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Structure Base Virtual Screening for Identifying Inflammatory Inhibitors

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Abstract. Phospholipase A2 (PLA2) is an enzyme that induces inflammation, making PLA2 activity an effective approach to reduce inflammation. Therefore, investigating natural compounds for this PLA2 inhibitory activity has important therapeutic potential. The objective of this study was to investigate the potential inhibitors for inflammatory diseases through a virtual screening approach. Out of 10,000 compounds from zinc database, only five compounds were selected based on the lowest free energy binding and further used for molecular interaction analysis. These five compounds were Metacetamol (-11.43 kcal/mol), 7-Methoxybenzofuran-2-carboxylic acid (-10.22 kcal/mol), 6-nitro-4H-1,3-benzodioxine-8-carbaldehyde (-10.08kcal/mol), 4-(2-Amino-1,3-thiazol-4-yl)benzene-1,3-diol (-9.86 kcal/mol), and 1-Ethyl-1H-indole-3-carbaldehyde (-9.53 kcal/mol). These findings also provide insight on valuable implications for the use of these five compounds in treating inflammation, and may help researchers develop more natural bioactive compounds in daily foods as anti-inflammatory agent.

1. Introduction

Inflammation is the body protective response to injury and infection. It is a body defensive system reaction by removing the injurious stimuli and initiating the healing process. Inflammation is also known as a complex process involving many cell types as well as different components of blood. Thus a variety of inflammation target proteins were studied to understand the signaling pathway of inflammation disorder [1].

Phospholipase A2 (PLA2) is one of the target proteins commonly studied for treating the inflammation. The excess amount and activity of PLA2 are usually resulting from the snake bite, insect bite or any other venomous animals. Thus, the uncontrolled production of this PLA2 will affect the various toxic action such as the coronary artery disease, acute coronary syndrome and neurotoxic action [2]. PLA2 hydrolyzes the sn-2 ester bond of glycerophospholipids for the liberation of free fatty acids and lysophospholipids molecules [3]. It also contributes to the initiations of inflammation which subsequently induces the secretion of arachidonic acid and leads to the formation of inflammation mediators [4].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is a widely used treatment for this inflammatory condition in order to reduce swelling and inflammation. Despite their wide usage, the prolonged use of these drugs may cause several side effects such as gastrointestinal toxicity, hepatoxicity, and asthma [5,6]. Therefore, there is a great interest in the development of new remedies that can act as the anti-inflammatory agent with less side effects. Thus the objective of this study is to screen new compounds that can be proposed as anti-inflammatory agent.

2. Methodology

2.1. Receptor preparation

Energy minimization of receptor was generated using Schrödinger's Protein Preparation Wizard software. This minimization step is important to ensure the receptor with the right molecular parameters and confidently used for molecular docking. Missing hydrogen atom and metal ionization state were added automatically during minimization step. The receptor of Lp-PLA2 (PDB ID:5YE7_A) was prepared for docking simulation using Autodock Tools (ADT) software package [7]. Hydrogen atoms were added to the receptor using ADT. Default Kollman chargers and solvation parameters were assigned to the model. The grid binding box was centered at 45 Å x 25 Å x 20 Å and the size created with the dimensions of 26Å x 28Å x 30Å according to coordinates X, Y, and Z, with the default spacing of 1 Å. AutoGrid was used to create the affinity grids which focus on the active sites.

2.2 Ligand preparation

The 3D structures of 10,000 compounds were obtained from ZINC database. Raccoon software is a graphical interface for preparing the ligands (http://autodock.scripps.edu/resources/raccoon). This software was used to split multiple-molecule ligand files and add the Gasteiger-Marsili charges, merged the non-polar hydrogen atoms onto their respective heavy atoms and generate the "pdbqt" docking input format for each compound [8].

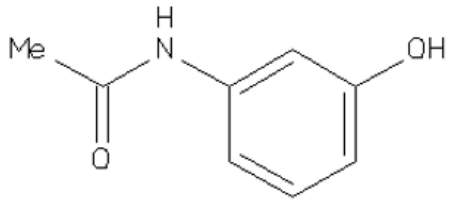
2.3 Virtual screening and molecular docking

Both virtual screening and docking simulation were performed using ADVina [8]. ADVina was used due to its accuracy and speed. The input files that were required for performing this step were; pdbqt files to represent receptor and ligand and also config.txt file. The ligand pose with the lowest predicted free energy of binding was used for the subsequent analysis.

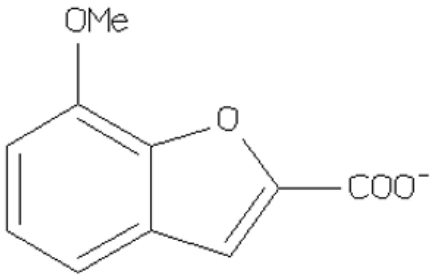
3. Results and discussions

Virtual screening and molecular docking study were carried out on ZINC database to find the compounds that can strongly bind with receptor 5YE7_A. 5YE7_A is a target protein used for identifying the anti-inflammatory inhibitors. The chemical structures of the compounds were taken from ZINC database. The three-dimensional structure of 5YE7_A was obtained from Protein Data Bank with its inhibitor. Hydrogen atoms were added to the protein. The existing ligand was re-docked into the active pocket of the 5YE7_A with parameters; grid binding box was cantered at 45 Å x 25 Å x 20 Å and the size created with the dimensions of 26Å x 28Å x 30Å according to coordinates X, Y, and Z, with the default spacing of 1 Å. The RMSD value for control docking is 0.894 Å. Therefore, parameters used for control docking can be further utilized in virtual screening of Lp-PLA2 inhibitors.

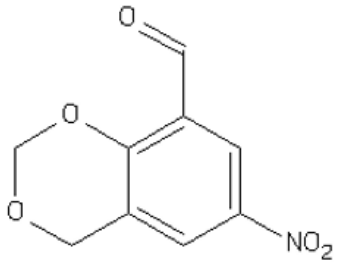
The docking results showed that five plants showing lower free energy binding such as Metacetamol (-11.43 kcal/mol), 7-Methoxybenzofuran-2-carboxylic acid (-10.22 kcal/mol), 6-nitro-4H-1,3-benzodioxine-8-carbaldehyde (-10.08kcal/mol), 4-(2-Amino-1,3-thiazol-4-yl)benzene-1,3-diol (-9.86 kcal/mol), and 1-Ethyl-1H-indole-3-carbaldehyde (-9.53 kcal/mol). Figure 1 shows the 2D structure of the five selected compounds.



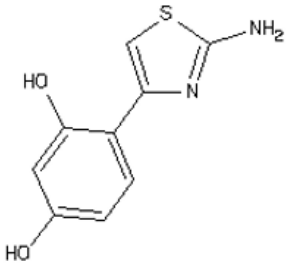
Metacetamol



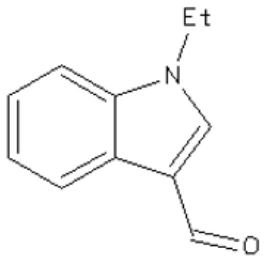
7-Methoxybenzofuran-2-carboxylic acid



6-nitro-4H-1.3-benzodioxine-8-carbaldehyde



4-(2-Amino-1,3-thiazol-4-yl)benzene-1,3-diol



1-Ethyl-1H-indole-3-carbaldehyde

Figure 1. 2D structure of five selected compounds

Figure 2 shows that these five compounds bound in the same binding region. However, these five compounds had slightly different binding orientations which means each substituent from the compound may interact with different residues. Compound with hydroxyl (OH) substituent will tend to interact with polar residues while for region that has more aromatic rings will tend to be buried in the hydrophobic site.

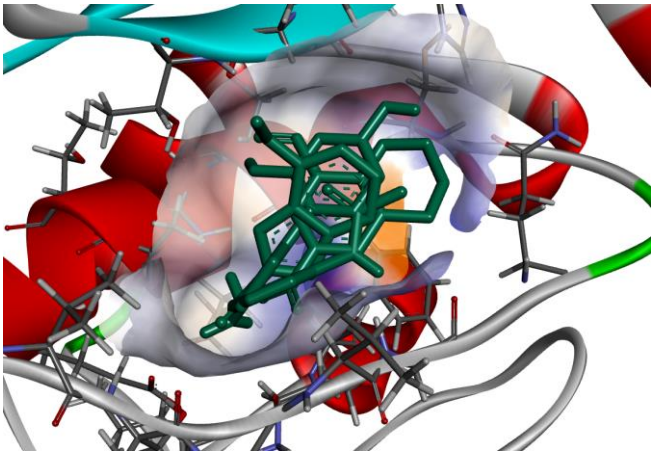


Figure 2. Surface representations of the binding site of receptor with the bound ligands.

Based on the results, all of the selected compounds have formed hydrogen bond and hydrophobic interactions, except for and 1-Ethyl-1H-indole-3-carbaldehyde, it only formed hydrophobic interactions (Figure 3). The residues Phe86, Tyr98 and His281 has interact with the aromatic ring of 1-Ethyl-1H-indole-3-carbaldehyde forming hydrophobic contacts. The molecular interactions will affects the functional value of lipoproteins such as amount of cholesterol increased in the phospholipid membranes has a significantly dissimilar effect on membrane binding, the membrane penetration, and the activity of purified Lp- PLA2 [9,10].

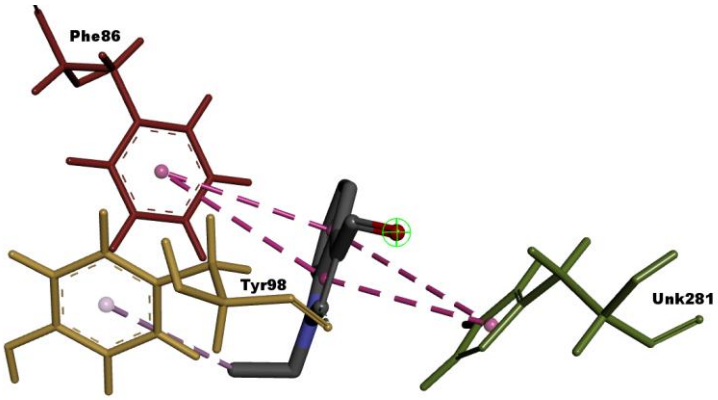


Figure 3. Hydrogen bond and hydrophobic interactions between Lp-Pla2 and 1-Ethyl-1H-indole-3-carbaldehyde. Purple dash line indicates hydrophobic interaction.

Compound 6-nitro-4H-1,3-benzodioxine-8-carbaldehyde has the highest number of molecular interactions which consist of six and one number of hydrogen bond and hydrophobic interactions. Residues involved in hydrogen bond formation are Thr284, Ser152, Val139 and Lys140 (Figure 4). This compound is the bulkier structure compared to the others, thus it is proposed that the huge the structure the more molecular interactions will be formed and probably contributed to the stability of the dock conformation.

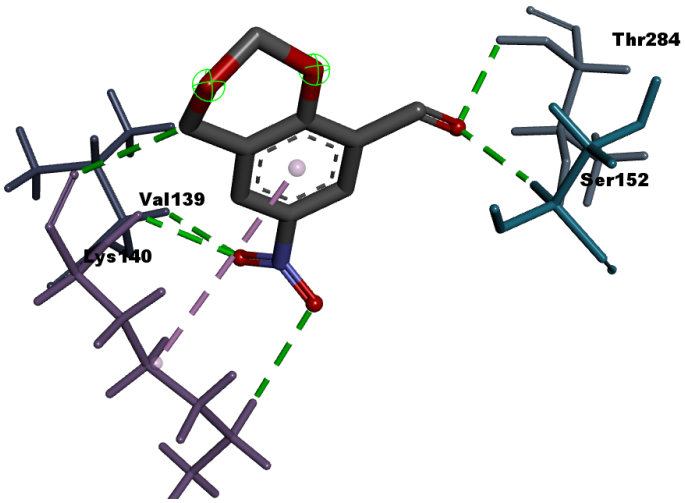


Figure 4. Hydrogen bond and hydrophobic interactions between Lp-Pla2 and 6-nitro-4H-1,3-benzodioxine-8-carbaldehyde. Green dashed lines and purple dash line indicate the hydrogen bond and hydrophobic interaction, respectively.

The other ligands such as Metacetamol, 7-Methoxybenzofuran-2-carboxylic acid, and 4-(2-Amino-1,3-thiazol-4-yl)benzene-1,3-diol also have hydrogen bonds and hydrophobic contacts as shown in Figures 5a, b and c. All these there compound have almost similar value of binding energy and formed molecular interactions with same residues Lys140 and Ala155, respectively.

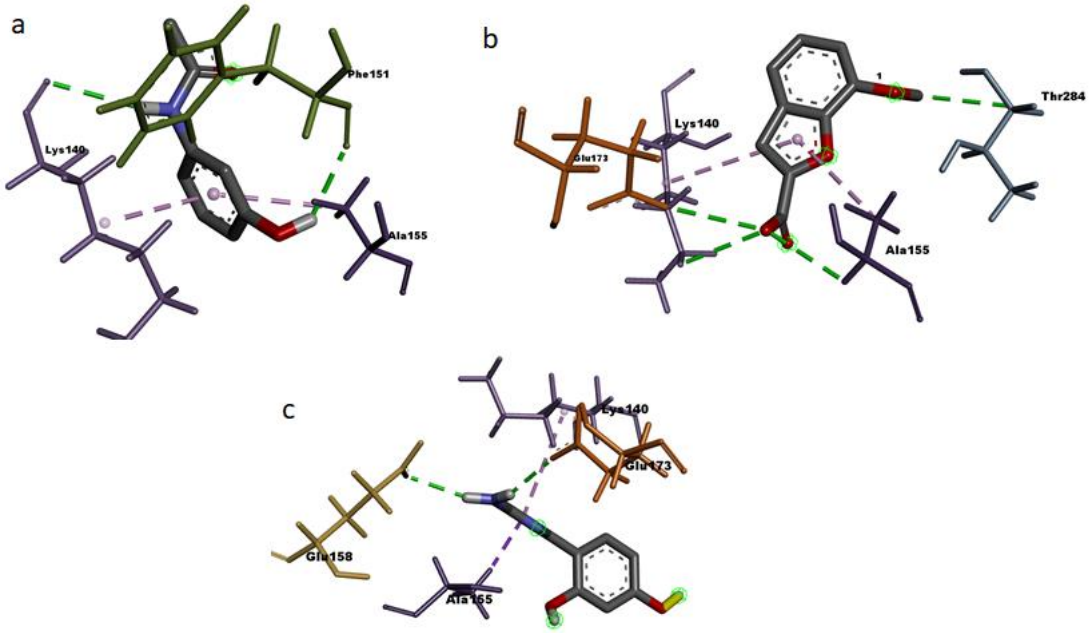


Figure 5. Hydrogen bond and hydrophobic interactions of Lp-Pla2 towards Metacetamol, 7-Methoxybenzofuran-2-carboxylic acid and 4-(2-Amino-1,3-thiazol-4-yl)benzene-1,3-diol. Green dashed lines and purple dash line indicate the hydrogen bond and hydrophobic interaction, respectively.

4. Conclusion

Docked ligands have formed molecular interactions with residues Lys140, Phe151, Glu173, Ala155, Thr284, Val139, Ser152, Glu158, Phe86, Thr98 and Val281 through hydrogen bonds and hydrophobic interactions. All the selected compounds could be a good potential as lead compounds for treating inflammation. However, an additional *in vitro* or *in vivo* study needs to be carried out to further evaluate the inhibitory potential of these compounds as anti-inflammatory agents.

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