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RESEARCH ARTICLE

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Virtual Screening of Indonesian Herbal Compounds with Neuraminidase Inhibitor Activity against N2 Influenza Virus Protein: An *in silico* Study

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Background: Neuraminidase inhibitor (NAI) is one of anti-influenza drugs recommended for use by the World Health Organization (WHO). However, after treatment with NAI drugs in human, resistance to influenza antiviral drugs is begun to rise. Therefore, identification of compounds from Indonesian herbal plants as natural inhibitors of the influenza virus neuraminidase protein needs to be conducted for the development of new anti-influenza drugs.
Materials and methods: The crystal structure of the neuraminidase protein complex used in this study was obtained from the Protein Data Bank (PDB). Structure-based pharmacophore modeling was performed using LigandScout version 4.4.5 software. Indonesian herbal plant compounds were collected from the HerbalDB database. Protein and ligand processing was carried out using Autodock 4.2 software. The 3D interaction visualization was carried out with Autodock software, while 2D interaction visualization was carried out with LigPlot software. To determine the toxicity and drug-likeliness of the ligand, the test ligands that had the best docking results were predicted using SwissADME and AdmetSAR.
Results: From the virtual screening results, 24 hits were found, and five compounds had the best binding energy among the 24 tested compounds, these were pollenitin (ΔG=-7.22 kcal/mol), OPC-4:0 (ΔG=-7.11 kcal/mol), 6-hydroxykaempferol (ΔG=-7.08 kcal/mol), 5,8-dihydroxy-7,4'-dimethoxyflavone (ΔG=-7.07 kcal/mol), and 3,5,6,7-tetrahydroxy-4'-methoxyflavone (ΔG=-6.95 kcal/mol). The best five compounds were then chosen for further analysis.
Conclusion: OPC-4:0 is found to be the best compound for the NAI based on its binding energy, pharmacokinetics, toxicity, and drug-likeliness. Thus, OPC-4:0 might be a potential candidate as a NAI of HxN2 virus.

Keywords: *influenza, molecular docking, neuraminidase, resistance, virtual screening*

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Introduction

Avian influenza virus (AIV) is a zoonosis virus belonging to Orthomyxoviridae family with negative sense of ssRNA genome.[1,2] According to the Global Initiative on Sharing All Influenza Data (GISAID) database, influenza A viruses with N2 neuraminidase protein have been detected in humans, the environment, and animals in Indonesia, including the H1N2, H2N2, H3N2, H9N2, H5N2, and H4N2 viruses.[3]

The H2N2 subtype influenza virus, etiological agent of second pandemic in the 20th century, emerged in humans in Southeast Asia in 1957 and resulted in more than 1 million deaths.[4] To date, the threat of re-introduction and transmission of the H2N2 virus in humans still potentially exists. The reason is the virus and other influenza A viruses with a combination of the various hemagglutinin and neuraminidase genes continue to circulate in poultry and swine.[5]

AIV H9N2 is a low pathogenic avian influenza (LPAI) virus which is found endemic in Europe, Asia, and Africa. It is known as a zoonotic virus that has been found to infect humans. Meanwhile, the H3N2 influenza virus causes seasonal influenza in humans. The H9N2 virus was first isolated from turkeys in 1966 in North America, but since 1988 the H9N2 virus has been isolated from humans and other mammals such as pigs and dogs.[6] To 2022, cases of H9N2 infection in humans have been reported as many as 72 cases, of which two cases were found fatal.[7,8] In February 2021, a case of human infection with H9N2 was found in Cambodia with one poultry sample isolated in the patient's environment also being detected positive for H9N2.[9]

In Indonesia, the highly pathogenic avian influenza (HPAI) H5N1 virus has been found endemic from 2003 to the present with the highest mortality rate due to H5N1 virus infection in humans in the world with 168 deaths cases.[10] Meanwhile, since 2016 the H9N2 virus has been found circulating in Indonesia causing disruption to egg production and quality.[11] Gene reassortment may occur due to the co-circulation of subtype H9N2 and H5N1 viruses.[12]

Currently, the options for treating influenza viruses are still very limited and antiviral drug resistance is a problem that is quite difficult to overcome. The global development of new drugs for influenza viruses is divided into three categories, these were M2 ion channel blockers, neuraminidase inhibitors (NAIs) and RNA-dependent RNA polymerase (RdRp) inhibitors.[13] NAI is a small chemical compound that binds to the active site and residue on the viral NA enzyme. Neuraminidase is surface antigenic glycoprotein of influenza virus, and it has role to catalyze the cleavage of neuraminic acid residue. That process facilitates the release of virions from the host cell surface. There are currently only two licensed NAIs worldwide: oseltamivir and zanamivir. Meanwhile, peramivir and laninamivir is licensed and used only in Japan, and several other countries.[14]

The World Health Organization (WHO) has recommended treatment with a NAI (oseltamivir) because the majority of currently circulating influenza viruses have been reported to be resistant to adamantane-class drugs such as amantadine and rimantadine. Resistance is known to begin after treatment of H1N1, H3N2, H7N9 and H5N1 with NAI in humans.[15] Herbal antiviral drugs have been explored and their clinical applications demonstrated ideal antiviral activity. Therefore, the identification and exploration of compounds in medicinal plants based on the database of Indonesian herbal plants as natural inhibitors of the influenza virus neuraminidase protein may need to be conducted for the development of new anti-influenza drugs. In 1990s, a technology was developed to accelerate the drug discovery process by enabling synthesis and screening of large libraries of compounds in a short time.[16] *In silico* study, a platform for screening the activity of potential therapeutics against the molecular targets, is a technique for speeding up and reducing the cost of drug development and find novel drug to combat the virus diseases.[17] One of them is structure-based screening methods which are considered as effective tools for the identification of active compounds in drug discovery.

Considering that oseltamivir resistance has been widely reported in Indonesia,[18-20] several studies were developed to find new candidates as neuraminidase inhibitors.[21-23] However, there have been no studies reporting the use of Indonesian herbs as NAI. Therefore, this study was conducted to find novel NAI candidates from Indonesian herbal plant compounds from Indonesian Herbal Database (HerbalDB) using structure-based screening methods.

Materials and methods

Retrieval of Protein Structure Data

The crystal structure of the neuraminidase protein (N2/ Influenza A virus (A/RI/5+/1957(H2N2))) in complex with oseltamivir used in this study was obtained from the Protein Data Bank (PDB) (4K1K) (https://www.rcsb.

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org/structure/4K1K). The criteria applied in this protein collection were that the protein had a resolution of <2.5Å, the Ligand Structure Quality Assessment led to "Better" with a score close to 1 and the protein had no mutations based on the protein structure summary in the PDB.[24]

Structure-based Pharmacophore Modeling

Structure-based pharmacophore modeling was performed using LigandScout software (version 4.4.5) (https://ligandscout.software.informer.com/3.1/) by importing protein crystal structures from the PDB, performing extraction, identification, and interpretation of ligands, and creating pharmacophore.

Virtual screening of Herbal Compounds from the HerbalDB Database

After the pharmacophore was modeled, virtual screening was carried out using LigandScout software with the maximum number of compounds that were omitted was 3. Indonesian herbal plant compounds were collected from the HerbalDB database (http://herbaldb.farmasi.ui.ac.id/v3/) to carry out virtual screening. The hits compound was then used for further molecular docking process.

Retrieval of Hits Compound

Compounds that became hits in the virtual screening results were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) by downloading files with the sdf extension. Compounds obtained in the sdf format were then prepared and converted into pdb format by using MarvinSketch software (https://chemaxon.com/marvin).

Protein-Ligand Processing and Molecular Docking

The protein-ligand complex was processed by separating the protein from the native ligand, removing contaminant ligands and water. The processed protein was then stored in the PDBQT extension. Native ligand was also processed before docking and stored in the PDBQT extension. Protein and ligand processing was carried out using Autodock 4.2 software (https://autodock.scripps.edu/).[25] Validation of the docking method between 4K1K protein and native ligand (oseltamivir) was carried out by redocking the native ligand with three different sizes of gridboxes, these were 40×40×40, 50×50×50 and 60×60×60. The criterion for the results of the docking method validation was mainly determined by the lowest root-mean-square deviation (RMSD) value, which was <2Å, followed by the lowest binding energy (ΔG) and inhibition constant. The gridbox that had the lowest RMSD value, and the best ΔG value was used for the docking of the test compounds. The 3D interaction visualization was carried out with Autodock 4.2 software while 2D interaction visualization was carried out with LigPlot+ v.2.2.4 software (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/).

Toxicity dan Drug-likeliness

To determine the toxicity and drug-likeliness of the ligands, the test ligands that had the best docking results were predicted using SwissADME (http://www.swissadme.ch/) and AdmetSAR (http://lmmd.ecust.edu.cn/admetsar1/predict/). Statistics for various parameters were generated by submitting canonical SMILES from the best test ligands on the web server.

Results

Retrieving Protein through the PDB Database

From the searching results of the neuraminidase protein of the HxN2 influenza virus in the PDB, the crystal structure of the Neuraminidase protein in complex with oseltamivir with PDB ID 4K1K was obtained. 4K1K is a neuraminidase protein of influenza A virus (A/RI/5+/1957(H2N2)) with a resolution of 1.60 Å (<2.5Å), no mutations were found and the binding affinity annotation information as follows IC50: min: 0.11, max: 170 (nM) against six tests. This protein had two chains, namely the A and B chains with a sequence length of 388 amino acids obtained from the influenza A virus (A/RI/5+/1957(H2N2)) (Figure 1). Meanwhile, the native ligand was oseltamivir, which had a Quality Assessment towards number 1 which led to the "Better" indicator based on the structure summary in the PDB.

Figure 1. Structure of the neuraminidase protein A chain 4K1K.

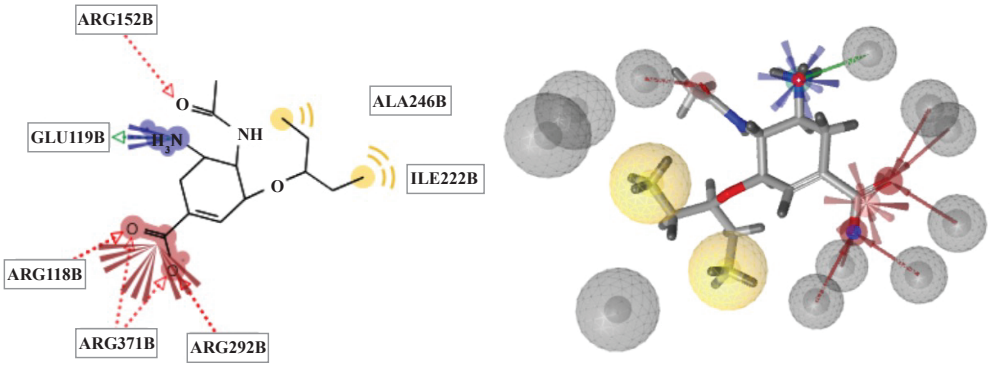

Figure 2. Pharmacophore model built with native ligand molecule and 4K1K protein. The pharmacophore model obtained had 7 H bond acceptors (red arrows), 1 H bond donor (green arrows), and 2 hydrophobic interactions (yellow circle).

Structure-based Pharmacophore

The pharmacophore model was obtained through structure-based pharmacophore modeling using LigandScout software. The pharmacophore model obtained has 7 H bond acceptors, 1 H bond donor, 2 hydrophobic interactions, 1 negative ionizable and 1 positive ionizable (Figure 2).

Table 1. 24 hits compounds from HerbalDB virtual screening for 4K1K pharmacophore model.

Compound	Molecular Formula	PubChem CID
3,5,6,7-tetrahydroxy-4'-methoxyflavone	$C_{16}H_{12}O_7$	21633676
3-methylpentyl glucosinolate	$C_{13}H_{25}NO_9S_2$	131751970
3-o-beta-d-glucopyranosyl sitosterol	$C_{35}H_{60}O_6$	70699351
5,8-dihydroxy-7,4'-dimethoxyflavone	$C_{17}H_{14}O_6$	15289454
5-hete	$C_{20}H_{32}O_3$	5280733
5-methylthiopentylglucosinolate	$C_{13}H_{25}NO_9S_3$	9548727
6-hydroxykaempferol	$C_{15}H_{10}O_7$	5281638
7-(methylsulfinyl)heptyl glucosinolate	$C_{15}H_{29}NO_{10}S_3$	124079390
8-hydroxyapigenin 8-(2",4"-disulfatoglucuronide)	$C_{21}H_{18}O_{18}S_2$	44258586
8-methylthiooctyl glucosinolate	$C_{16}H_{31}NO_9S_3$	46173877
Alliin	$C_6H_{11}NO_3S$	87310
Torvanol A	$C_{20}H_{20}O_{10}S$	5321987
Ent-copalyl diphosphate	$C_{20}H_{36}O_7P_2$	5280897
Epijasmonic acid	$C_{12}H_{18}O_3$	7251183
Gluconapin	$C_{11}H_{19}NO_9S_2$	9548620
L-cystein s-oxide	$C_6H_{11}NO_3S$	9576089
L-theanine	$C_7H_{14}N_2O_3$	439378
L-tryptophan	$C_{11}H_{12}N_2O_2$	6305
N-pentyl glucosinolate	$C_{12}H_{23}NO_9S_2$	131752362
OPC-4-0	$C_{14}H_{22}O_3$	5716900
Pollenitin	$C_{16}H_{12}O_7$	44259965
Shikimic acid	$C_7H_{10}O_5$	8742
Sinigrin	$C_{10}H_{18}KNO_{10}S_2$	23693101
Turgorin	$C_{13}H_{15}O_{13}S$-	442990

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Table 2. Docking validation results of native ligands (oseltamivir).

Indicator	Gridbox		
	40x40x40	50x50x50	60x60x60
ΔG	-8.16 kcal/mol	-8.25 kcal/mol	-8.17 kcal/mol
RMSD	1.08 Å	1.06 Å	0.55 Å
Inhibition constant	1.05 μM	904.18 nM	1.03 μM

Virtual Screening of Indonesian Herbal Compounds

Virtual screening of 1,377 compounds collected from the HerbalDB database was carried out by omitting a maximum of 3 features in the pharmacophore model. The omit feature was automatically done by the LigandScout software from the advanced option. From the screening results, a total of 24 hits were obtained (Table 1).

Molecular Docking

Validation of the docking method between 4K1K protein and the native ligand was carried out by redocking the native ligand with three different sizes of gridboxes, these were 40×40×40, 50×50×50 and 60×60×60. The RMSD score, ΔG value and inhibition constant from the native ligand which was redocked on the crystal structure can be seen in Table 2.

Table 3. Binding energy and inhibiting constant values of the 24 hits compounds.

Compound	ΔG (kcal/mol)	Inhibition constant (μM)
3,5,6,7-tetrahydroxy-4'-methoxyflavone	-6.95	7.98
3-methylpentyl glucosinolate	-6.07	35.31
3-o-beta-d-glucopyranosyl sitosterol	-6.84	9.73
5,8-dihydroxy-7,4'-dimethoxyflavone	-7.07	6.63
5-hete	-6.19	28.84
5-methylthiopentylglucosinolate	-6.55	15.87
6-hydroxykaempferol	-7.08	6.49
7-(methylsulfinyl)heptyl glucosinolate	-5.71	65.74
8-hydroxyapigenin 8-(2",4"-disulfatoglucuronide)	-6.50	17.14
8-methylthiooctyl glucosinolate	-4.54	467.88
Alliin	-5.53	87.66
Ent-copalyl diphosphate	-6.73	11.62
Epijasmonic acid	-6.11	33.13
Gluconapin	-5.65	72.60
L-cystein s-oxide	-5.52	89.73
L-theanine	-5.31	127.43
L-tryptophan	-6.48	17.88
N-pentyl glucosinolate	-5.54	86.31
OPC-4-0	-7.11	6.12
Pollenitin	-7.22	5.14
Shikimic acid	-5.36	117.91
Sinigrin	-5.54	87.61
Torvanol A	-6.67	12.85
Turgorin	-5.57	83.01

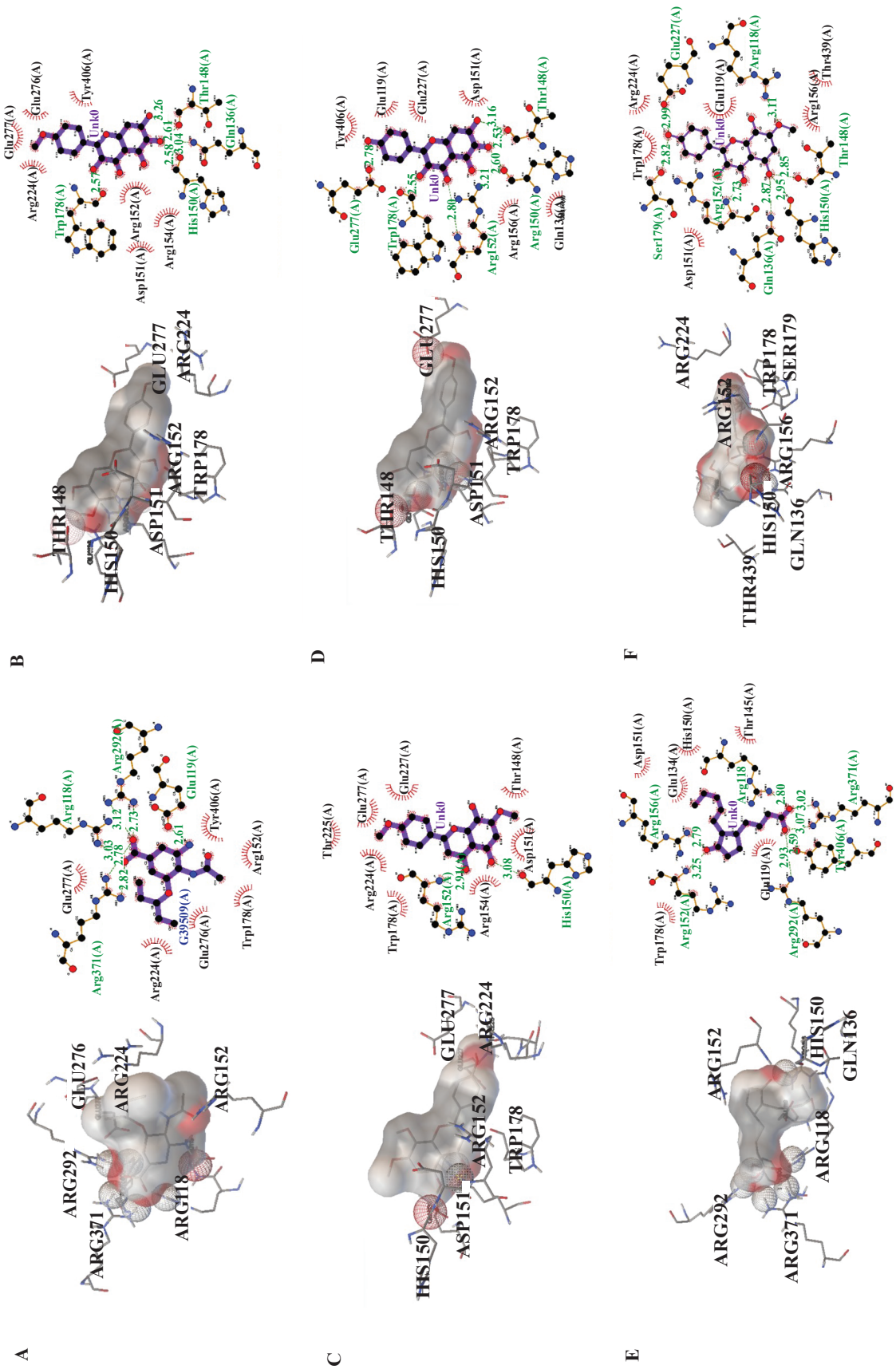

Figure 3. The representation of 3D (left) and 2D (right) interactions between the ligands and 4K1K protein. (A) Native ligand; (B) 3,5,6,7-tetrahydroxy-4'-methoxyflavone; (C) 5,8-dihydroxy-7,4'-dimethoxyflavone; (D) 6-hydroxykaempferol; (E) OPC-4:0; and (F) pollenitin.

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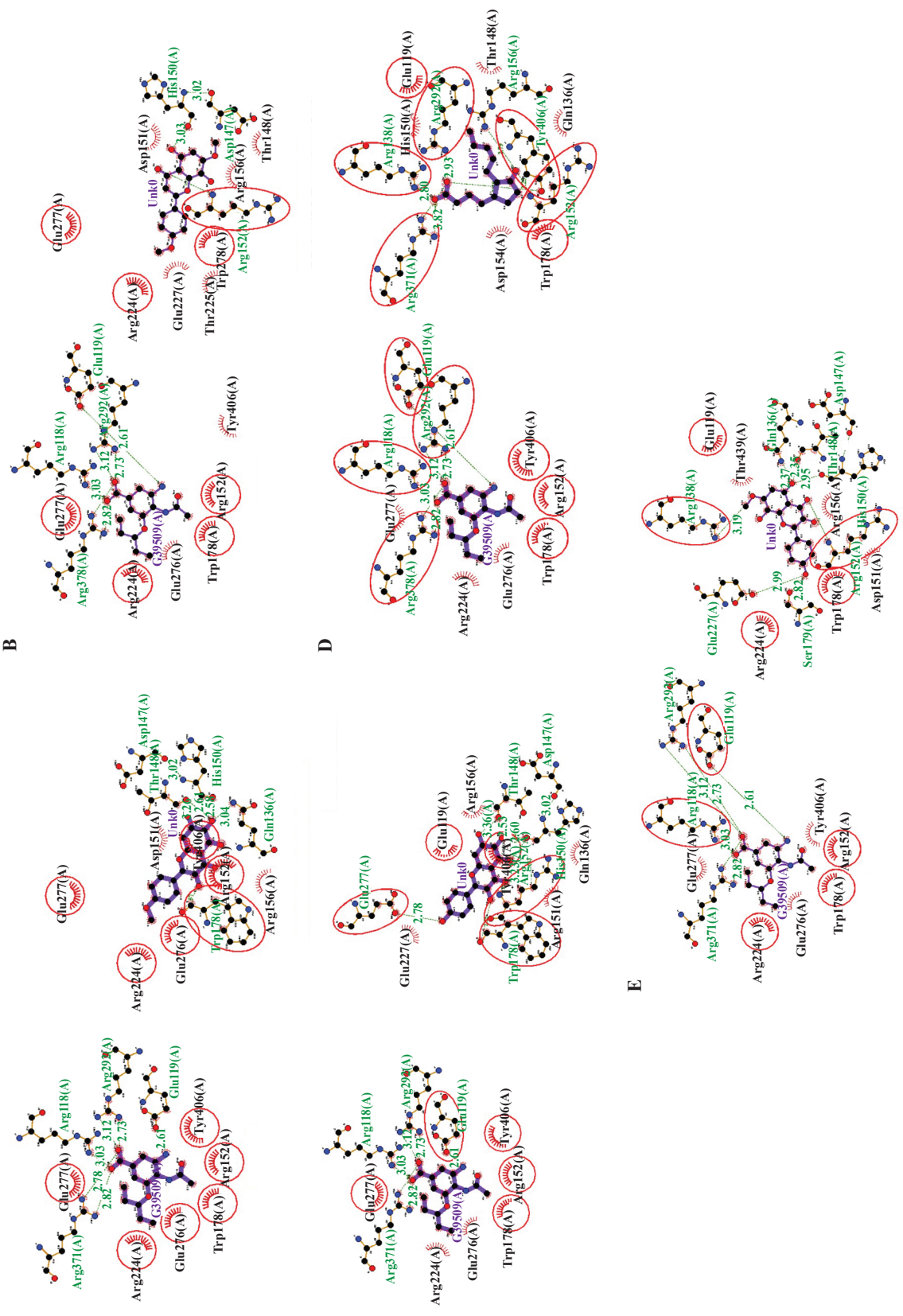

Figure 4. Overlapping residues between the native ligand (left) and the tested ligand (right) when interacting with the 4K1K protein. (A) 3,5,6,7-tetrahydroxy-4'-methoxyflavone; (B) 5,8-dihydroxy-7,4'-dimethoxyflavone; (C) 6-hydroxykaempferol; (D) OPC-4:0; and (E) pollenitin.

Table 4. Comparison of the interactions between native ligand and five best test ligands.

Residue	Native Ligand	Test Ligand				
		1	2	3	4	5
Glu277	√	√	√	√ H (2.78)	-	-
Arg371	√ H (2.82; 2.78)	-	-	-	√ H (3.02; 3.07)	-
Arg118	√ H (3.03)	-	-	-	√ H (2.80)	√ H (3.11)
Arg292	√ H (3.12; 2.73)	-	-	-	√ H (2.93)	-
Glu119	√ H (2.61)	-	-	√	√	√
Tyr406	√	√	-	√	√ H (2.59)	-
Arg152	√	√	√ H (2.91)	√ H (2.58)	√ H (3.25)	√ H (2.73)
Trp178	√	√ H (2.57)	√	√ H (2.55)	√	√
Glu276	√	√	-	-	-	-
Arg224	√	√	√	-	-	√
Asp151	-	√	√	√	√	√
Arg156	-	√	√	√	√ H (2.79)	√
His150	-	√ H (2.58)	√ H (3.08)	√ H (3.21; 2.60)	-	√ H (2.95)
Gln136	-	√ H (3.04)	-	√	√	√ H (2.87)
Thr148	-	√ H (3.26)	√	-	√	-
Thr148	-	√ H (2.61)	-	√ H (2.53; 3.16)	-	√ H (2.85)
Thr225	-	-	√	-	-	-
Glu227	-	-	√	√	-	√ H (2.99)
Thr439	-	-	-	-	-	√
Ser179	-	-	-	-	-	√ H (2.82)

*1: 3,5,6,7-tetrahydroxy-4'-methoxyflavone; 2: 5,8-dihydroxy-7,4'-dimethoxyflavone; 3: 6-hidroxykaempferol; 4: OPC-4-0; 5: Pollenitin; H: Hydrogen bond (ΔG).

From the validation results of the docking method, the 60×60×60 gridbox had the lowest RMSD value which showed better results among other gridboxes, therefore 60×60×60 gridbox was used for docking of all the hits ligands using Autodock software. The structures of 24 test ligands were retrieved from the PubChem database, which were then cleaned using MarvinSketch software.

From the results of the molecular docking of 24 test compounds, the ΔG score was obtained (Table 3). Five compounds that had the best ΔG values among the 24 tested compounds were pollenitin, -4:0, 6-hydroxykaempferol, 5,8-dihydroxy-7,4'-dimethoxyflavone, and 3,5,6,7-tetrahydroxy-4'-methoxyflavone, which were then selected for further analysis. To provide a better visualization of the interactions between the five best tested ligands and the neuraminidase 4K1K protein, 2D visualization was performed using LigPlot+ v.2.2.4, meanwhile 3D visualization was performed using AutoDock software. Overview of 2D and 3D interactions between the ligands and the protein were presented in Figure 3.

Furthermore, after describing the 3D and 2D interactions between the ligand and the protein, the interaction of overlapping residues or shared residues between the native ligand and the protein was then compared with the residues found in the interaction of the tested ligands and the protein. A description of the residues that had the same properties as the native ligand and the tested ligands when interacting with the 4K1K protein were presented in Figure 4 and Table 4.

Admetox dan Drug-likeliness

Toxicity of pollenitin, OPC-4:0, 6-hydroxykaempferol, 5,8-dihydroxy-7,4'-dimethoxyflavone, and 3,5,6,7-tetrahydroxy-4'-methoxyflavone were predicted with AdmetSAR. All compounds were predicted to have no carcinogenic and toxic properties. Drug-likeliness of the five

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Table 5. Analysis and prediction results of pharmacokinetics, toxicity and drug-likeliness.

Ligand	Drug likeness				Pharmacokinetics						Toxicity		
	MW	HBA	HBD	LogP	GI Abs	CYP Inhibitor					AMES	Carcinogenesis	AOT
						CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4			
3,5,6,7-tetrahydroxy-4'-methoxyflavone	316.26 g/mol	7	4	1.69	High	Yes	Yes	No	No	Yes	-	-	III
5,8-dihydroxy-7,4'-dimethoxyflavone	314.29 g/mol	6	2	2.48	High	Yes	No	Yes	Yes	Yes	-	-	III
6-hydroxykaempferol	302.24 g/mol	7	5	1.28	High	Yes	No	No	Yes	Yes	-	-	II
OPC-4-0	238.32 g/mol	3	1	2.68	High	No	No	No	No	No	-	-	III
Pollenitin	316.26 g/mol	7	4	1.74	High	Yes	No	No	Yes	Yes	-	-	III

*MW: Molecular weight; HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donor; GI Abs: Gastrointestinal absorption; AOT: Acute oral toxicity.

test compounds were analyzed using SwissADME (Table 5). Based on the Lipinski's rule of five, the molecular mass of a compound that could be used as a drug is <500g/mol, in which the five test compounds complied with the rules of molecular weight. Based on the number of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), the five test compounds also fulfilled the Lipinski's rule of five, namely having HBD<5 and HBA<10. Furthermore, the logP value which indicated the lipophilicity of a compound had a logP value limit in the range -0.4-5.6. Oral drugs must have a LogP <5, ideally between 1.35 and 1.8 for good oral and intestinal absorption according to Lipinski's rule of five. Thus, from the drug-likeliness analysis, the five tested ligand compounds fulfilled the Lipinski's rule of five.[26]

In the pharmacokinetic analysis, the five tested ligand compounds had high gastrointestinal (GI) absorption. However, only OPC-4:0 did not have CYP inhibitors, while the other four tested ligand compounds had CYP inhibitors, in which the inhibition of this enzyme could lead to toxicity or other negative side effects. The five tested ligands did not show any AMES toxicity, which indicates the mutagenicity and genetic toxicity of chemical compounds and carcinogenesis, but the acute oral toxicity (AOT) of 6-hydroxykaempferol showed higher results than the other four tested ligand compounds with AOT being included in category II, which means that the compound had a greater LD_{50} value of 50mg/kg but less than 500mg/kg, while the other four tested ligand compounds showed AOT results in category III, namely compounds with an LD_{50} value greater than 500 mg/kg but less than 5,000 mg/kg.

Discussion

Resistance is known to begin after treatment of H1N1, H3N2, H7N9 and H5N1 with NAI in humans.[17] Resistance to oseltamivir and zanamivir is a major concern in antiviral (influenza) research and offers a challenge for new drug researcher to design novel NAIs that are expected to outperform existing NAI drugs.[27,28]

NAI drugs, which work by binding to the viral NA active site, results in the major issues in terms of resistance, efficacy, and cost. Apart from that, side effects are also found in the nervous, respiratory, and digestive systems.[29] Therefore, it seems necessary to develop novel NAIs to combat influenza virus.

Several *in silico* studies to find novel NAIs have been reported before using various computational approaches towards N1 protein of H5N1 and H1N1 influenza.[30-32] In drug discovery, structure-based screening methods were considered as effective tools for the identification of active compounds.[33-35] Various studies have attempted to discover the potential of small molecule as antivirals through bioinformatics studies, including hit-and-lead discovery approaches, and analog synthesis. Virtual screening is capable of sorting large libraries of bioactive molecules which are expected to be potential candidate compounds as novel NAIs.

Protein preparation was carried out by obtaining the crystal structure of the neuraminidase protein with the inhibitor complex oseltamivir (4K1K), which was a neuraminidase protein of influenza A virus (A/RI/5+/1957(H2N2)). The protein complex was then extracted into LigandScout software to obtain pharmacophore features. The pharmacophore features associated with H_2O were removed. After obtaining the pharmacophore features, virtual screening is carried out using the ligand database. Omitted pharmacophore feature can be performed to optimize the virtual screening process on the ligand database with maximum 3 feature omitted in this study.

The results of this study indicated that OPC-4:0 was the best candidate among the other test compounds as an inhibitor of the N2-influenza protein. Based on the PubChem database, OPC-4:0 or (9r,13r)-1a,1b-dihomo-jasmonic acid, which can be found in common wheat, corn, and eggplant, is a member of the class of compounds known as cyclic ketones.[36] It contains ketones conjugated into cyclic moieties and considered as an octadecanoid lipid molecule which practically insoluble in water.

OPC-4:0 in the PubChem database is also described as a natural compound which is found in the sunflower plant (*Helianthus annuus*).[36] It has potential antimicrobial effects against different Gram positive and negative bacterial strains based on several previous studies. The antimicrobial activity of methanolic seed extract of *H. annuus* has been studied against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholera* with high antimicrobial activity was observed in *S. typhi*. Meanwhile, the moderate activities were found against *S. aureus* and *V. cholera*, and lower activity was found against *B. subtilis*.[37]

Besides the antimicrobial activity, jasmonic acid and its derivates have been reported to have the potential anti-inflammatory and anticancer activity as well. A study reported the inhibition of pro-inflammatory mediators (nitric oxide, interleukin-6 and tumor necrosis factor-α) production

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in vitro.[38] Although there are no reports that clearly report antiviral activity and explain the antiviral mechanism of OPC-4:0, especially as an influenza virus NAI, the results of this study highlight the potential for OPC-4:0 to be studied and investigated further. *In vitro* testing may be necessary to determine the activity and mechanism of OPC-4.0 in inhibiting the neuraminidase protein of the HxN2 influenza virus which is known that some of these viruses are capable of infecting humans and animals.

Conclusion

From this study, OPC-4:0 is found to be the best compound for the neuraminidase inhibitor based on its binding energy, pharmacokinetics, toxicity, and drug-likeliness. Thus, OPC-4:0 might be a potential candidate as a neuraminidase inhibitor of HxN2 virus.

Authors Contributions

DN and F were involved in concepting and planning the research, DN performed the data acquisition/collection, DN and F calculated the experimental data and performed the analysis, DN, F, NLPID drafted the manuscript and designed the figures, DN and F aided in interpreting the results. All authors took part in giving critical revision of the manuscript.

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