Bioactive Compounds Screening from Zingiberaceae Family as Influenza A/Swine Flu Virus Neuraminidase Inhibitor through Docking Approach

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Abstract: Problem statement: Influenza A/H1N1 is a disease caused by infection of influenza a virus subtype H1N1. It is a major health problem in tropical and subtropical countries. This virus constantly mutates and consequently will be developed into new drug-resistant strains. Approach: In this research, we have conducted docking study to screen bioactive compounds from Zingiberaceae family, which has a role as neuraminidase inhibitor of influenza a virus. Results: The docking result identified that 1, 2-di-O-β-D-glucopyranosyl-4-allylbenzene (BGA) compound has the affinity and ability to inhibit neuraminidase. There are fourteen residues contact of BGA compound to neuraminidase and eight residues contact of enzyme that formed hydrogen bond with catalytic site. Conclusion/recommendation: The docking result showed that BGA has better binding energy and affinity compared with other bioactive compounds and the standard compounds.

Keywords: Neuraminidase inhibitory, molecular docking, influenza A/H1N1, bioactive compounds, neuraminidase inhibitor, initial screening process, screen bioactive, subtropical countries, catalytic site, hydrogen bond, conducted docking study, β-D-glucopyranosyl-4-allylbenzene (BGA), surface glycoprotein

INTRODUCTION

Influenza A/H1N1 virus is one of subtype influenza a virus of the Orthomyxoviridae family and one of the main causes of influenza in humans (Van Reeth, 2009). Influenza remains a major cause of morbidity and mortality (Morens et al., 2010). The Lastest influenza pandemic infected 25-30% of the world’s population and killing at least 20-50 million world-wide, including more than half a million people in the United States (Thompson et al., 2009). The utilization of antiviral drugs has increased dramatically along with the spread of pandemic influenza A/H1N1 which began in April 2009. The virus is always mutated, forming new strain that are resistant to existing antiviral drug, which also has the potential to infect humans by the means of animal to human transmission or human to human transmission (Moss et al., 2010).

Influenza viruses have a single-stranded nucleic acid, which consists of eight gene segments that encode 11 proteins. The envelope of influenza viruses is composed of complex carbohydrates and two glycoproteins, namely hemagglutinin and neuraminidase (Bantia et al., 2011). Neuraminidase is a surface glycoprotein, which has an important activity for the replication of influenza A and B virus. This enzyme is responsible for the catalytic cleavage of Sia(α2-6)Gal or Sia(α2-3)Gal protein ketosidic bond that exists between the terminal sialic acid and amino acid residues. Bond cleavage has some very important effects. First, it allows the release of virus from infected cells. Second, it prevents the formation of viral aggregates after its release from the host cells (Skehel, 2009; Lai et al., 2010). The neuraminidase is important in viral replication, releases and pathogenicity. Those facts made neuraminidase as a potential antiviral target (Durrant and McCammon, 2010).

The extensive studies of herbs from diversity of medicinal plants present a great opportunity as potential source of good-quality compounds to be developed as anti-influenza drugs. Lead compounds from herbs such as those from Zingiberaceae family have the potential to inhibit neuraminidase and are expected to be further investigated as alternative drugs to inhibit the development of influenza virus A (Grienke et al., 2009) They exist in plants that contain glicoside active compounds as well as polyphenols (Ryu et al., 2009a; Jeong et al., 2009; Arora et al., 2010) and xantho...
(Ryu et al., 2009b), which can be developed as inhibitors of hemagglutinin and neuraminidase biological activity (Sung et al., 2010).

In this research, we have screened bioactive compounds based on their interactions with neuraminidase, using computer software applications (in silico method) (Vidal et al., 2011) to determine the best drug lead compounds (Wang et al., 2009). The interaction profiles of the bioactive compounds with neuraminidase were determined by docking experiments (Sandhya et al., 2010; Lee et al., 2009) as an initial screening process for selecting lead compounds, which can bind to the enzyme active site. Analysis was based on Gibbs Free energy values, inhibition constant (Elmi et al., 2009), conformation of the structure, affinity and hydrogen bonding of enzyme and ligands (Krüger and Gohlke, 2010).

MATERIALS AND METHODS

Neuraminidase enzyme is used in this study. Neuraminidases sequences were obtained from complete sequences of influenza A/H1N1 virus and the data time range is selected from January 2009 until September 2009. Complete sequences were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/genomes/flu/). Multiple sequence alignment method was using ClustalW program (www.ebi.ac.uk/Tools/clustalw2/index.html website). Homology modeling was performed using the Swiss Model server, which can be accessed through http://swissmodel.expasy.org/SWISS-MODEL.html. The selected three-dimensional structure was the one with highest similarity toward neuraminidase sequences from 2HTY chain F (PDB Code).

Optimization and minimization of three-dimensional structure of the enzyme were employed using the software of MOE 2008.10, with addition of hydrogen atoms. Protonation was employed with protonate 3D programs. Furthermore, partial charges and force field was employed with MMFF94x. Solvation of enzymes was performed in the form of a gas phase with a fixed charge with a RMS gradient of 0 05 kcal / A0 mol. Other parameters were in accordance with default values in the software.

Docking simulation: The docking process was begun with the docking preparation, that was employed using a docking program from MOE 2008.10 software. Docking simulations were performed by the Compute-Simulation dock program. Placement method was conducted using a triangle matcher with 1,000,000 repetition energy readings each position and other parameters were in accordance with existing default in the MOE software. Furthermore, scoring functions used London DG, refinement of the configuration repetition forcefield with 1,000 populations. The first repetition of 100 times and the second setting was shown only for one of the best results.

RESULTS

Neuraminidase sequence > gi|284999325|gb| ADC32390.1|neuraminidase [Influenza A virus (A/Ancena/15/2009 (H1N1))] was used as target sequence which has a 91.429% similarity with template of 2HTY chain F (PDB Code) and value of Superimpose 0.88 A°.

The bioactive ligands were isolated from species Alpinia galangal, Alpinia officinarum (Lakshmipathy and Kannabiran, 2009; Erukainure et al., 2011; Kiranmayee et al., 2010; Duru and Onyedineke, 2010; Jinsong et al., 2010), Curcuma longa, Curcuma aromatica, Curcuma xanthorrhiza and Zingiber zerumbet (Bhuiyan et al., 2009; Aja et al., 2010). There are a total 99 bioactive compounds, including 13 of flavonoids, 47 of the diarylheptanoids, 18 of the glycosides, 10 phenolics and 11 zerumbones including 13 of flavonoids, 47 of the diarylheptanoids, 18 of the glycosides, 10 phenolics and 11 zerumbones (Kiing et al., 2009; Islas-Osuna et al., 2010; Suthindhiran and Kannabiran, 2009; Vani et al., 2009; Gayosso-Garcia et al., 2010).

The docking results showed that there are 10 out from 99 bioactive ligands that have low Gibbs Free energy. Furthermore, the docking studies of the 10 compounds were compared with ZNM (Zanamivir) ligand as standard 1 and ILG (Isoliquartigenin) ligand as standard 2. From the 10 of best ligands, 4 ligands have a lower Gibbs energy than ZNM ligand standard. The analysis of the docking results is shown in Table 1. The BGA compound has of the lowest AG value with AG = -8.1176 kcal/mol and it is lower than the ΔG of ILG ligand (-7.7494 kcal/mol) isolated from Glucyrrhiza uralensis (Gong et al., 2009) and the ΔG of Zanamivir (-5.4946 kcal/mol).
Table 1: Docking results and minimized energy of the complexes enzyme ligands

| Ligan | Compound 2* | Compound 3* | Compound 9* | Compound 10* | Std 1* | Std 2* |
|-------|-------------|-------------|-------------|-------------|-------|-------|
| G (kcal/mol) | -5.9891 | -8.1176 | -6.2940 | -6.5707 | -5.4946 | -7.4944 |
| MR | 442.461 | 474.4590 | 353.3500 | 354.3550 | 334.3290 | 550.5130 |
| Log P | -1.554 | -3.2280 | 3.4220 | -0.1880 | -6.8550 | -1.3630 |
| pKi (μM) | 11.896 | 14.2300 | 11.2090 | 10.4820 | 15.7490 | 13.7390 |
| H Don | 5.000 | 5.0000 | 1.0000 | 2.0000 | 7.0000 | 5.0000 |
| H Acc | 4.000 | 8.0000 | 3.0000 | 4.0000 | 6.0000 | 7.0000 |

*) Name of compounds/legends: 1. Compound 2: 1-O-(6-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-4-allylbenzene (RGA); 2. Compound 3: 1,2-di-O-β-D-glucopyranosyl-4-allylbenzene (BGA); 3: Compound 9: 1E,4Z,6E)-1,7-bis (3,4-dimethoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (DPH); 4. Compound 10: 5Z,7 (E)-6-methoxy-8-(4-hydroxy-3-methoxyphenyl)-5-{[(2E)--(4-hydroxy-3-methoxyphenylocta-5,7-dienoic acid (MPP)}; 5. Std 1: Zanamivir (ZNM); 6. Std 2: Isoliquartigenin (ILG)

Table 2: Hydrogen bonds of neuraminidase residue with ligands

| Ligand | Amino acid residue | Total of H bond |
|--------|-------------------|----------------|
| Compound 2 (RGA) | Glu119, Trp179, Trp179, Glu278, Arg118, Glu119, Arg152, Arg293 | 9 |
| Compound 3 (BGA) | Asp151, Ser247, Glu277, Glu277, Asn344, Arg118, Ser247, Arg293, Arg293, Asn295, Arg152, Arg368, Arg368 | 14 |
| Compound 9 (DPH) | Asp151, Arg118, Glu136, Arg368, Lys432 | 5 |
| Compound 10 (MPP) | Glu119, Glu278, Glu119, Arg152, Arg152, Arg156 | 6 |
| Std 1 (ZNM) | Glu119, Asp151, Ser247, Glu277, Glu277, Arg152, Ser247, Arg293, Arg293, Asn295, Arg368, Arg368 | 13 |
| Std 2 (ILG) | Val116, Gly147, Asp151, Glu277, Glu278, Glu136, Arg156, Arg156, Asp368, Arg430, Thr438 | 12 |

*) Bold: catalytic site

Table 3: Score residue contact of neuraminidase with ligands

| Catalytic site (score %) | Ligan | Arg118 | Asp151 | Glu278 | Arg293 | Arg368 |
|-------------------------|-------|--------|--------|--------|--------|--------|
| Compound 2 (RGA) | 40.5 | - | 23.7 | 33.9 | - |
| Compound 3 (BGA) | 64.0 | 35.4 | 42.2 | 45.3; 24.3; 11.4 | 76.7; 23.2 |
| Compound 9 (DPH) | 26.5 | 12.7 | - | - | 21.5 |
| Compound 10 (MPP) | - | - | 74.0 | - | - |
| Std 1 (ZNM) | - | 31.7; 45.9 | 27.1 | 39.7; 13.6 | 81.3; 83.2 |
| Std 2 (ILG) | - | 63.7 | 27.0 | 75.1 | 34.3 |

The docking result indicates that the BGA ligand has pKi value of 14.430 μM, suggesting that BGA ligand has stronger affinity for neuraminidase than standard 2 (pKi = 13.739 μM). However, when compared with the standard ZNM, pKi value of BGA ligand is lower than ZNM ligand. The results suggest that BGA ligand interacts strongly and effectively with neuraminidase (Table 1).

The hydrogen bonding network between neuraminidase residues and BGA or standard ligands is shown in Tab. 2. The BGA, ZNM and ILG ligands form 14, 13 and 12 hydrogen bonds to neuraminidase, respectively. BGA ligand binds to the catalytic site at the Arg118, Asp151, Glu278, Arg293 and Arg 368 residues and forms as many as 8 hydrogen bonds with the active site. BGA has a higher number of hydrogen bonds to the active site than ZNM standard (7 hydrogen bonds) and ILG standard (4 hydrogen bonds) (Table 2).

Thus, based on the hydrogen bonds, it suggests that the BGA ligand has strong interaction with neuraminidase than those of standards. All three ligands interact with Asp151, Glu78, Arg293 and Arg368 of the catalytic site; however, BGA ligand has additional interaction with the Arg118 residue. The active site binding scores of BGA ligand for neuraminidase are 64% for Arg118; 42.2% for Glu278; 45.3%, 24.3%, 11.4% for Arg 293; and 76.7%, 23.2% for Arg368 (Table 3).

DISCUSSION

The conformation of BGA ligand bound to the neuraminidase is shown in Fig. 1. Because the BGA ligand has more residue contacts on the active site than ZNM and ILG, it suggests that BGA interacts more tightly to neuraminidase than ZNM or ILG ligand.

The conformation of BGA is similar to that of ZNM when entering and filling the cavity of neuraminidase binding site. The structure of BGA was superimposed the structure of ZNM as shown in Fig. 2A and BGA filled more surface area of the cavity of at the ligand binding site more than ILG (Fig. 2B).

BGA ligand has many interactions with residue contacts at the catalytic site that are similar to those of ZNM ligand (Fig. 1A and 2A). In addition, BGA ligand has better interactions to the catalytic site than ILG ligand (Fig. 1A and 2B). The chemical structure of BGA ligand is shown below:

The difference of the charge within the binding site of BGA, which occurs between the NH3 clusters binding site of arginine 293 and arginine 368 residues, allowed the interaction with functional group of the ligand.
Fig. 1: The neuraminidase residue contact with ligands, (A) shows neuraminidase contact residues with BGA in 3D; (B) shows neuraminidase contact residues with BGA in 2D; (C) shows neuraminidase contact residues with ZNM in 3D; (D) shows neuraminidase contact residues with ZNM in 2D; (E) shows neuraminidase contact residues with ILG in 3D; (F) shows neuraminidase contact residues with ILG in 2D

Fig. 2: A. The conformation of BGA (blue) with ZNM (green), B. Conformation of BGA (blue) with ILG (red)

It will eventually increase the affinity and stability of the enzyme-ligand complex. Stability and affinity of the ligand complex with the active site was influenced by the distance of the hydrogen bond and complex score. The value of complex score is shown in Table 3.

Fig. 3: The chemical structure of 1, 2-di-O-β-D-glucopyranosyl-4-allylbenzene (BGA). Taken from (Duru and Onyedineke, 2010; Jinsong et al., 2010)

It was indicated that the BGA could change the conformation of neuraminidase cavity upon entering the binding site of neuraminidase (Fig. 3).

Our results suggest that O-glycoside group such as BGA can interact with neuraminidase. This further confirms the results of Sung et al. (2010) that O-glycoside group is hydrophilic with a polar functional group, which can inhibit neuraminidase. It is in line with the polar hydrophobic characteristics of neuraminidase.

**CONCLUSION**

In conclusion, BGA ligand was found to be the most potent inhibitor of neuraminidase out of 99
compounds evaluated from Zingiberaceae family. The complex between BGA ligand and neuraminidase produces 14 hydrogen bonds with 8 hydrogen bonds at the neuraminidase catalytic site. BGA has the lowest binding energy (-8.1176 kcal/mol) and highest pKi (14.430 μM) compared to the two standards (ZNM and ILG). Thus, BGA can be considered to be lead compound for inhibition neuraminidase activity. In the future, BGA will be investigated further as a potential antiviral drug for treating influenza caused by A/H1N1 virus.

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