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In Silico Analysis and Identification of Possible Inhibitor of H5N1 Virus

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ABSTRACT

Fingerroot (*Boesenbergia pandurata* (Roxb.) belongs to the family Zingiberaceae (Ginger). *B. pandurata* has pharmacological benefits such as neuroprotective, chemoprotective, anti-inflammatory, anti-angiogenic, antioxidant, an inhibitor of protease enzyme NS2B/NS3 dengue virus, Japanese encephalitis virus and swine flu virus (H1N1). This study aims to determine the most effective compounds from *B. pandurata* as neuraminidase inhibitors of H5N1 virus. The amino acid sequence for neuraminidase of avian influenza A virus subtype H5N1 of A/China/GD02/2006 (ABX57872.1) was retrieved from protein sequence database at NCBI. Then, modeled by Swiss Model. Analyse of molecular docking was performed using PyRx and the interactions between neuraminidase inhibitors of H5N1 and *B. pandurata* active compound was analyzed by PyMol software and LigPlot+ software. From the 30 active compounds which have been docked, 4-hydroxypanduratin A, rubranine, boesenbergin B, boesenbergin A, 5,7-dimethoxyflavone, and tectochrysin had an equal or smaller free binding energy than control compound. 4-hydroxypanduratin A proved to be the most potent active compound as a neuraminidase inhibitor (NA 1) because it has the most negative binding energy and the same amino acid binding residue with the control compound. Therefore, 4-hydroxypanduratin A is predicted to be used as inhibitors of neuraminidase in the H5N1 virus.

Keywords: *Boesenbergia pandurata, H5N1, neuraminidae, 4-hydroxypanduratin A.*

INTRODUCTION

Avian Influenza A (H5N1) is one of the types of influenza virus which is the highest cause of pathogenic death in humans [1]. This virus is still a major health problem in the world [2] and also classified as highly pathogenic avian influenza (HPAI) which has caused a large number of deaths in humans and birds [3]. The threat of HPAI virus H5N1 needs further research [4]. This virus belong to the family orthomyxoviridae which is enclosed by an envelope with a genome that composed of 8 different RNAs where each of RNAs enclosed by a single-stranded helical capsid [5].

The influenza envelope layer consists of two major glycoproteins: hemagglutinin (HA) and neuraminidase (NA) [6]. There are 16 serotypes hemagglutinin (H1-H16) and 9 serotypes neuraminidase (N1-N9) [7]. Neuraminidase (NA) is a target protein that acts as a sialidase that divides sialic acid from cellular glycoproteins. Viral glycoproteins are expressed on infected cells and collected into virions. This mediates the aggregation of newly born viral particles on the surface of the infected cell and allows the release of the virus [8].

Inhibition of neuraminidase (NA) is a way to limit the spread of infection from influenza virus [9]. Oseltamivir (tamiflu) and zanamivir (relenza) are neuraminidase inhibitors that are developed based on knowledge of the structure of the neuraminidase [10]. Oseltamivir is the first neuraminidase inhibitor drug used for treatment [11]. Nevertheless, it revealed that

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the use of oseltamivir was unusable due to viral resistance to the drug [12]. Then, a new metabolite compounds from plants that can inhibit the activity of the virus are needed.

Fingerroot (*Boesenbergia pandurata* (Roxb.)) belong to the Zingiberaceae family (Ginger) [13]. *B. pandurata* has pharmacological benefits such as neuroprotective, chemoprotective [14], anti-inflammatory [15], anti-angiogenic [16] antioxidant [17], inhibition of protease enzymes NS2B/NS3 in dengue virus [18], inhibition of japanese encephalitis virus [19], and inhibition of the swine flu virus (H1N1) [2]. *B. pandurata* contain several secondary metabolite compounds such as flavonoids, essential oils (EOs), and miscellaneous compounds [13].

Treatment of Influenza A virus infection is still limited and the threat of a new pandemic from this infection still requires the development of the newest therapeutic agents [20]. Therefore, it is necessary to make a drug taken from a natural compounds of a plant that can be used against Avian Influenza A (H5N1).

MATERIALS AND METHODS

Protein Preparation and Modelling

The amino acid sequence for neuraminidase of avian influenza A virus subtype H5N1 of A/China/GD02/2006 (ABX57872.1) was retrieved from protein sequence database at NCBI [21]. Molecular modeling of the target protein were determined using the Swiss Model website where the Swiss Model will show the best model of the sequnce of protein that we provide based on the percentage of the sequence identity [22]. Later on, the sequences are putted into the Swiss sequence target and modelled to obtain the appropriate model. The model has been compared also to the neuraminidase (NA 1) protein model of some literature [6-8].

Ligan Preparation

30 natural ligand compounds from *B. pandurata* and ligand control zanamivir structure collected from PubChem database (Table 1) [23]. The ligands is based on the similarity functions as antivirals. The ligands antiviral activity were analyzed by PASS Online Prediction [24]. The ligand-like resemblance to the drug was selected by druglikeness based on Lipinski's rule of five (ROF) [25]. The preparation of the ligand involves adding hydrogen bonds, neutralizing the charge groups and removing the structure of the ligands. The optimized ligand is used for molecular docking.

Active Binding Site Prediction

Binding site prediction of the target protein is performed to find out which protein residues will be docked with ligand queries. The binding site prediction of the H5N1 viral neuraminidase (NA 1) protein was performed using the Zhang Cofactor website [26].

Molecular Docking

The protein target and prepared ligands were docked by PyRx software [27] and molecular docking was performed and resulted as a binding affinity score. Visualization performed using the PyMOL software [28] and Ligplot^{+} software [29].

Table 1. Active compound from *B. pandurata* and Zamamivir as control

Compounds	*CID*	*Molecular formulae*
(-)-nicolaioidesin B	637029	$C_{26}H_{30}O_4$
(+)-Krachaizin A	11729201	$C_{26}H_{30}O_4$
(+/-)-isopanduratin A1	44444913	$C_{26}H_{30}O_4$
β-Ocimene	5281553	$C_{10}H_{16}$
(2R)-8-geranylpinostrobin	23656470	$C_{26}H_{30}O_4$
4-hydroxypanduratin A	636530	$C_{25}H_{28}O_4$
5,6-Dehydrokawain	5273621	$C_{14}H_{12}O_3$
5,7-dimethoxyflavone	88881	$C_{17}H_{14}O_4$
Alpinetin	4053302	$C_{16}H_{14}O_4$
Boesenbergin A	23643133	$C_{26}H_{28}O_4$
Boesenbergin B	6313827	$C_{26}H_{28}O_4$
Camphor	2537	$C_{10}H_{16}O$
Cardamonin	641785	$C_{16}H_{14}O_4$
Flavokawain A	5355469	$C_{18}H_{18}O_5$
Flavokawain B	5356121	$C_{17}H_{16}O_4$
Zanamivir	60855	$C_{12}H_{20}N_4O_7$
Geraniol	637566	$C_{10}H_{18}O$
Helichrysetin	6253344	$C_{16}H_{14}O_5$
Isopanduratin A	10069916	$C_{26}H_{30}O_4$
Methyl Cinnamate	637520	$C_{10}H_{10}O_2$

Neral	643779	$C_{10}H_{16}O$
Panduratin A	6483648	$C_{26}H_{30}O_4$
Panduratin C	6483647	$C_{26}H_{30}O_5$
Panduratin D	24864268	$C_{28}H_{30}O_4$
Pinocembrin	68071	$C_{15}H_{12}O_4$
Pinostrobin chalcone	5316793	$C_{16}H_{14}O_4$
Rotundaflavones Ia	101863268	$C_{26}H_{30}O_4$
Rubranine	42607681	$C_{25}H_{26}O_4$
Sakuranetin	73571	$C_{16}H_{14}O_5$
Tectochrysin	5281954	$C_{16}H_{12}O_4$
Uvangoletin	6483649	$C_{16}H_{16}O_4$

RESULTS AND DISCUSSION

Potential Analysis of the B. pandurata Active Compound as NA 1 Protein inhibitors.

The analysis of the capability of inhibition of *B. pandurata* active compounds on NA 1 protein was done by molecular docking method, which simulated the binding of *B. pandurata* active compounds on NA 1 protein, then compared with control compound known to inhibit NA 1 protein. This study used active compounds from *B. pandurata* as ligands obtained based on literature study results. Results from the literature study found 75 types of active compounds from the *B. pandurata* plant, which are included in flavonoids, essential oils (EO), and various compounds [13].

The active compounds of *B. pandurata* having a free binding energy equal to or less than the control are 4-hydroxypanduratin A, rubranine, boesenbergin B, boesenbergin A, 5,7-dimethoxyflavone, and tectochrysin. The six compounds have the lowest energy compared with other compounds (Table 2). The binding energy of the active compounds were -9.7 kcal/mol for 4-hydroxypanduratin A, -9.0 kcal/mol for rubranine, -8.5 kcal/mol for boesenbergin B, -8.4 kcal/mol for boesenbergin A, -7,8 kcal/mol for 5,7-dimethoxyflavone, and -7,7 kcal/mol for tectochrysin.

Predicted compounds that have the potential to become neuraminidase inhibitors (NA1) in avian influenza A from the six compounds that have free binding energy equal to or less than the control are 4-hydroxypanduratin A. This compound has the most negative binding affinity (ΔGbind) energy of -9.7 kcal/mol compared to the others. The more negative the affinity binding value (ΔGbind) indicates a good degree of stability between the ligand and the target protein, so that the bonds formed will be stronger [30].

Binding Side Analysis Between Ligand of Active Compound of B. pandurata and Protein NA 1

4-hydroxypanduratin A, rubranine, boesenbergin B, boesenbergin A, 5,7-dimethoxyflavone, and tectochrysin had different binding sites with target protein (Figure 1). 4-hydroxypanduratin A compound has the side binding at the center and slightly to the right, rubranine has a lower left binding side, boesenbergin B has a central and slightly inward binding side of NA1 protein, boesenbergin A and 5,7-dimethoxyflavone have the same binding sides with rubranine, and tectochrysin has the side binding at the center and slightly to the left. 4-hydroxypanduratin A compound has a NA1 protein binding position similar to the control.

Compounds that have the ability to have the same or almost the same binding side as the control indicate that the compound can act like a control [31]. 4-hydroxypanduratin A has a binding position that is almost the same as the control. It shows that the compound is indicated to be capable of acting like a control.

Analysis of Interaction Between Ligand of B. pandurata active compound and NA 1 Protein

4-hydroxypanduratin A, rubranine, boesenbergin B, boesenbergin A, 5,7-dimethoxyflavone, and tectochrysin had different interactions with protein NA 1 (Table 3). 4-hydroxypanduratin A has the same three hydrophobic bonds as the controls: Arg205, Asn275, and Ile407, while rubranine and boesenbergin A have one hydrophobic bond similar to that of Ile407. The other three compounds, boesenbergin B, 5,7-dimethoxyflavone, and tectochrysin, do not have the same hydrophobic bonds as controls.

Table 2. Potential inhibitor of active compound in *B. pandurata* against NA 1 protein

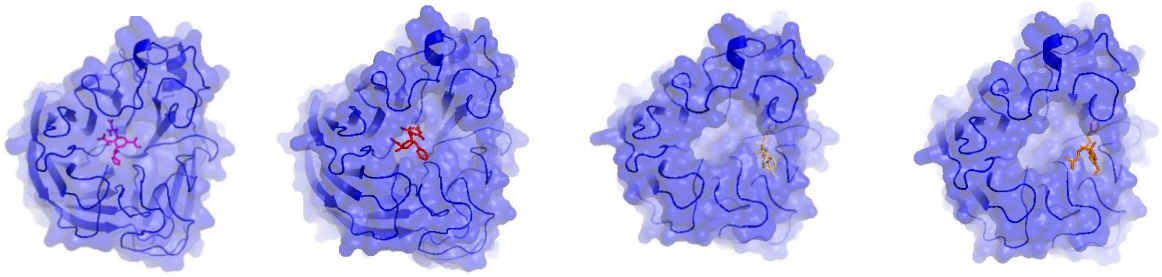
Compounds	*Molecular formulae*	*M.W (g/mol)*	*Binding Affinity score (kcal/mol)*	*Log P*	*Hydrogen bond donor*	*Hydrogen bond acceptors*	*Target Protein*
4-hydroxypanduratin A	$C_{25}H_{28}O_4$	392	-9.7	4.708601	3	4	NA 1
Rubranine	$C_{25}H_{26}O_4$	390	-9.0	5.246600	1	4	NA 1
Boesenbergin B	$C_{26}H_{28}O_4$	404	-8.5	6.207601	1	4	NA 1
Boesenbergin A	$C_{26}H_{28}O_4$	404	-8.4	6.207601	1	4	NA 1
5,7-dimethoxyflavone	$C_{17}H_{14}O_4$	282	-7.8	3.319999	0	4	NA 1
Tectochrysin	$C_{16}H_{12}O_4$	268	-7.7	3.016999	1	4	NA 1
Zanamivir	$C_{12}H_{20}N_4O_7$	331	-7.7	5.120199	8	11	NA 1
Cardamonin	$C_{16}H_{14}O_4$	270	-7.6	3.002499	2	4	NA 1
Helichrysetin	$C_{16}H_{14}O_5$	286	-7.5	2.708099	3	5	NA 1
Panduratin D	$C_{28}H_{30}O_4$	430	-7.6	7.052203	1	4	NA 1
Pinostrobin chalcone	$C_{16}H_{14}O_4$	270	-7.6	3.002499	2	4	NA 1
Flavokawain B	$C_{17}H_{16}O_4$	284	-7.6	3.305498	1	4	NA 1
Panduratin C	$C_{26}H_{30}O_5$	422	-7.4	5.717201	3	5	NA 1
Sakuranetin	$C_{16}H_{14}O_5$	286	-7.4	2.812899	2	5	NA 1
Alpinetin	$C_{16}H_{14}O_4$	270	-7.3	3.107299	1	4	NA 1
Panduratin A	$C_{26}H_{30}O_4$	404	-7.1	6.207601	1	4	NA 1
Pinocembrin	$C_{15}H_{12}O_4$	256	-7.1	2.804299	2	4	NA 1
Uvangoletin	$C_{16}H_{16}O_4$	272	-7.1	2.921899	2	4	NA 1
Flavokawain A	$C_{18}H_{18}O_5$	314	-6.9	3.314099	1	5	NA 1

4-hydroxypanduratin A had the same binding sites with a control of 99% because it had the same 3 amino acid hydrogen bonds with the controls: Asp131, Arg132, and Tyr324, while rubranine, boesenbergin B, boesenbergin A, 5,7-dimethoxyflavone, and tectochrysin had a binding ability of 33% because the compounds had only one amino acid hydrogen bond residue equal to the control. Even though these compounds have similar amino acids with control, they will not cause resistance to their use. This is because of the differences in compounds and constituents [33]. In addition, the 4-hydroxypanduratin A compound has a shorter hydrogen bond length compared to the controls and also with the other. 4-hydroxypanduratin A has a hydrogen bond length of Asp131 (3.00 Å), Arg132 (2.02 Å), and Tyr324 (2.12 Å), rubranine has a hydrogen bond length Tyr324 (3.13 Å), boesenbergin B has a hydrogen bond length of Tyr324 (3.08Å), boesenbergin A has a hydrogen bond length Tyr324 (3.03 Å), 5,7-dimethoxyflavone has a hydrogen bond length Tyr324 (2.89 Å), and tectochrysin has a hydrogen bond length of Tyr324 (2.88 Å), and and tectochrysin have a binding ability of 33% because they have only 1 amino acid hydrogen bond residu equal to the control (Table 3). The active compound is predicted have a stronger ability to inhibit the target protein if it has more amino acid binding residues equal the control compound [32].

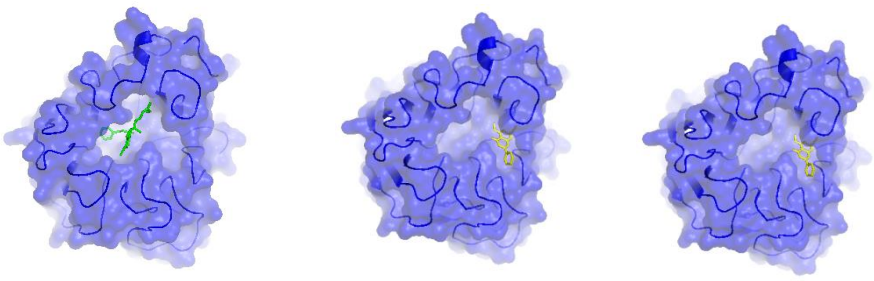
Table 3. Interaction between active compound of *B. pandurata* and NA 1 protein

Compounds	*Interaction*
4-hydroxypanduratin A	Hydrogen bond (Length): **Asp131** (3.00 Å), **Arg132** (2.02 Å), Arg152 (2.77 Å), **Tyr324** (2.12 Å)
	Hydrophobic bond: **Arg205**, **Asn275**, **Ile407**
Rubranine	Hydrogen bond (Length): **Tyr324** (3.13 Å), Arg273 (3.21 Å)
	Hydrophobic bond: Trp379, Arg348, **Ile407**
Boesenbergin B	Hydrogen bond (Length): **Tyr324** (3.08Å)
	Hydrophobic bond: Ser160, Arg205, Tyr382
Boesenbergin A	Hydrogen bond (Length): **Tyr324** (3.03 Å)
	Hydrophobic bond: Trp379, Arg348, **Ile407**, Lys412
5,7-dimethoxyflavone	Hydrogen bond (Length): **Tyr324** (2.89 Å)
	Hydrophobic bond: Asn346, Arg348
Tectochrysin	Hydrogen bond (Length): **Tyr324** (2.88 Å)
	Hydrophobic bond: Arg348, Trp379, Ile407
Zanamivir	Hydrogen bond (Length): **Asp131** (3.10 Å), **Arg132** (3.28 Å), **Tyr324** (3.35 Å)
	Hydrophobic bond: **Arg205**, **Asn275**, **Ile407**

In addition, the compound has the same binding site as controls on the amino acid residues with the length of the hydrogen bond residue in 4-hydroxypanduratin A: Asp131 (3.00 Å), Arg132 (2.02 Å), Tyr324 (2.12 Å), shorter if compared to Zanamivir compounds whose length hydrogen bond residues are: Asp131 (3.10 Å), Arg132 (3.28 Å), Tyr324 (3.35 Å). The shorter the length hydrogen bond can make the interaction between the compound and the target protein faster [32]. Therefore, 4-hydroxypanduratin A compound is predicted have the ability to bind to NA 1 protein so that it can inhibit the protein activities.



Zanamivir – NA 1 | 4-hydroxypanduratin A – NA 1 | Rubranine – NA 1 | Boesenbergin A – NA



5,7-dimethoxyflavone – NA | Boesenbergin B – NA 1 | Tectochrysin – NA 1

CONCLUSION

Some of the active compounds contained in *B. pandurata* are 4-hydroxypanduratin A, rubranine, boesenbergin B, boesenbergin A, 5,7-dimethoxyflavone, and tectochrysin are predicted to have the ability to inhibit neuraminidase in the H5N1 virus, especially the 4-hydroxypanduratin A.

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