when the RMS gradient of 0,05 kcal/mol/Å².

3.2 Creating and Preparation of the Fragment Libraries
The biogenic database was obtained from ZINC15 [20]. We used Osiris DataWarrior to screened and created the fragment libraries. The RO3 was applied to reduce the amount of the potential fragment. Moreover, The RO3 also helped to create libraries that only consist of fragment-like properties and simple molecules that easily to elaborated [21,22]. In general, RO3 would reduce the complexity of the molecule in fragment library in which have following criteria [17]:

- the compounds molecular weight are less than 300g/mol,
- have the number of hydrogen bond acceptor less than or equal with three,
- have less than or equal with three hydrogen bond donor, and
- have the number of cLogP 3

Furthermore, some rules are added in RO3 as well. These rules are:

- A number of rotatable bonds are less than or equal with three, and
- have 60 Å² surface area.

The RO3 screening results in 13.085 compounds from 198.621 compounds. Toxicity screening could also be used to avoiding reactive, unstable, or toxic fragments [21,23]. We used toxicity parameter such as mutagenic, tumorigenic, irritant and reproductive-effect compound as a parameter
in Osiris DataWarrior to screen and create fragment libraries. After the toxicity screening process was conducted, we obtained 1,541 compounds in our fragment libraries.

The additional factor that must be considered to screened and creating libraries of compounds is drug-likeness. Some compounds fail to develop as a drug due of poor drug-likeness and pharmacokinetic properties [24]. Drug-likeness score could be used as a parameter to design fragment libraries. We used the drug-likeness score of the CAST as the standard ligand for screening the fragment database. This screening process resulted in 52 compounds in fragment libraries.

Fragment-based drug design method involves molecules that have a molecular weight less than 300 g/mol. Molecules such CAST which has molecule weight 189.210 g/mol could be involved in this method. CAST itself has inhibition activity towards processing α-glucosidase I [17,21]. Fragment linking between CAST and another potential fragment could build a bigger molecule with wider interaction area in the processing α-glucosidase I active site. Both energies minimized and partial charge were applied to both CAST and fragment libraries using an MMFF94x force field. The RMS gradient of the minimization was performed until it reaches 0,001 kcal/mol/Å.

3.2 Molecular docking Analysis of CAST and Fragment Libraries
Molecular docking is a method to predict and determine the favorable interaction between ligand and the receptor (e.g. enzyme or protein) [25]. Gibbs free energy binding energy (ΔG) was obtained from the result of molecular docking that used to predict which ligand-enzyme conformation that favourable. In this study, the molecular docking was performed using MOE 2014.09 software. First, we docked CAST towards processing α-glucosidase I to obtain the ΔG and their binding interaction. This simulation resulted that CAST has a binding interaction with three residues (Glu771, Asp392, and Trp391, as shown in figure 1.). The Glu771 itself is one of the catalytic residues in processing α-glucosidase I. The docking simulation also showed the ΔG of CAST is -28.7224 kcal/mol with an inhibition constant (pKi) of 20.9238.

![Figure 1. Binding interaction between CAST and the active site of processing α-glucosidase I protein.](image)

Fragment libraries are used in second docking. There are 52 fragment molecules that docked towards processing α-glucosidase I. The potential fragment that chose have the small ΔG and have
binding interaction around CAST’s binding interaction [17,26]. The molecular docking simulation from 52 fragment result N-(2-methylindolin-1-yl)propionamide fragment is the primary candidate to be linked with CAST. This fragment has -26.271 kcal/mol with pKi 19.138 and distance 3.65 Å to CAST. Furthermore, the N-(2-methylindolin-1-yl)propionamide has interaction with Glu429 residue in the active site of processing α-glucosidase I. Interaction of processing α-glucosidase I with N-(2-methylindolin-1-yl) propionamide is showed in figure 2.

Figure 2. Binding interaction N-(2-methylindolin-1-yl) propionamide and α-glucosidase I protein.

3.3 Analysis of Fragment Linking and Molecular Docking of Ligand Formed from CAST and Potential Fragment

Fragment linking was used to linked two or more fragment with a linker to generated higher affinity binding compound with wider area interaction in the active site. The fragments should bind in the different area in binding site and close enough to be linked [17]. In this study, we linked CAST with N-(2-methylindolin-1-yl)propionamide using Link Multiple Fragments feature in MOE 2014.09. Fragment linking between CAST and another potential fragment could build a bigger molecule with wider area interaction in the active site of processing α-glucosidase I. To generated ligands with drug-likeness and good bioavailability properties, some parameter can be applied in Descriptor Filter such as molecular weight (less than 500g/mol, according to Lipinski’s RO5), have total polar surface area (TPSA) less than 140 Å², and have rotatable bonds less than 10 [21,27]. This work resulted in 127 ligands. Each ligand was given code name LRS 1 to LRS 127.

All the ligands that were generated from fragment linking then docked towards active site of I. We chose five ligands out of 127 ligands that have small ΔG result than other ligands and have interaction with catalytic residues of processing α-glucosidase I. Table 1 showed the ΔG and inhibition constant (pKi) of the five best ligands and two standard ligands that used in our work. The docking result showed ligand LRS 28 (N-(S)-(S)-4-methyl-5-((1S,3S,6S,7R,8R,8aR)-1,6,7,8-tetrahydrooctahydroindolizin-3-yl)pentylindolin-1-yl)propionamide) has the lowest ΔG among the others. Ligand LRS 28 has -52.0572 kcal/mol with pKi 37.9229. The molecular interaction between LRS 28 and processing α-glucosidase I can be seen in figure 3.
Table 1. ΔG energy and pKi value from the selected linked compounds and standard ligands

| Ligands | Linker | ΔG (kcal/mol) | pKi  |
|---------|--------|---------------|------|
| LRS 28  |        | -52.0572      | 37.9229 |
| LRS 85  |        | -52.0232      | 37.8981 |
| LRS 24  |        | -51.181       | 37.2846 |
| LRS 47  |        | -49.5897      | 36.1254 |
| LRS 22  |        | -49.5482      | 36.0951 |
| *CAST   | -      | -28.7224      | 20.9238 |
| *Bu-CAST| -      | -34.3206      | 25.0020 |

Note: *Standard Ligands
3.4 Analysis of Drug Scan

The toxicity and drug-likeness analysis of the best ligands was performed using Osiris DataWarrior 4.2.2 and T.E.S.T (Toxicity Estimate Software Tool) [28,29]. Toxicity parameter such as mutagenicity, the tumorigenic and irritant effect was predicted using Osiris Data Warrior 4.2.2, while developmental toxicity was predicted using T.E.S.T. The toxicity prediction from best five ligands as well the standard ligands are shown in table 2.

Table 2. Toxicity prediction by Osiris DataWarrior and T.E.S.T Software

| Ligands | Mutagenic | Tumorigenic | Irritant | Developmental Toxicity |
|---------|-----------|-------------|----------|------------------------|
| LRS 28  | None      | None        | None     | None                   |
| LRS 85  | None      | None        | None     | Yes                    |
| LRS 24  | None      | None        | None     | Yes                    |
| LRS 47  | None      | None        | None     | None                   |
| LRS 22  | None      | None        | None     | None                   |
| *CAST   | None      | None        | None     | None                   |
| *Bu-CAST| None      | None        | None     | Yes                    |

Note: *Standard Ligands

The drug-likeness properties are related to the Lipinski’s RO5 and Veber rules. Ligands that have properties fit with these rules could be considered as potential drug candidates with good oral bioavailability [27,30,31]. RO5 states that drug candidates would easily absorb by the body if they have the following criteria:

- The molecular weight (MW) of the ligands is less than 500 g/mol
- clogP is less than 5.0
- Have less than 5 hydrogen bond donor and no more 10 hydrogen bond acceptor
Unlike RO5, Veber rules only state 2 criteria for candidate drugs to have good oral bioavailability. These criteria are had no more 10 rotatable bonds and the polar surface area equal to or less than 140 Å². We used Osiris DataWarrior to analysis the drug-likeness. The result drug-likeness prediction is shown in Table 3.

**Table 3. The molecular properties prediction by Osiris DataWarrior**

| Ligands  | Molecular Weight | Number of H-Donor | Number of H-Acceptor | cLogP  | Polar Surface Area (Å²) | Number of Rotatable bonds |
|----------|------------------|-------------------|----------------------|--------|------------------------|--------------------------|
| LRS 28   | 461.601          | 5                 | 8                    | 1.5144 | 116.5                  | 8                        |
| LRS 85   | 486.611          | 6                 | 9                    | -0.3757| 128.53                 | 7                        |
| LRS 24   | 475.628          | 5                 | 8                    | 1.7327 | 116.5                  | 8                        |
| LRS 47   | 477.624          | 5                 | 8                    | 1.7327 | 116.5                  | 8                        |
| LRS 22   | 487.639          | 5                 | 8                    | 2.323  | 128.53                 | 8                        |
| *CAST    | 189.210          | 4                 | 5                    | -1.8813| 84.16                  | 0                        |
| *Bu-CAST | 259.301          | 3                 | 6                    | -0.4879| 90.23                  | 4                        |

Note: *Standard Ligands

Table 2 has shown that one of two standard ligands and some of the modified ligands have no toxic properties. There is no risk of being mutagenic, tumorigenic and irritant for all modified ligands and the standard. However, the standard ligand of Bu-CAST and the modified ligand such as LRS 85 and LRS 24 have developmental toxicity properties. The result in table 3, all the standards have a good result and fit the criteria from both RO5 and Veber rules. Table 3 also indicates that among five modified ligands, LRS 47 violated one of Veber rules and LRS85 violate one of the RO5, for having six hydrogen bond donors and higher TPSA value than the stated rules (at 141 Å²), respectively. Based on table 2 and table 3, among five modified ligands, LRS 28 and LRS 22 have the best result. These ligands showed to be negative on mutagenic, tumorigenic, irritant, and developmental toxicity properties. These ligands also fit the RO5 and also Veber rules. Ligand LRS 47, even though didn’t fit with one of two criteria in Veber’s rules, it has no mutagenic, tumorigenic, irritant, and developmental toxicity properties.

**Conclusion**

In this work, 127 ligands have been formed from fragment linking between CAST and N-(2-methylindolin-1-yl) propionamide. Molecular docking studies showed, best five ligands have shown their ∆G were smaller than the standard. Ligand LRS 28 showed the smallest ∆G at -52,0572 kcal/mol among all the modified ligands. Computational toxicity and drug-likeness test also showed the good result as well as the standard. Based on all the simulation that have been done, ligand LRS 28, LRS 22, and LRS 47 were the potential candidate to inhibit processing α-glucosidase I. We conclude these ligands are also potential ligands to developed as a drug. Therefore, in vitro and in vivo test is needed to further determine its inhibitory activity towards processing α-glucosidase I and bioactivity as dengue therapy.

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Conflict of Interest
We declare that there is no conflict of interest in this research nor publication.

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