J. Chosun Natural Sci.
Vol. 8, No. 3 (2015) pp. 209 – 213
http://dx.doi.org/10.13160/ricns.2015.8.3.209

Molecular Docking Study of Naturally-derived Neuraminidase Inhibitors Isolated from *Phellinus Baumii*

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Abstract

Influenza A virus (H1N1) causes and spreads infectious diseases and becomes a major health threat in humans. Among the subtypes of influenza virus, neuraminidase (NA) plays an important role in viral life cycle and becomes an attractive therapeutic target. Currently two NA inhibitors namely Zanamivir and Oseltamivir are available for treating infectious diseases. Recently five naturally derived polyphenols extracted from *Phellinus baumii* was reported as inhibitors against NA. Molecular docking is powerful tool in computer aided drug designing which aids in exploring and elucidating the properties of the molecules from their 3D structure. Hence, in the present study, molecular docking was carried out on reported polyphenols isolated from ethanolic extract of fruiting bodies of *Phellinus baumii*. The objective of this work was to study the interaction and to propose the binding mode of these compounds within the binding site of H1N1 neuraminidase. The results showed these compounds had better binding energy and H-bond interactions with the important active site residues of the receptor which authenticate these compounds contributes to inhibitory activity of neuraminidase to treat influenza infection.

Keywords: H1N1 Neuraminidase, Polyphenols, Molecular Docking

Introduction

Influenza A viruses are single stranded RNA virus with an eight segmented genome belonging to the family of Orthomyxoviridae. The subtypes of Influenza A virus are classified based on the combination of the virus coat glycoproteins on its surface namely Hemagglutinin (HA) and Neuraminidase (NA) which are essential for the infectious cycle of influenza virus. There are 16 different HA antigens (H1-H16) and 9 different NA antigens (N1-N9) for Influenza A virus[1,2]. Hemagglutinin is a lectin that binds sialic acid on host cell membrane. During initial stage of infection, HA facilitates attachment of an influenza virus onto an epithelial cell through HA-sialic acid interaction. Neuraminidase is a sialic acid hydrolase that will cleave the sialic acid on the host cell membrane, releasing the newly formed viral particle from the HA-sialic acid bondage at the end of infectious cycle. Since, the neuraminidase is responsible for the release of mature virons from infected cells and the inhibition of neuraminidase enzymatic activity stops virus infection and becomes an attractive therapeutic target. There are two neuraminidase inhibitors currently available to treat influenza infection namely Zanamivir and Oseltamivir[3-5]. But the long time usage of these drugs is restricted due to the high toxicity and there is a possibility that these drugs can lead to development and spread of drug-resistant mutants[6]. Hence, there is a need to develop novel inhibitors against neuraminidase. Recently B.S. Hwang et' al has identified naturally derived NA-inhibitors isolated from ethanolic extract of an eatable mushroom, *Phellinus baumii*[7]. These compounds are polyphenols, identified by spectroscopic methods and found to inhibit non-competitively the neuraminidase activity of H1N1, H5N1 and H3N2 influenza virus.

Therefore, this study was initiated to examine whether these reported polyphenols namely hispidin, hypholomine B, inoscavin A, davallialactone and phelligridin D could possibly bind in the same active site region as zanamivir and to investigate its interaction with active site residues using *in silico* docking studies.

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(Received: July 27, 2015, Revised: September 17, 2015, Accepted: September 25, 2015)

Since the process of drug discovery involves the prediction of binding mode of the small molecules within the binding site of receptor and estimating their binding affinity[8], molecular docking was performed. Autodock, a flexible docking program which predicts the structure of protein ligand complex with greater accuracy and higher speed[9] was used for docking.

2. Experimental Section

2.1. Protein Preparation

For the present study, the X-ray crystal structure of 2009 pandemic H1N1 neuraminidase complexed with Zanamivir was downloaded from PDB database (3TI5). During protein preparation, all heteroatoms including co-crystallized ligands, ions and water molecules present in the crystal structure were removed and subsequently hydrogen atoms and charges such as Kollman and Gasteiger charges were assigned to the protein structure. ADT atom type was assigned for each atom and the non-polar hydrogen atoms were merged. Finally macromolecule was saved in PDBQT file format using Autodock.

2.2. Ligand Preparation

The structure of ligand molecules were drawn using ChemSketch software and 3D structure were optimized using SYBYLX2.0 software. The energy minimization of all the molecules was performed using Tripos force field in SYBYL. The molecules were prepared by defining the number of rotatable bonds and assigning ADT type using Autodock and the molecule was saved in PDBQT format for subsequent analysis. The structures of polyphenols used in this study are shown in Fig. 1.

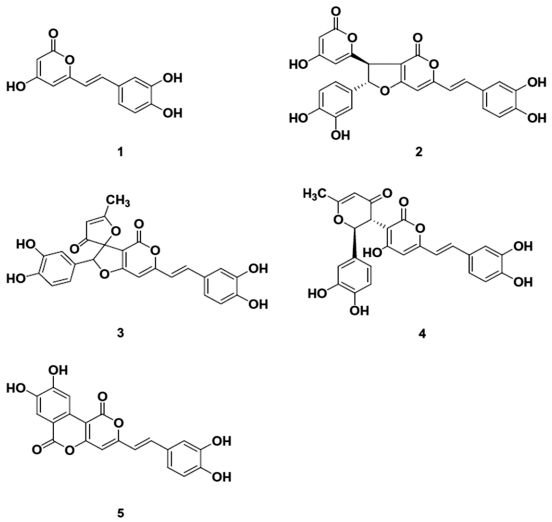

Fig. 1. Chemical structure of polyphenols (1-hispidin, 2-hypholomine B, 3-inoscavin A, 4-davallialactone and 5-phelligridin D) isolated from the fruiting bodies of *Phellinus baumii*.

2.3. Molecular Docking

Molecular docking was performed by considering the protein to be rigid and ligand as flexible where all rotatable bonds of the ligand were considered. Before performing docking, the grid calculations were used to define the grid maps. The grid box was generated and centered on the binding site residues with 56×56×56 grid points in XYZ direction with 0.375Å spacing. The binding site was selected based on the binding pocket of zanamivir, a co-crystal ligand molecule in the target protein structure and from the reported study on active site residues of neuraminidase[10]. Lamarckian genetic algorithm which combines both genetic and local search algorithm was employed for docking with default parameters. During docking experiment, for each ligand molecule 10 poses were generated and scored using Autodock scoring function. H-bond interaction, binding energy, π-π interactions and orientation of the docked compound within the active site are critical for the analysis of receptor-ligand complex and its interaction[11]. The docking results were further analyzed using PyMOL and LIGPLOT software.

3. Results and Discussion

To study the interaction and the binding mode of polyphenols obtained from ethanolic extract of fruiting bodies of *Phellinus baumii* with the target H1N1 neuraminidase (PDB code: 3TI5), molecular docking was performed using Autodock 4.2 version.

3.1. Validation of Autodock Software

To validate the autodock software, re-docking was performed on structure of H1N1 neuraminidase (3TI5) utilizing the co-crystallized ligand molecule zanamivir into the binding pocket of neuraminidase. It was reported that if the RMSD of the best conformation is <2.0Å from the bound ligand in the experimental crystal

structure then the used scoring function is successful[12]. Therefore, the docked mode of zanamivir was compared to the crystal structure of bound ligand-protein complex. The RMSD of the docked pose of zanamivir with the co-crystal zanamivir was found to be 1.215Å which authenticates the accuracy of the software.

3.2. Molecular Docking

10 different conformations were generated for each compound during the docking experiment. Among the generated conformations, the conformation which has better interaction with active site residues with lowest binding energy was considered as active conformation for each compound. We found that all the five phenolic compounds showed better binding energy in the range of -5.63 to -6.66kcal/mol and strong H-bond was also observed. The binding mode of compound hispidin is shown in Fig. 2. The binding energy was -5.62 kcal/mol and has H-bonds with ARG371, GLU227, ARG292, ASP151 and THR225 residues. The binding energy of compound hypholomine B was found to be -5.87kcal/mol and forms H-bond with ARG152, GLU227, ASN247 residues is depicted in Fig. 3. Fig. 4 represents the binding of inoscavin which has highest binding energy -7.54 kcal/mol when compared to other compounds. This compound has H-bond interaction with SER246, TRP178, ARG152 and ASN247. The binding energy of the compound davallialactone was -6.3kcal/mol and its binding mode are depicted in Fig. 5. The residues such as ASP198, SER246, GLU227 and TRP178 form H-bond interaction with davallialactone. Phelligridin D has H-bond interaction with TRP178 and ASN221 residues whose binding energy was found to be -6.66kcal/mol. The binding of Phelligridin D with

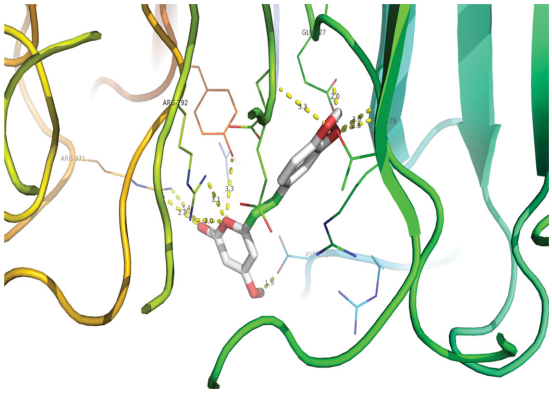

Fig. 2. Binding of hispidin within the active site of H1N1 neuraminidase.

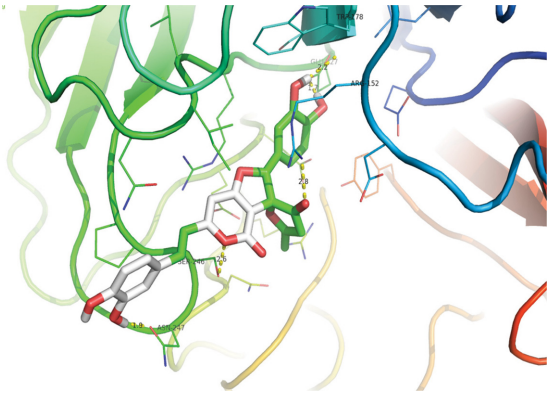

Fig. 4. Binding of inoscavin A within the active site of H1N1 neuraminidase.

Fig. 3. Binding of hypholomine B within the active site of H1N1 neuraminidase.

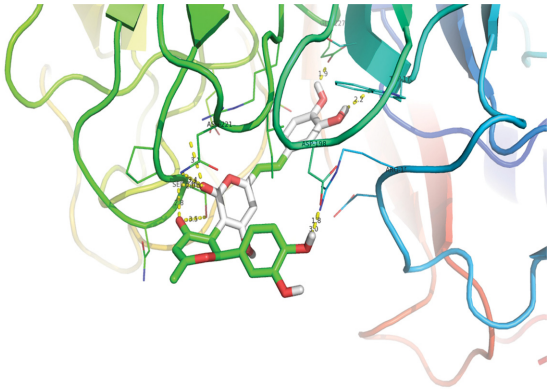

Fig. 5. Binding of davallialactone within the active site of H1N1 neuraminidase.

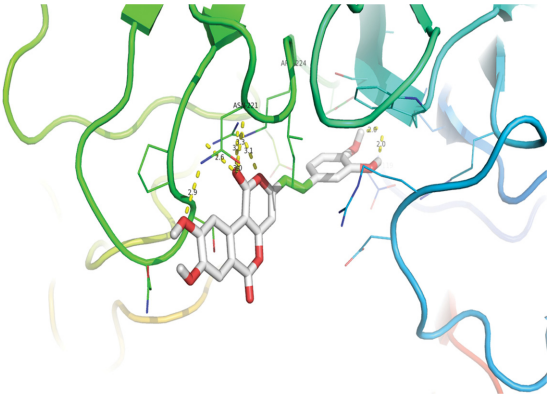

Fig. 6. Binding of phelligridin D within the active site of H1N1 neuraminidase.

H1N1 is shown in Fig. 6. The LIGPLOT software was used to calculate the hydrophobic interaction of the protein-ligand complex structures. The interaction of protein-ligand complex as well as its hydrophobic interaction of all the compounds is shown in Fig. 7.

The binding energy of the polyphenols ranging from -5.63 to -6.66 kcal/mol were comparable to the co-crystal ligand zanamivir (-6.57 kcal/mol) which confirms that the inhibitory binding energy of these five polyphenols were similar to zanamivir. The important active site residues of neuraminidase such as ARG118, GLU119, ASP151, ARG152, ARG156, TRP178, SER179, ASN198, ILE222, ARG224, GLU227, SER246, HIS274, GLU276, GLU277, ARG292, ASN294, ARG371, TYR406 and GLU425 has been reported in Yen et' al. The docking study revealed that the polyphenols compound binds within these active site residues and forms H-bond interaction with many of these important active site residues which validates these compounds acts as better inhibitor molecules against neuraminidase enzymatic activity.

4. Conclusion

The results of *in silico* docking study exhibited the binding interaction of polyphenolic compounds which acts as inhibitors against H1N1 neuraminidase. This study also clearly indicated the binding site of these compounds were similar to the zanamivir, a drug cur-

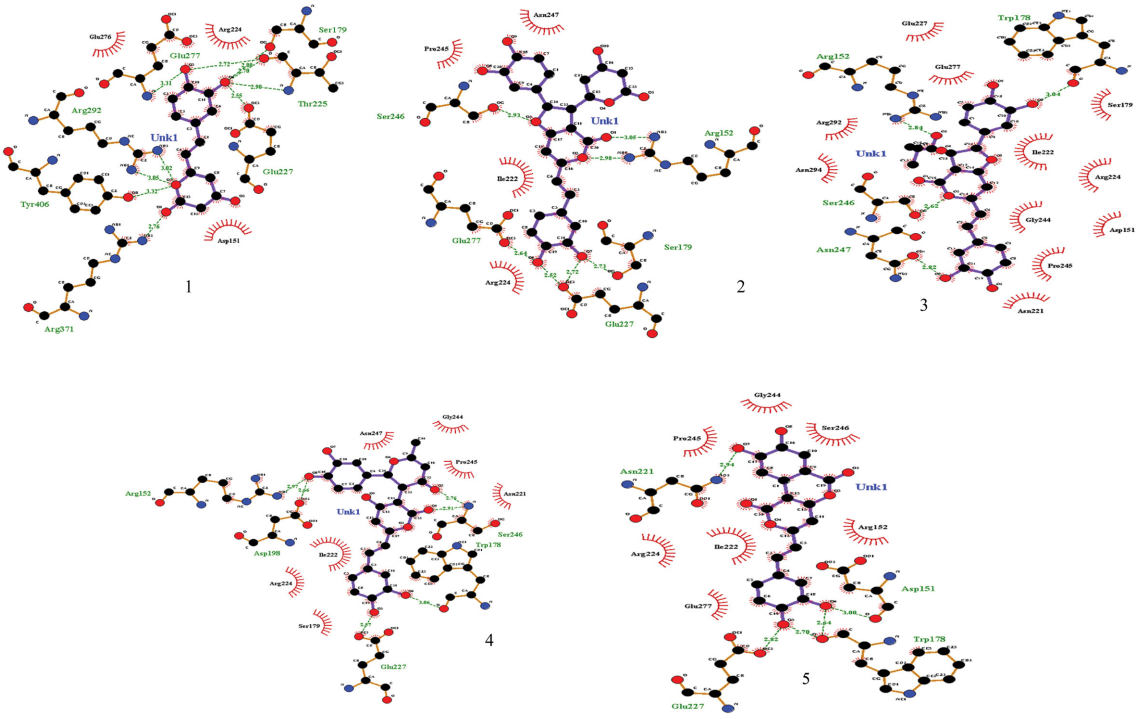

Fig. 7. Protein-Ligand interaction of all compounds (1-hispidin, 2-hypholomine B, 3-inoscavin A, 4-davallialactone and 5- phelligridin D) generated using LIGPLOT.

rently used for treatment of influenza infection and also suggest that all polyphenols compounds had strong H-bond interaction with the active site residues.

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