

Research Article

The Mutation Effect of Neuraminidase and Screening Bioactive Compound to Inhibiting Neuraminidase in H5N1

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ABSTRACT

Mutation that occur in the virus help to avoid the host's immune system and are passed on to the next generation and synthetic compounds begin to cause influenza viruses to be resistant. The purpose of this study was to determine the effect of mutations and inhibiting compounds for neuraminidase on the H5N1 virus. All DNA neuraminidase 1 (NA1) sequences from the H5N1 virus in 2016 were downloaded through NCBI and converted into protein sequences in ExPASy. Amino acids are aligned with Clustal Omega in EMBL-EBI and HOPE servers to predict mutation effects. Amino acid sequence from Subang is used to represent neuraminidase in Indonesia. Protein molecular modeling is done using Swiss Model and Fugue, the results of subsequent modeling in Ramachandra. Eight natural compounds from plants and two control compounds, zanamivir and oseltamivir were used as ligands, docked using PyRx and visualized with PyMOL and LigPlot⁺. As many as 11 amino acid sequences used there are 30 regions in sequences that are mutated and alter the function of neuraminidase which initially as exo-alpha-sialidase activity becomes hydrolase activity. The docking results show that rosmarinic acid, rosmanol, chlorogenic acid, oleonolic acid, carnosic acid, caryophyllene and capsaicin have binding affinity between zanamivir and oseltamivir. Visualization shows that rosmarinic acid, chlorogenic acid, rosmanol, and capsaicin compounds are predicted to replace zanamivir and oseltamivir. Because mutations in neuraminidase alter the function of neuraminidase from exo-alpha-sialidase activity into hydrolase activity and rosmarinic acid, chlorogenic acid, rosmanol, and capsaicin compounds are predicted to be inhibitors for NA1.

Keywords: Neuraminidase (NAI), mutation, bioactive compounds, exo-alpha-sialidase

Introduction

Influenza virus belong to the Orthomyxoviridae family, which has eight single-stranded RNA negative-sense segments. Orthomyxoviridae is divided into five genera: influenza A, influenza B, influenza C, isavirus, and thogotovirus [1]. From all of genera only influenza A [2], B and C viruses can infect humans [3]. Influenza A such as bird flu (H5N1) has a risk of zoonotic infections, host switches, and a significant cause of viral pandemic [4]. Until March 2011 cases of bird flu due to influenza A H5N1 strains killed as many as 18.72% of people in Indonesia [5].

Eight genes of influenza A virus encode 10 proteins: hemagglutinin (HA), neuraminidase (NA), matrix proteins M2 and M1, nonstructural protein NS1 and NS2 proteins, nucleocapsid, and three polymerases, PB1 (basic polymerase 1), PB2 (basic polymerase 2), and PA (acid polymerase) proteins [6]. Hemagglutinin (HA) and neuraminidase (NA) are two important glycoproteins that are responsible for influenza virus infection [7]. Hemagglutinin is located on the surface of the virion and is needed for infection, while neuraminidase serves to breakdown sialic acid (neuraminic acid) from infected cells [8].

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Both of these proteins have become targets in drug design research. Compared with hemagglutinin, neuraminidase is more used as a target to be inhibited because it has a high mutation rate [9] and has effectiveness in preventing the virus so that it does not spread in the host's body [10].

There are three factors that cause viruses to evolve, namely: large populations, short generation times, and high mutation rates. Every mutation that occurs helps the virus to avoid the host's immune system and is passed on to the next generation [9]. Mutations that occur in neuraminidase can cause changes in structure and function [11]. Some neuraminidase inhibitors such as oseltamivir (Tamiflu®, Roche, Basel, Switzerland) and zanamivir (Relenza®, Philadelphia, PA, USA), are antiviral drugs that are useful for the treatment of various forms of influenza [12]. The World Health Organization (WHO) recommends the use of oseltamivir or zanamivir for the treatment of the H5N1 virus. Oseltamivir and zanamivir are reversible competitive inhibitors of neuraminidase, thus preventing virions from being released from infected cells [13].

However neuraminidase inhibitors such as oseltamivir and zanamivir are approved for the treatment and prevention of influenza, more effective preventive drugs will be useful in slowing the spread of H5N1 influenza [12]. In addition, both drugs also cause resistension of the H5N1

virus [14]. Therefore, a better drug is needed that can prevent the spread of the virus.

Chemical compounds resulting from secondary metabolites in some plants have been used to treat several diseases such as influenza [15]. The effectiveness of this compound in reducing symptoms caused by influenza needs to be reviewed further. This efficacy comes from the specific binding mechanism or not to amino acid residues [12]. If these compounds selectively bind to specific targets then they have the potential to be used more widely in the prevention or treatment of H5N1 influenza. Therefore this study aims to find out the effect of mutations and inhibiting compounds for neuraminidase on the H5N1 virus.

Material and Methods

Mutation prediction

All DNA sequences from neuraminidase 1 (NA1) in 2016 (Additional file: Table S1) were downloaded through NCBI and converted into protein sequences in ExPASy. The amino acid sequence of the H5N1 virus is aligned using Multiple Sequence Alignment carried out using Clustal Omega in EMBL-EBI [16] (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) to find mutated regions. HOPE server [17] (www.cmbi.ru.nl/hope/) to predict mutation effects on NA1.

Table 1. Amino acid sequences of neuraminidase in Indonesia

Region	Year	Sequence
Subang	2016	QKQEFKMNPQNQKIVTIGSICMVIGIISLM- LQVGNIISIWVSHSIQTGNQHKTEPIRNTNFFTE- NAVASVTLAGNSSLCPKIGWAVH- SKDNSIRIGSKGDVFVIREPFISCSHLECRTFFLTQGALLNDKHSN- GTVKDRSPHRTLMSCPIGEAPSPYNSRFESVAWSA- SACHDGTSWLIIGISGPDNGAVAVLKYNGIITDTIKSWRNNIL- RTQESECVCVNGSCFTIMTDGPNNGQASYKIFKIE- KGKVVKSVELNAPNYHYEECSYCPDAGEIMCVCRDNWHGSRN- PWVSFNQNLEYQIGYICSGVFGDNPRPNDGTG- SCDPVSSNGAYGVKGFSEFKYNGVWIGRTKSTHSRSGFEMI- WDPNGWTGTDSEFSMKQDIVAITDWSGYSGS- FVQHPELTGLDCIKPCFWVELIRGRP KENTI- WTSGSSISFCGVNSDTVSWSPDGAELPFTIDKFIQKTPCFY

Majalengka	2016	QKQEFKMNPQNQKIVTIGSICMVIGIISLM- LQVGNIISIWVSHSIQTGNQHKTEPIRNTNFFTE- NAVASVTLAGNSSLCPIKGWAVH- SKDNSIRIGSKGDVVFVIREPFISCSHLECRTFFLTQGALLNDKHSN- GTVKDRSPHRTLMSCPIGEAPSPYNSRFESVAWSA- SACHDGTSWLIIGISGPDNGAVAVLKYNIGIITDTIKSWRNNIL- RTQESECVCVNGSCFTIMTDGPNNGQASYKIFKIE- KGKVVKSVELNAPNYHYEECSYCPDAGEIMCVCARDNWHGNSR- PWVSFNQNLEYQIGYICSGVFGDNPRPNDGTG- SCDPVSSNGAYGVKGFSEFKYGNVWIGRTKSTHSRSGFEMI- WDPNGWTGTDSEFSMKQDIVAITDWSGYSGS- FVQHPELTGLDCIRPCFWVELIRGRPKESTI- WTSGSSISFCGVNSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Kuningan	2016	QKQEFKMNPQNQKIVTIGSICMIIGIISLM- LQIGNIISIWVSHSIQTGNQHQTPIRNTNSLTENAVA- SATLAGNSSLCPIKGWAVH- SKDNSIRIGSKGDVVFVIREPFISCSHLECRTFFLTQGALLNDKHSN- GTVKDRSPHRTLMSCPIGEAPSPYNSRFESVAWSA- SACHDGTSWLIIGISGPDNGAVAVLKYNIGIITDTIKSWRNNIL- RTQESECACVNGSCFTIMTDGPSNGQASYKIFKIE- KGKVVKSVELDAPNYHYEECSYCPDAGEIICVCARDNWHGNSR- PWVSFNQNLEYQIGYICSGVFGDNPRPNDGTG- SCGPVSSNGAYGVKGFSEFKYGNVWIGRTKSTHSRSGFEMI- WDPNGWTGTDSEFSMKQDIVAVTDWSGYSGS- FVQHPELTGLDCIRPCFWVELIRGRPKESTI- WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Pati	2016	QKQEFKMNPQNQKIITIGSICMAIGIISLM- LQIGNIISIWVSHSIQTGNQHQTPIRNTNSLTENAVASVTLTGNSSL CPIKGWAVHNSKDNSIRIGSKGDVVFVIREPFISCSHLECRT- FFLTQGALLNDKHSNGTVKDRSPYRTLMSCPIGEAP- SPYNSRFESVAWSASACHDGTSWLIIGISGPDNGA- VAVLKYNIGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGP- SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSYCPDAGE- IICVCARDNWHGNSRNPWVSFNQNLEYQIGYICSGVFGDNPRP- NDGTGSCGPVSSNGAYGVKGFSEFKY- NGVWIGRTKSTHSRSGFEMIWDPNGWTGTDSDFSIKQDI- VALTGWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTI- WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Sleman	2016	QKQEFKMNPQNQKIITIGSICIAIGIISLM- LQIGNIISIWVSHSIQTGNQHQTPIRNTNSLTENAVASVTLTGNSSL CPIKGWAVHNSKDNSIRIGSKGDVVFVIREPFISCSHLECRT- FFLTQGALLNDKHSNGTVKDRSPYRTLMSCPIGEAP- SPYNSRFESVAWSASACHDGTSWLIIGISGPDNGA-

		VAVLKYNIGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGP-SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSCTPDAGE-IICVCRDNWHGSGNRPWVSFNQNLEYQIGYICSGVFGDNPRP-NDGAGSCGPVSSNGAYGVKGFSFKYG-NGVWIGRTKSTHSRSGFEMIWDPNGWTGTDSEFSIKQDIVALT-DWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTI-WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Sukoharjo	2016	QKQEFKMNPQNKIITIGSICMAIGIISLM-LQIGNIISIWVSHSIQTGNQHQTEPIRNTNSLTENAVASVTLTGNSSLCPIKGWAVHSDKNSIRIGSKGDVVFVIREPFISCSHLECRT-FFLTQGALLNDKHSNGTVKDRSPYRTLMSCPIGEAP-SPYNSRFESVAWSASACHDGTSWLIIGISGPDNGA-VAVLKYNIGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGP-SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSCTPDAGE-IICVCRDNWHGSGNRPWVSFNQNLEYQIGYICSGVFGDNPRP-NDGTGSCGPVSSNGAYGVKGFSFKYG-NGVWIGRTKSTHSRSGFEMIWDPNGWTGTDSEFSIKQDIVALT-DWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTI-WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Kediri	2016	QKQEFKMNPQNKIITIGSICMVIGIISLM-LQIGNIISIWVSHSIQTGNQHQTEPIRNTNSLTENAVASVTLAGNSSLCPIKGWAVHSDKNSIRVGSKGDVVFVIREPFISCSHLECRT-FFLTQGALLNDKHSNGTVKDRSPYRTLMSCPIGEAP-SPYNSRFESVAWSASACHDGTSWLIIGISGPDNGA-VAVLKYNIGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGP-SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSCTPDAGE-IICVCRDNWHGSGNRPWVSFNQNLEYQIGYICSGVFGDNPRP-NDGTGSCGPVSSNGAYGVKGFSFKYG-NGVWIGRTKSTHSRSGFEMIWDPNGWTGTDSEFSMKQDIVA-ITDWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTI-WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Banyuwangi	2016	QKQEFKMNPQNKIITIGSICMVIGIISLM-LQIGNIISIWVSHSIQTGNQHQTEPIRNTNSLTENAVASVTLAGNSSLCPIKGWAVHSDKNSIRIGSKGDVVFVIREPFISCSHLECRT-FFLTQGALLNDKHSNGTVKDRSPYRTLMSCPIGEAP-SPYNSRFESVAWSASACHDGTSWLVIGISGPDNGA-MAVLKYNIGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGP-SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSCTPDAGE-IICVCRDNWHGSGNRPWVSFNQNLEYQIGYICSGVFGDNPRP-NDGTGSCGPVSSNGAYGVKGFSFKYGNIGWIGRTKSTHSRSGFEMIWDPNGWTGTDSEFSMKQDIVAITDWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTI-

		WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Denpasar	2016	QKQEFKMNPQNQKIITIGSICMVIGIISLM- LQIGNIISIWVSHSIQTGNQHQTEPIRNTNSLTENAVASVTLAGNSSL CPIKGWAVHNSKDNSIRIGSKGDFVIREPFISCSHLECRT- FFLTQGALLNDKHSNGTVKDRSPYRTLMSCPIGEAP- SPYNSRFESVAWSASACHDGTSWLVIGISGPDNGA- VAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDGP- SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSYCPDAGE- IICVCRDNWHGNSRNPWVSFNQNLEYQIGYICSGVFGDNPRP- NDGTGSCGPVSSNGAYGVKGFSFKYGNIGWIGRTKSTHSRSGFEM- IWDPNGWTGTDSEFSMKQDIVAITDWSGYSGS- FVQHPELTGLDCIRPCFWVELIRGRPKESTI- WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Lampung Utara	2016	QKQEFKMNPQNQKIVTIGSICMVIGIISLM- LQIGNIISIWVSHSIQTGNQHQTEPIRNTNFLTENAVASVTLAGNSSL CPIKGWAVH- SKDNSIRIGSKGDFVIREPFISCSHLECRNFFLTQGALLNDKHSN- GTVKDRSPHRTLMSCPIGEAPSPYNSRFESVAWSA- SACHDGTSWLIIGISGPDNGAVAVLKYNGIITDTIKSWRNNIL- RTQESECACVNGSCFTVMTDGPSNGQASYKIFKIE- KGKVVKSVELDAPNYHYEECSYCPDAGEIICVCRDNWHGNSR- PWVSFNQNLEYQIGYICSGVFGDNPRPNDGTG- SCGPVSSNGAYGVKGFSFKYGNVWIGRTKSTHSRSGFEMI- WDPNGWTGTDSEFSMKQDIVAITDWSGYSGS- FVQHPELTGLDCIRPCFWVELIRGRPKESTI- WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY
Lamongan	2016	QKQEFKMNPQNQKIVTIGSICMVIGIISLM- LQIGNIISIWVSHSIQTGNQHQTEPIRNTNSLTENAVASVTLAGNSSL CPIKGWAVHNSKDNSIRIGSKGDFVIREPFISCSHLECRT- FFLTQGALLNDKHSNGTVKDRSPHRTLMSCPIGEAP- SPYNSRFESVAWSASACHDGTSWLIIGISGPDNGA- VAVLKYNGIITGTIKSWRNNILRTQESECACVNGSCFTVMTDGP- SNGQASYKIFKIEKGKVVKSVELDAPNYHYEECSYCPDAGE- IMCVCRDNWHGNSRNPWVSFNQNLEYQIGYICSGVFGDNPRP- NDGTGSCGPVSSNGAYGVKGFSFKY- NGVWIGRTKSTHSRSGFEMIWDPNGWTGTDSEFSIKQDIVA- ITDWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTI- WTSGSSISFCGVSSDTVSWSWPDGAELPFTIDKFIQKTPCFY

Protein Preparation and Protein Modeling

DNA sequence from Subang, West Java (KY614882.1), which have been converted into protein sequence are used to represent neuram-

inidase proteins in Indonesia. Protein molecular modeling is carried out using the Swiss Model [18] and Fugue [19]. The results of the next modeling in Ramachandra to see the best modeling.

Ligand preparation

Eight natural plant compounds and two control compounds, zanamivir and oseltamivir were used as ligands. The ligands used are compounds that have the ability as medicinal compounds based on the Lipinski's rule of five [20], antiviral based on the PASS Online [21], the ability to be absorbed by the intestine based on the HIA (Human Absorption Intestine) test on Pre-ADMET [22] and toxicity with PROTOX-II Web server [23].

Molecular Docking

The target protein and ligands are docked using PyRx software [24] so that binding affinity values are generated. Then the docking results are visualized with PyMol [25] to see the position of the ligand on the protein and Ligplot⁺ [26] to see the chemical bond between ligand on the protein.

Result and Discussion

Mutation analysis in amino acid sequences of NA1

Neuraminidase 1 (NA1) from the H5N1 virus is very important for the process of releasing the virus from its host cell [8] and the protein is targeted by antiviral [10]. Information about the effects of mutations on NA1 is very important in the process of drug design and development [9]. Comparing and classifying structures of proteins is very important in understanding the function of proteins [17].

A total of 11 sequences from NAI in 2016 carried out multiple alignment using Clustal Omega in EMBL-EBI [16]. Based on the results of multiple sequence alignment (MSA) there are 30 regions in the sequence that have mutations (Table 2). These changes cause changes to the secondary and tertiary structures which result in changes in the function of the protein [17]. Mutation analysis was carried out using the HOPE server and the results of the analysis showed that mutations occurred at 11 amino acid positions causing a change in function on NA1 proteins.

Table 2. Mutations in the amino acid sequence of neuraminidase (NA1)

No.	Original Amino Acid	Mutation amino acid	Location
1.	Valine (V)	Isoleucine (I)	14
2.	Metionin (M)	Isoleucine (I)	21
3.	Valine (V)	Isoleucine (I), Alanine (A)	22
4.	Valine (V)	Isoleusin (I)	32
5.	lysine (K)	Glutamine (Q)	51
6.	Phenylalanine (F)	Serine (S)	60
7.	Phenylalanine (F)	Leucine (L)	61
8.	Valine (V)	Alanine (A)	69
9.	Alanine (A)	Threonine (T),	72
10.	Isoleucine (I)	Valine (V)	94
11.	Threonine (T)	Asparagine (N)	117
12.	Histidine (H)	Tyrosine (Y),	141
13.	Isoleucine (I)	Valine (V)	178
14.	Valine (V)	Methionine (M)	189
15.	Aspartic Acid (D)	Glycine (G)	200
16.	Valine (V)	Alanine (A)	218
17.	Isoleucine (I)	Valine (V)	227
18.	Asparagine (N)	Serine (S)	233

19.	Asparagine (N)	Aspartic Acid (D)	256
20.	Methionine (M)	Isoleucine (I)	275
21.	Threonine (T)	Alanine (A)	318
22.	Aspartic Acid (D)	Glycine (G)	322
23.	Valine (V)	Isoleucine (I)	343
24.	Glutamic Acid (E)	Aspartic Acid (D)	372
25.	Methionine (M)	Isoleucine (I)	375
26.	Isoleucine (I)	Valine (V), Leucine (L)	382
27.	Aspartic Acid (D)	Glycine (G)	384
28.	Lysine (K)	Arginine (R)	405
29.	Asparagine (N)	Serine (S)	420
30.	Asparagine (N)	Serine (S)	435

The initial function of NA1 is as α -exo-sialidase activity which catalyzes α -(2 $\rightarrow$ 3), α -(2 $\rightarrow$ 6), α -(2 $\rightarrow$ 8)-glycosidic linkage terminal sialic residues in oligosaccharides, glycoproteins, glycolipids, colominic acid and synthetic substrates, change function into hydrolase activity which catalyzes the hydrolysis of various bonds, for example C-O, C-N, C-C, phosphate anhydride bonds, etc. [27].

Docking of Natural Compounds, Oseltamivir and Zanamivir as NA1 Inhibitors

The H5N1 virus is not easy to overcome because of the ability of viruses that begin to resist synthetic drugs [14]. Oseltamivir-resistant H5N1 virus was found in Vietnam [28], this alarming situation was followed by findings elsewhere, with resistance to amantadine [29]. Thus, the H5N1virus gene responsible for resistance to oseltamivir, peramivir, zanamivir, and laninamivir was also found [30]. This causes new drugs to be needed from natural compounds [31].

There are eight ligands that have been tested on swine flu [15], namely capsaicin, carnosic acid, caryophyllene, chlorogenic acid, gingerol, oleonolic acid, rosmanol, and rosmarinic acid and two control compounds, oseltamivir and zanamivir (Table 3). Ten of these compounds have been tested for drug sensitivity with lipinski [20], Human Intestine Absorption (HIA) with Pre-ADMET [21], toxicity with PROTOX-II [22], and antiviral activity with PASS Online [23] to be used as drugs will be seen the ability to inhibit NA1 through docking.

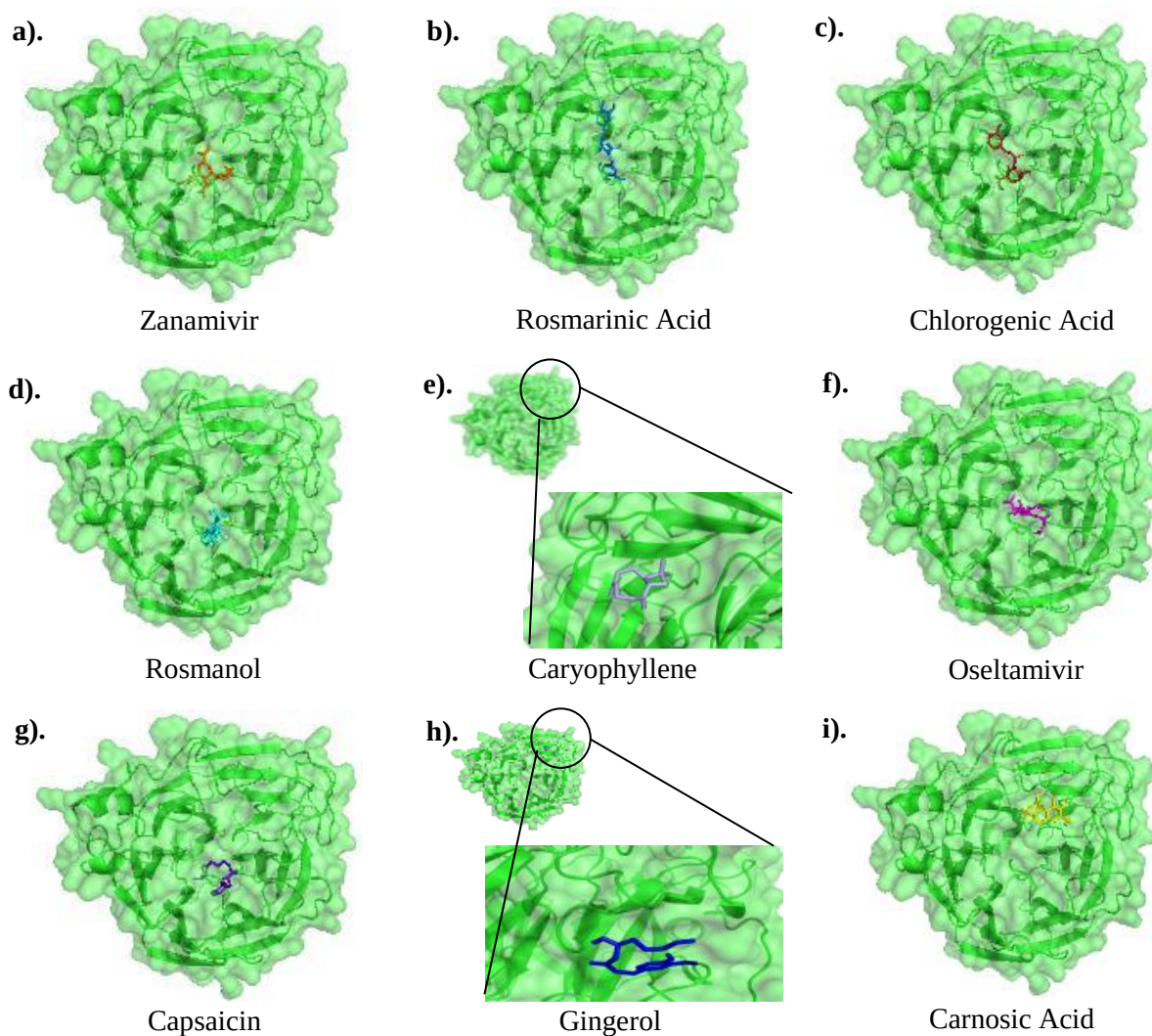
The drug-likeness test shows that all compounds include drug compounds because they meet Lipinski rules [20]. The PASS Online test results show that capsaicin compounds, carnosic acid, caryophyllene, chlorogenic acid, gingerol, oleonolic acid, rosmanol, and rosmarinic acid have inhibitory activities against rhinovirus viruses, adenoviruses, coronaviruses, and picornaviruses. Based on that, these compounds are predicted to be able to inhibit NA1 that has not or has mutated, besides that these compounds have been tested to neuraminidase from swine flu and have inhibitory ability. The HIA test results of rosmarinic acid and chlorogenic acid compounds are quite capable of being absorbed, while rosmanol, oleonolic acid, carnosic acid, caryophyllene, and capsaicin can be absorbed perfectly [32]. These things indicate that these natural compounds can be absorbed by the body without leaving a residue that can harm the human body when consuming it Toxicity test with PROTOX-II capsaicin compound entered in class 2 with toxicity dose LD50 47 mg/kg which was fatal when swallowed. Gingerol compounds enter in class 3, the dose of toxicity is 250 mg/kg toxic when swallowed. Carnosic acid compounds, chlorogenic acid, oleonolic acid, and rosmanol with toxicity doses, respectively LD50 450 mg/kg, LD50 1190 mg/kg, LD50 2000 mg/kg, and LD50 450 mg/kg, which are dangerous if swallowed. Whereas Caryophyllene and rosmarinic acid were included in class 5 with a toxicity dose of LD50 1050 mg/kg for caryophyllene and LD50 5000 mg/kg for rosmarinic acid. Class 5 may be dangerous if swallowed [22].

Table 3. Potential Natural compounds in inhibition NA1

		Molecular	Hydrogen	Hydrogen		Molar Re-	ΔG	
Zanamivir	60855	332	9	11	-3.79	76.893684	-7.8	4.07
Rosmarinic Acid	5281792	360	5	8	1.76	89.796974	-7.6	62.49
Rosmanol	13966122	346	3	5	3.03	90.567368	-7.5	88.07
Chlorogenic Acid	1794427	354	6	9	0.65	82.518768	-7.2	20.43
Oleonolic Acid	10494	456	2	3	7.23	132.68155	-7.0	96
Carnosic Acid	65126	332	3	4	4.08	91.684372	-6.1	92.52
Caryophyllene	5281515	204	0	0	4.73	66.742981	-5.9	100
Capsaicin	1548943	305	2	4	3.79	88.951469	-5.8	92.49
Oseltamivir	65028	312	3	6	1.29	84.156075	-5.5	87.16
Gingerol	442793	294	2	4	3.23	82.752571	-5.4	91.97

Visualization of the interaction between ligand and NA1 shows several positioning positions on NA1 (Figure 1). Based on the location of controls, namely zanamivir and oseltamivir, there are four natural compounds that have the same at-

tachment, rosmarinic acid, chlorogenic acid, rosmanol, and capsaicin. These compounds are predicted to be able to replace control drugs, zanamivir and oseltamivir to inhibit NA1.



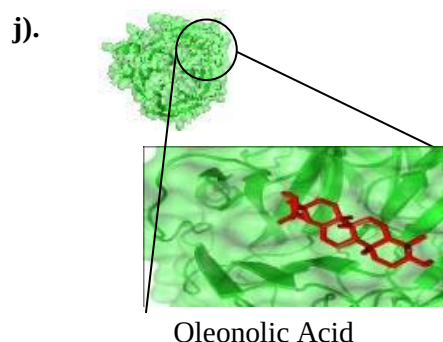


Figure 1. Interaction between ligands on NA1. a) Zanamivir and NA1, b) Rosmarinic acid and NA1, c) Chlorogenic acid and NA1, d) Rosmanol and NA1, e) Caryophyllene and NA1, f) Oseltamivir on NA1, g) Capsaicin on NA1, h) Gingerol on NA1, i) Carnosic acid on NA1, and j) Oleonolic acid on NA1.

This is because the attachment of natural compounds in the same position to the control compounds is predicted to be able to mimic the NA1 inhibition process by control compounds [33].

Interactions that occur between control compounds and some natural compounds have chemical bonds that have similarities in some amino acid residues from NA1 which bind through hydrogen bonds. Natural compounds that have the same bond with control are: rosmarinic acid, rosmanol, chlorogenic acid, and capsaicin. Rosmarinic acid has the same hydrogen bond as zanamivir, which is a residue of amino acids Tyr330 and Arg354 and Tyr330 with oseltamivir. Rosmanol has the same hydrogen bond as zanamivir, which is an amino acid residue of

Arg138 and Tyr388 and has no similarity to oseltamivir. Chlorogenic acid has the same hydrogen bond as zanamivir, which is an amino acid residue of Trp165 and Arg354 and has no similarity to oseltamivir. Capsaicin has the same hydrogen bond as zanamivir, which is an amino acid residue of Asn233 and has nothing in common with oseltamivir. In addition, the hydrogen bond length of the rosmarinic acid, rosmanol, chlorogenic acid, and capsaicin compounds has a shorter hydrogen bond length compared to the control. Therefore, the four compounds are thought to interact with NA1 like control compounds because they have bonds with the same amino acid residues and short hydrogen bonds [33].

Table 4. Chemical bond between ligands and NA1

Compound	Interaction
Zanamivir	Hydrogen bond (length): Arg138 (2.83 Å), Trp165 (2.92 Å), Asn233 (3.33 Å), Asn281 (3.07 Å), Tyr330 (3.01), Tyr338 (3.11 Å), Arg354 (3.23), Hydrophobic bond: Arg104, Glu105, Asp137, Ile209, Arg211, Glu214, Glu263, Glu264
Rosmarinic Acid	Hydrogen bond (length): Arg104 (2.85 Å), Glu214 (2.76 Å), Arg279 (3.21), Tyr330 (3.00 Å), Arg354 (2.80 Å) Hydrophobic bond: Glu105, Asp137, Glu264, Tyr388, Ile413, Pro417, Lys418
Rosmanol	Hydrogen bond (length): Arg138 (2.83 Å), Tyr388 (3.07 Å), Hydrophobic bond: Asp137, Ile209, Arg211, Glu214, Asn233, Glu263, Glu264, Arg279, Tyr330

Chlorogenic Acid	Hydrogen bond (lenght): Trp165 (2.81 Å), Glu214 (3.30 Å), Glu264 (2.27 Å), Arg279 (2.98 Å), Arg354 (3.06 Å), Arg426 (2.70 Å) Hydrophobic bond: Arg104, Glu105, Val135, Asp137, Ser166, Glu263, Arg211, Tyr330, Tyr388, Pro414, Thr424
Oleonolic Acid	Hydrogen bond (lenght): - Hydrophobic bond: Pro79, Ile80, Tyr339, Asn341, Trp344, Trp361
Carnosic Acid	Hydrogen bond (lenght): Lys148 (2.98 Å), His352 (2.77 Å) Hydrophobic bond: Asn311, Pro312, Tyr330, Ser353, Arg354, Pro417
Caryophyllene	Hydrogen bond (lenght): - Hydrophobic bond: Pro79, Ile80, Tyr339, Gly340, Trp344, Trp361
Capsaicin	Hydrogen bond (lenght): Ser166 (3.21 Å), Glu214 (3.13 Å), Asn 233 (3.19 Å) Hydrophobic bond: Glu105, Asp137, Arg138, Arg142, Trp165, Ile209, Arg211, Thr212, Glu264, Arg279, Tyr330, Tyr388
Oseltamivir	Hydrogen bond (lenght): Asn281 (3.20 Å), Tyr330 (2.81 Å), Asp137 (3.02 Å) Hydrophobic bond: Arg104, Val135, Arg211, Asn233, Glu263, Arg279, Tyr388
Gingerol	Hydrogen bond (lenght): Tyr339 (2.85 Å), Asn341 (3.30 Å), Trp344 (2.90 Å) Hydrophobic bond: Ile80, Gly340, Trp361, Pro363

Conclusion

Mutations in neuraminidase (NA1) cause a change in function from exo-alpha-sialidase activity to hydrolase activity. Mutated amino acid residues are docked with eight natural compounds and two control compounds. Natural compounds such as rosmarinic acid, rosmanol, chlorogenic acid and capsaicin are predicted to inhibit the activity of the NA1 enzyme on H5N1 compared to carnosic acid, caryophyllene, gingerol, and oleonolic acid.

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