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Original Article

Identification of Linked NS2B-NS3 Protease Conformation of the Zika Virus with Allosteric Bioactive Compound Antagonists

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ABSTRACT

The quickly spreading of diseases that are triggered by Zika virus such as Guillain-Barre syndrome and microcephaly in infants making Zika virus becomes one of the International Public Health Emergency concern. The aim of this study was to analyze the best candidate ligands which can be used as inhibitors of linked NS3-NS2B protease of Zika virus in silico. Inhibitory activity assay of 10 bioactive compounds against linked NS3-NS2B protein of Zika virus (PDB ID: 5H4I) was done by Autodock Flexible Receptor (ADFR). The best molecule as a candidate competitive inhibitor of linked NS3-NS2B protease catalytic triad was PHA-690509 (-11.71 kcal/mol) with the inhibition constant (Ki) value was 0.002553 µM. Understanding in silico virtual screening approaches will be important in improving the discovery of effective inhibitors of Zika viral targets.

Keywords: NS3-NS2B protease, Zika virus, inhibitors, molecular docking.

1. INTRODUCTION

The widely-spread of Zika virus during a short time makes it a particular concern of WHO to declare ZIKV (Zika Virus) as a Public Health Emergency due to an outbreak in the world in 2016 [1]. ZIKV infection can trigger neurological disorder known as Guillain-Barre syndrome [2]. ZIKV fetal infection is also associated with an increase in cases of abnormalities and microcephaly in infants [3]. ZIKV is directly infecting human neural progenitor cells (hNPCs), which will increase cell death and disrupt cell cycle. This resulted in a growth of hNPC disorder more severe [4].

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The transmissions of Zika virus including by mosquito *Aedes sp*., sexual, transplacental transmission and blood transfusions [1]. Despite knowing the media transmission of this virus, it does not rule out the existence of another media transmission of the virus that has not been found. Urgency in making the drug candidates is very high due to there is no vaccine or antiviral Zika available to date [5].

Genome of Zika virus (Family *Flaviridae*) is translated into a single polyprotein and will be cleaved by host-proteases (proteolytic cleavage) into three structural proteins (capsid, pre-membrane/membrane, and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [6]. NS3 protein is an essential component required for the proteolytic process of polyprotein to perform the process of viral replication [7].

NS3 protease has a catalytic triad (His51, Asp75 and Ser135), where NS2B, an essential cofactor, attached to the side of that catalytic triad for the process of catalysis, folding and protease activity [5, 6]. Catalytic triad (His51, Asp75 and Ser135) is located in the gap between the two β-barrels, in which two β-barrels are surrounded by NS2B [8].

Crystal form of NS3-NS2B protease generally is an 'open conformation' wherein the flexible part NS2B shows only limited interaction with NS3 protease. NS3 protease is generally in an inactive state and by binding with NS2B protein, will create enhancement of enzymatic activity 3,300-6,600 time [9]. When inhibitor is attached to the catalytic triad, it will induce a conformational change of NS2B, produce a more solid and 'closed' form [6, 7].

Cn716 ligand is an inhibitor containing boronic acid group and its form resembles a peptide. Boron atom will form a covalent bond with Oγ side of the catalytic Ser135. Peptide boronic acid cn716 is not cytotoxic to Huh7 cells (human liver cells). Other peptides which also have boronic acid groups (bortezomib) are used as a treatment of multiple myeloma. Previous study reported that cn716 ligand attached to the side of catalytic triad affected conformation change of NS3pro-NS2B ZIKV and configuration of the loop on the structure reveals that it changed into auto-inhibitory mode [8]. Here are 10 ligand candidates with description shown on Table 1.

Table 1: Ten bioactive compound candidates with descriptions

No	Ligand	Description
1.	Amodiaquine	Antimalarial drugs can inhibit the replication and DENV infection type 2 with unknown mechanism [10].
2.	Berberine	Alkaloid from *Hydrastis Canadensis*, family Berberidaceae, *Argemone mexicana, Xanthorhiza simplicissima, Phellodendron amurense, Coptis chinensis, Tinospora cordifolia,*and *Eschscholzia californica* [6, 11].
3.	Bisabolol	Substitute of essential oil from *Populus balsamifera*. Found in German chamomile flower [12].
4.	4-nitrophenyl 4-guadininobenzoat	Active side of protease inhibitors of Dengue virus (DENV) and have not yet been targeted to the ZIKV catalytic triad [13].
5.	Niclosamide	Food and Drug Administration (FDA) approved drug to fight worm infection in humans. One of the combination drug candidates which are targeted to ZIKV NS1 protein [6, 14].
6.	NITD008	Adenosine analog inhibitor produces antiviral activity to DENV NS5 RNA-dependent RNA polymerase (RdRP) protein by *in vitro* and *in vivo* [15].
7.	Prochlorperazine	FDA approved drug and shows protective effect against DENV infection (effective *in vivo* or *in vitro*) [6].
8.	Quecertin	Flavonoid molecule which found in many food variations, such as apple, berries, Brassica vegetable, onion, nuts, flower and seeds [13, 16].
9.	Quinacrine	Antimalarial compound shows high anti-prion activity to cell [6, 17].
10.	PHA690509	Cyclin-dependent kinase inhibitor and suppress production of particle ZIKV NS1 at submicromolar concentration [14].

2. MATERIALS AND METHODS

2.1 Receptor Model

Receptor protein structure (PDB ID: 5H4I, in .pdb file format) was obtained from Protein Data Bank [18]. This protein has 231 residues and a catalytic triad side. Water molecule and fragments those exist at receptor were removed using Discovery Studio 2016 Client [19]. Protein structure was refined using Kobamin [20] and compared with the structure of protein before refinement using Protein Structure Validation Suite [21].

2.2 Ligand Preparation

The structure of the ligands were obtained from PubChem [22] and DrugBank [23] in .sdf file format. Ligands were converted into .pdb format using GUI OpenBabel [24]. If there is no ligand structure in PubChem, ligand can be drawn manually using ChemSketch [25], and optimized into 3D structure using Avogadro [26]. Pharmacokinetic properties of all the ligands were tested using SwissADME [27].

2.3 Molecular Docking

In Silico Docking Running Autogrid Flexible Recept 1.0 (AGFR) [28] by using pocket fills (AutoSite) number 5 and 7. Size of box site parameters appears automatically and its specificity was increased by choosing flexible side to catalytic triad residue (B: HIS51, ASP75, SER135). Result of AGFR map in .zipfile was resumed in AutoDock Flexible Receptor (ADFR) [29] using Command Prompt. The interaction of AGFR result was visualized usingAutoDock Tools [30] and PyMOL Viewer [31]. SetAntialiasing value was used to determine the quality of the image where the default value was 0. From binding energy, inhibition constant (Ki) can be calculated using equation [32]:

$$\Delta G = -RT \ln K_A \qquad K_A = K_i^{-1} = \frac{[EI]}{[E][I]}$$

$$K_i = e^{\left(\frac{\Delta G}{RT}\right)}$$

3. RESULTS AND DISCUSSION

Table 2: Pharmacokinetic properties by Swiss ADME

No.	Compounds	Molecular weights (g/mol)	#H-bond donors	Consensus Log P	#H-bond acceptors	GI absorption	Solubility in water
1	Amodiaquine	355.86	2	4.22	3	High	Moderately soluble
2	Berberine	336.36	0	2.53	4	High	Moderately soluble
3	Bisabolol	222.37	1	3.74	1	High	Soluble
4	4-nitrophenyl 4-guanidino benzoate	300.27	2	1.14	5	High	Soluble
5	Niclosamide	327.12	2	2.99	4	High	Moderately soluble
6	NITD008	290.27	4	-0.75	6	Low	Very soluble
7	Prochlorperazine	373.94	0	4.06	2	High	Moderately soluble
8	Quercetin	302.24	5	1.22	7	High	Soluble
9	Quinacrine	399.96	1	5.13	3	High	Moderately soluble
10	PHA690509	333.45	3	2.51	2	High	Soluble

In this study, ten ligands were observed using SwissADME and they follow the rules of Lipinski, including: molecular weight is not more than 500 Da, hydrogen bond donor is not more than 5, the hydrogen bond acceptor is not more than 10, and log p-value is not more than 5. GI absorption pharmacokinetic test for all ligands except NITD008 show high values as shown on Table 2.

Pharmacokinetic studies showed that NITD008 GI absorption is in low level in plasma when taken orally. But the value of this absorption can be enhanced by covalently linking isobutyrate acid to the 3' and 5' hydroxyl groups of ribose via ester linkages to NITD008 [32]. Ramachandran plot results showed the percentage of considerable value between the most favored regions before (87.7%) and after refinement (92.9%). Therefore, the linked NS2B-NS3 protease with refinement was used in this study.

Table 3: Result of protein binding energy docking (kcal/mol) with related residue interactions

No.	Ligand	Binding Energy (kcal/mol)	Hydrophobic interactions with protein residues
1	Amodiaquine	-10.63	His51, Asp75, Asp83, Tyr130, Pro131, Ser135, Tyr150, Gly151, Asn152, Gly153, Tyr161.
2	Berberine	-7.14	His51, Asp75, Asp83, Phe84, Ser85, Leu86, Asn152, Gly153,Val154, Val155.
3	Bisabolol	-8.06	Asp75, Asp83, Asn152, Gly153,Val154, Val155, Tyr161.
4	4-nitrophenyl 4-guanidinobenzoat	-9.92	Asp83, Phe84, Ser85, Leu86, Asn152, Gly153, Val155.
5	Niclosamide	-9.30	His51, Lys54, Tyr130, Ala132, Ser135, Tyr150, Gly151, Tyr161.
6	NITD008	-8.87	Asp83, Phe84, Gly151, Asn152, Gly153, Val154, Val155, Tyr161.
7	Prochlorperazine	-11.09	His51, Asp75, Gly82, Asp83, Tyr130, Pro131, Ala132, Ser135, Gly151, Asn152, Tyr161.
8	Quercetin	-9.66	His51, Asp83, Phe84, Tyr130, Ala132, Ser135, Gly151, Asn152, Gly153, Tyr161.
9	Quinacrine	-10.80	His51, Tyr130, Ser135, Tyr150, Gly151, Asn152, Gly153, Val155, Tyr161.
10	PHA-690509	-11.71	His51, Asp75, Tyr130, Ser135, Tyr150, Gly151, Asn152, Gly153, Tyr161.

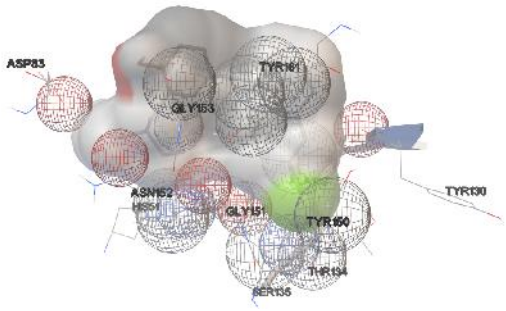

Fig 1: Docking result of protein and amodiaquine (-10.63 kcal/mol) binding interaction

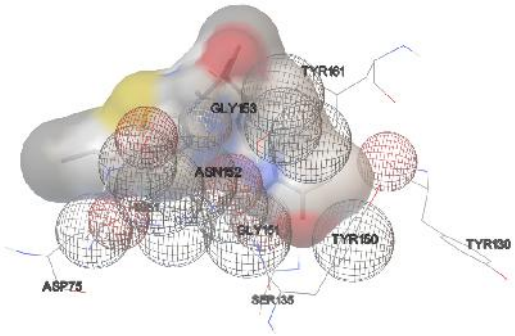

Fig 2: Docking result of protein and PHA690509 (- 11.71 kcal/mol) binding interaction

ADFR results (Table 3) of refined protein showed two best ligand conformations based on energy binding values. The focus point of this result is the interaction with catalytic triad residues (His51, Asp75 and Ser135) with the lowest binding energy to receptor protein which shows contribution of better proton transfer from hydrogen bonding and electrostatic reactions.

Further docking analysis was also done by comparing ADFR binding energy using grid full receptor and gridbox specific receptor as shown on Table 4. It is obvious that involved residues of both gridbox types would contribute discrepancy of receptor binding site stability. However, stability interaction which are found on receptor binding site is believed better with grid specific receptor than grid full receptor.

Binding energy of the residue using AutoDock Flexible Receptor (ADFR) shows that the binding energy value is significantly lower than docking using Autodock4 (AD4) as shown on Table 5. This study using receptor protein with refinement. The two best candidate bioactive compounds of AD4 result are prochlorperazine (-8.17 kcal/mol) and amodiaquine (-7.29 kcal/mol). Meanwhile, when using ADFR, two bioactive compounds with the lowest binding energies are PHA-690509 (-11.71 kcal/mol) and prochlorperazine (-11.09 kcal/mol). There is a possibility that eight other bioactive compounds could act as non-competitive inhibitor of protein NS3-NS2B Zika virus.

Amodiaquine has the third lowest binding energy value among other bioactive compounds after using refined receptor (Fig 1). Amodiaquine has diethylaminomethyl group which is a crucial chemical structure that contributes to binding with catalytic triad residues. This is proven by all docking interaction with His51, Asp75, Ser135 on amodiaquine lies in diethylaminomethyl group.

Berberine with protein without refinement only binds to Ser135 (-7.13 kcal/mol), whereas when it binds to refined protein, berberine has more interactions with the catalytic triad residues (His51 and Asp75) (-7.14 kcal/mol). This is similar to PHA-690509 when it binds to protein structure after refinement (Fig 2). It ties up more residues of catalytic triad and produces a lower binding energy compared to the binding with protein without refinement.

Niclosamide has the same binding energy of protein before and after refinement which is -9.3 kcal/mol, but there is more hydrophobic bonding interactions of surrounding proteins after refinement (His51 and Ser135) than before refinement (His51). It is a bit similar to quinacrine, which has the same interaction catalytic triad related residue.

4-nitrophenyl 4-guanidinobenzoat and quercetin are noncompetitive inhibitors that have the highest affinity value when attached to the pocket behind the catalytic triad substrate binding pocket [13]. But both molecules also have low binding energy when they bind to target (quercetin -8.78 kcal/mol and 4-nitrophenyl 4-guanidinobenzoat -9.92 kcal/mol). This proves that both molecules are possible to be competitive inhibitor. In using rigid receptor, prochlorperazine is a very potential bioactive compound. Due to the same specific gridbox with a different center, Prochlorperazine has a binding energy of refined protein -11.09 kcal/mol (Table 5).

ADFR result of NITD008 compound has higher binding energy value (-8.87 kcal/mol). However, the result of NITD008 by AD4 showed much bigger binding energy value (-4.22 kcal/mol) than docking with ADFR. It indicates that flexible binding site of receptor could improve the stability interaction with the ligand. Binding energy receptor with NITD008 showed is contributed by the workings of the adenosine analog, which works if it was activated first and joined to the RNA chain to stop the viral RNA synthesis. In activation process, adenosine analog must be phosphorylated by host cell's kinases to form the 5'-triphosphate, and compete with the natural substrate of the active site of viral RNA polymerase [32]. Although quinacrine has complete catalytic triad residue interactions (His51, Asp75 and Ser135), quinacrine with AD4 has a binding energy that is not too low as expected (-6.52 kcal/mol), so it is not counted as a potential candidate. However, by using ADFR, the binding energy increases into -10.8 kcal/mol, so quinacrine can be one of docking potential candidate. PHA-690509 has a close similarity with quinacrine by AD4 docking which is the binding energy that is not too low compared to others (-6.48 kcal/mol) and becomes one of the potential candidates when using ADFR (-11.71 kcal/mol).

Table 4: Comparison ADFR binding energy using grid full receptor and gridbox specific receptor

No.	Ligand	Involved residues by grid full receptor	Involved residues by grid specific receptor
1	Amodiaquine	His51, Asp75, Asp83, Asp129, Tyr130, Ser135, Gly151, Asn152, Gly153, Tyr161	His51, Asp75, Asp83 Tyr130, Ala132, Thr134, Ser 135, Tyr150, Gly153, Tyr161
2	Berberine	Asp83, Phe84, Tyr130, Pro131, Ala132, Thr134, Ser135, Tyr150, Gly151, Asn152, Gly153	Tyr130, Pro131, Thr134, Ser135, Tyr150, Gly151, Asn152, Gly153, Tyr161
3	Bisabolol	Phe84, Ala132, Ser135, Gly151, Asn152, Gly153, Tyr161	Asp83, Phe84, Tyr130, Ala132, Thr134, Ser135, Tyr150, Gly151, Asn152, Gly153
4	4-nitrophenyl 4-guanidinobenzoat	His51, Asp75, Asp83, Tyr130, Ala132, Thr134, Ser135, Tyr150, Asn152, Gly 153, Tyr161.	His51, Ser81, Gly82, Asp83, Tyr130, Ser135 Tyr150, Gly151, Asn152, Gly 153, Tyr161.
5	Niclosamide	Thr34, Gln35, Val36, His51, Lys54, Pro102, Pro131, Ala132, Gly133	His51, Lys54, Tyr130, Ala132, Thr134, Ser135, Tyr150, Tyr161
6	NITD008	Asp129, Tyr130, Ala132, Ser135, Gly 151, Tyr161.	His51, Tyr130, Ala132, Ser135, Tyr150, Gly151, Gly153, Tyr161.
7	Prochlorperazine	His51, Asp75, Asp83, Tyr130, Ala132, Ser135, Tyr150, Gly151, Asn152, Val155, Tyr161.	His51, Asp75, Ser81, Asp83, Phe84, Ala132, Ser135, Gly151, Asn152, Gly153, Tyr161.

8	Quercetin	His51, Tyr130, Ser135, Tyr150, Gly151, Asn152, Gly153, Tyr161	His51, Tyr130, Ala132, Ser135, Tyr150, Gly151, Asn152, Gly153, Tyr161
9	Quinacrine	His51, Asp83, Phe84, Asp129, Tyr130, Thr131, Ala132, Ser135, Tyr150, Gly151, Asn152, Gly153, Gly159, Tyr161	Val36, His51, Phe84, Asp129, Tyr130, Ala132, Ser135, Gly151, Gly153, Tyr161
10.	PHA-690509	His51, Tyr130, Ala132, Ser135, Tyr150, Gly151, Asn152, Gly153, Val154, Val155, Tyr161	Trp50, His51, Asp83, Tyr130, Ala132, Ser135, Tyr150, Asn152, Gly153, Tyr161

Table 5: Comparison of AD4 and ADFR results with inhibition constant value (Ki)

No.	Ligand	AD4 Binding energy (kcal/mol)	Ki (µM)	ADFR Binding energy (kcal/mol)	Ki (µM)
1	Amodiaquine	-7.29	4.471583	-10.63	0.015831
2	Berberine	-5.35	118.5959	-7.14	5.761481
3	Bisabolol	-5.46	98.48036	-8.06	1.217365
4	4-nitrophenyl 4-guanidinobenzoate	-7.06	6.595377	-9.92	0.052543
5	Niclosamide	-5.9	46.82409	-9.3	0.149791
6	NITD008	-4.22	800.3591	-8.87	0.309762
7	Prochlorperazine	-8.17	1.010883	-11.09	0.007277
8	Quercetin	-6.45	18.48711	-9.66	0.081529
9	Quinacrine	-6.52	16.42486	-10.8	0.011878
10	PHA-690509	-6.48	17.57335	-11.71	0.002553

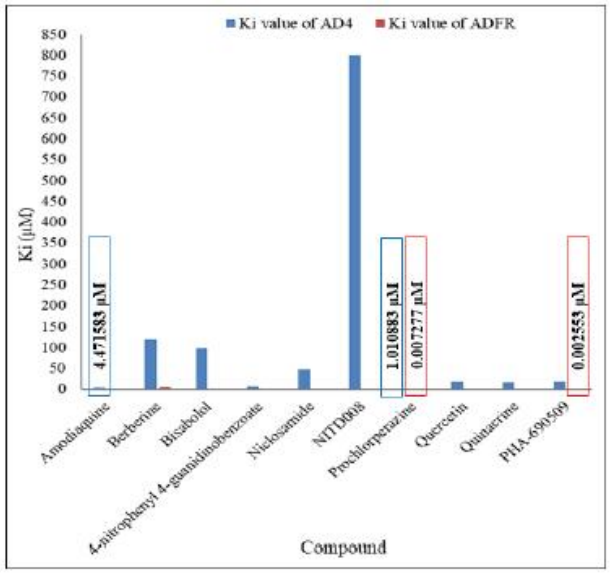

Fig 3: Inhibition constant values of allosteric bioactive compounds to NS3-NS2B protease

Meanwhile, the lowest value of inhibition constant (Ki) to NS3-NS2B protease was PHA-690509 (0.002553 µM), followed by prochlorperazine (0.007277 µM), with ADFR analysis. In contrast, the lowest value of inhibition constant to NS3-NS2B protease was prochlorperazine (1.010883 µM) and followed by amodiaquine (4.471583 µM) with AD4 analysis. As we know, the lower the Ki value, the stronger the chemical bonds between inhibitors and protein-inhibitor complex (Fig 3).

4. CONCLUSION

The best molecule as a candidate for Zika competitive inhibitor of NS3-NS2B catalytic triad using ADFR was PHA-690509 (0.002553 µM). The pharmacokinetically active compounds showed a remarkable docking profile against the active site of receptor residues of linked NS3-NS2B protease. It may lead a potential drug candidate for linked NS3-NS2B protease inhibition, particularly in the critical clinical treatment of ZIKV infection.

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