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Molecular docking of modified ipalbidine ligands into human cyclooxygenase-2 protein crystal structures

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Abstract. With many negative side-effects of nonsteroidal anti-inflammatory drugs such as gastrointestinal problems and the withdrawal of most selective COX-2 inhibitors from the market due to its increased risk of heart disease, there is a demand for an alternative analgesic (pain reliever) with less adverse side-effects. Nowadays, molecular docking is a major computational tool for drug development and drug design. This study focuses on the modification of potential analgesic ipalbidine in order to optimize its binding affinity to seven human COX-2 structures using Autodock Vina as a docking tool. Two different modified ligands were successfully enclosed in the COX-2 protein structure and were able to bind to the binding site. Based on the obtained binding affinities, it was found that by increasing the amount of hydrogen atoms on the ligand by converting the carbon double bond into a single bond, the binding affinity was reduced due to the hydrophobicity of the binding site liners. The second change was ipalbidine with inverted chirality. Inverted chirality showed higher binding affinities across the board, implying improved efficacy and bond strength. However, a change in the chirality of a drug may alter the selectivity or effect of the drug as a whole, and therefore clinical trials may be required to identify possible side-effects that may arise.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of drugs that reduce pain, fever or inflammation[1]. It is generally used to relieve pain from headaches, sprains, extreme menstrual cramps, arthritis, and other diseases that cause pain and inflammation. During injury, the damaged tissue produces prostaglandins[2] related to the physiological process of fever, pain, and inflammation. NSAIDs work by inhibiting Cyclooxygenases (COX) enzymes, which catalyze the production of prostaglandins and reduce inflammation and pain. There are two types of COX: COX-1 and COX-2[3]. NSAIDs that block both COX-1 and COX-2 have a common side effect that causes gastrointestinal problems, such as ulcers in the esophagus, stomach, or small intestines. COX-1 produces a type of prostaglandin that also helps to protect the stomach. Using NSAIDs that inhibit COX-1 reduces the type of prostaglandin that protects the stomach and allows gastric acids to damage the inner lining of the stomach causing gastrointestinal problems[4]. Selective COX-2 inhibitors (coxibs) aim to avoid this problem by only inhibiting COX-2, which is related to the production of prostaglandins responsible for pain and inflammation. However, these selective COX-2 inhibitors have increased the risk of heart attacks and stroke [5]. The high risk of heart disease and stroke caused Vioxx to be removed from the market.



Molecular docking is one of the techniques used in computer-aided drug design. It is a method in which ligand-receptor interaction can be maximized by calculating the ligand's optimal pose within the receptor. Knowing the preferred ligand-receptor orientation, it can be used to predict the binding affinity between pairs [6]. Molecular docking is important for the design of drugs because it is far more cost-effective than conducting drug development experiments [7].

The binding affinity of the ligand is determined by the 3D structure of the receptor from which the amount of attraction and repulsion between the ligand and the receptor is calculated [8]. Due to the second law of thermodynamics, there will be spontaneous reactions at constant temperature and pressure if the change in the free energy of Gibbs (free enthalpy) is negative. More negative binding energy also means a stronger bond between the ligand and the receptor. In protein-ligand binding, ligand is usually a molecule that produces a signal by binding the target protein to the site.

In this study, ipalbidine is modified to optimize its binding affinity as a potential inhibitor of COX-2. Ipalbidine is synthesized from *Ipomea alba* seeds. It is a non-addictive analgesic (pain reliever) and has the ability to prevent leukocyte bursting in the respiratory system [9]. Because it is a natural product, it is expected to have less side effects than other coxibs [10].

Although drugs have been developed to selectively inhibit COX-2 to replace non-selective NSAIDs and bypass negative side effects caused by NSAIDs on the gastrointestinal tract, research has shown that coxibs increase the risk of heart disease such as heart attack and stroke [11]. Due to these cardiovascular side-effects, the majority of COX-2 inhibitors have been removed from the market and the demand for pain killers and anti-inflammatory drugs with less adverse side-effects has increased [12].

The aim of this study is to modify ligand ipalbidine in order to improve its binding affinity, to determine its binding effect within the seven currently available human COX-2 protein structures to be used in future drug design studies. This study will help to develop new drugs that may have a potential to have less adverse side-effects than those currently available on the market due to the natural origin of *Ipomea alba*. The development of an alternative drug is also important, as not all drugs are compatible with every human or disease case.

This study limits the modifications to be made to ipalbidine to two modifications. One inverts the chirality of ipalbidine from (+)-ipalbidine to (-)-ipalbidine. The other changes the carbon double bond to a single bond, allowing more hydrogen to bind to the ligand. This study will also limit the ligands to be docked as flexible ligands and the receptor to a rigid structure. AutoDock Vina is the docking program used.

2. Methodology

In this study, multiple programs were used to prepare, modify, and dock the ipalbidine ligands into COX-2 receptors. These programs are AutoDock Tools, AutoDock Vina, Chimera, Visual Molecular Dynamics (VMD), VMD's add-on MultiSeq, and Avogadro. First, the human COX-2 protein structures were downloaded from the Protein Data Bank. These structures are 5FI9, 5FIA, 5IKQ, 5IKR, 5IKT, 5IKV, and 5KIR. These protein structures were produced through X-ray Crystallography and contain ligands. The COX-2 protein structures contain monomer A and B, and other extra ligands required to crystallize the protein. Monomer B and the ligands were removed by using the program Chimera. Choosing only chain A reduces the amount of space needed to search for the binding site and reduces the computation time when performing alignment. The ligand ipalbidine was downloaded from the PubChem database. Modifications to the ligand were done by using the program Avogadro. The first change to ipalbidine is to invert the ligand's chirality. A chiral molecule is defined as a molecule that is not superimposable on its mirror image [13]. The second modification is to change the carbon double bond to a single bond so that more hydrogen atoms can be attached to the ligand.

The ligand's flexibility was not modified and remained as flexible ligands downloaded from PubChem. Based on Figure 1, the red lines indicate parts of the ligand that are rigid and can not rotate; and the green lines indicate parts of the ligand that can rotate. Inverting the chirality of the ligand, the

lower half of the ligand where the nitrogen located is rigid and held constant while the upper half was allowed to rotate to optimize its binding affinity during the docking process.

Unnecessary parts of protein structures have been removed using the Chimera program. The parts to be removed from the protein are the extra ligands used to crystallize the protein and the B-chain part of the molecule. This step is in preparation for the next step of protein alignment.

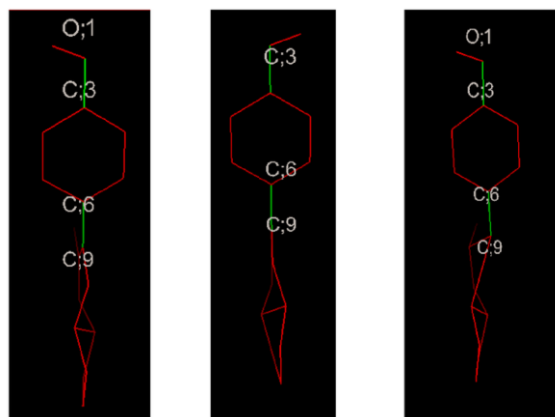


Figure 1. Flexibility of Modified Ipalbidine, Original Ipalbidine (left), Inverted Chirality (Middle), Double bond transferred to a single bond (right).

The protein structures were then structurally aligned by adding VMD to MultiSeq as shown in Figure 2. The purpose of alignment is to set the coordinates of protein structures in the same place in order to facilitate the process of locating the binding site of all protein structures and to facilitate the comparison of the results of the binding conformations.

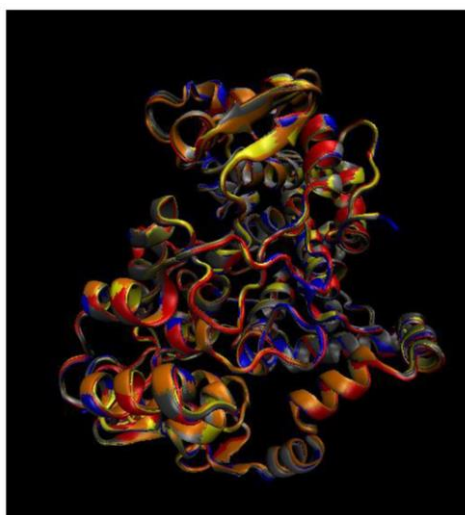


Figure 2. Multiple aligned proteins superimposed on top of each other

AutoDock Tools were used to determine the location of the receptor. In order to ensure that the search space is centered on the binding site, the protein previously bound to the binding site was chosen in AutoDock Tools and its coordinates were taken as the center of the binding site. In order to ensure that the search space is centered on the binding site, the protein previously bound to the binding site was chosen in AutoDock Tools and its coordinates were taken as the center of the binding site. To start the docking process, the receiver, ligand and search space must be entered into Autodock Vina. After docking, the results are shown in the command prompt or text file where the results are analyzed. The

results provide binding affinities and conformations of ligands. The desired ligand, modified ipalbidine, must be able to search for a binding site and produce negative values of binding affinities as more negative values of binding affinities indicate a higher binding strength and spontaneity of the reaction.

3. Result and Discussion

Figure 3 shows the molecular surface (white) of the 5FIA binding site. The binding affinity of each ligand is -6.9 kcal / mol for unmodified ipalbidine (green), -7.0 kcal / mol for inverted chirality (red), and -6.3 kcal / mol for modified double bond (blue).

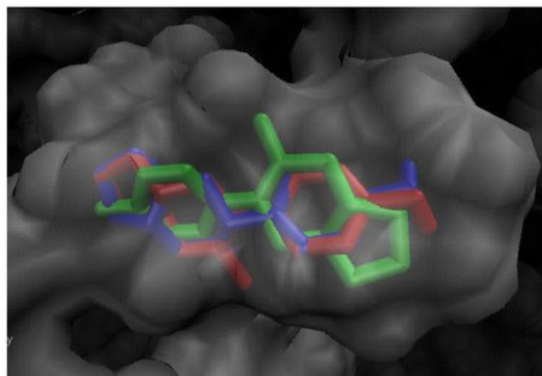


Figure 3. 5FIA binding site's molecular surface.

Figure 4 shows 5F19 binding site's molecular surface (white). The binding affinity of each ligand are, -8.7 kcal/mol for unmodified ipalbidine (green), -8.6 kcal/mol for inverted chirality (red), and -8.6 kcal/mol for modified double bond to a single bond (blue).

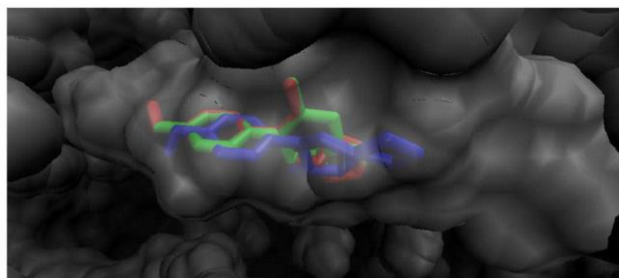


Figure 4. 5F19 binding site's molecular surface

Figure 5 shows 5IKQ binding site's molecular surface (white). The binding affinity of each ligand are, -7.5 kcal/mol for unmodified ipalbidine (green), -8.3 kcal/mol for inverted chirality (red), and -7.6 kcal/mol for modified double bond to a single bond (blue).

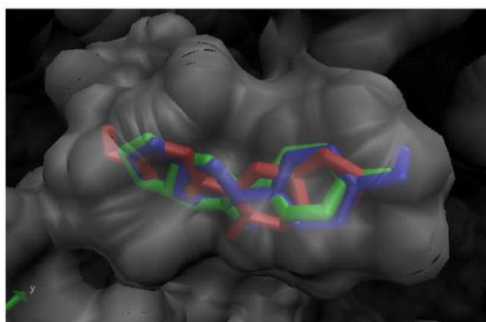


Figure 5. 5IKQ binding site's molecular surface

Figure 6 shows 5IKR binding site's molecular surface (white). All three ligands have the same binding affinity of -7.6 kcal/mol.

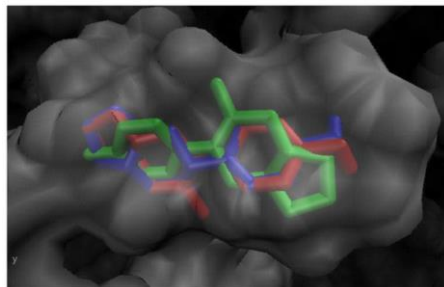


Figure 6. 5IKR binding site's molecular surface

Figure 7 shows 5IKT binding site's molecular surface (white). The binding affinity of each ligand are, -7.9 kcal/mol for unmodified ipalbidine (green), -8.0 kcal/mol for inverted chirality (red), and -8.1 kcal/mol for modified double bond to a single bond (blue).

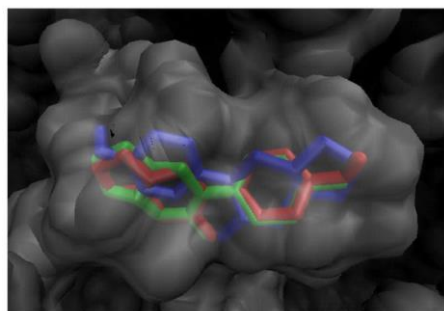


Figure 7. 5IKT binding site's molecular surface

Figure 8 shows 5IKV binding site's molecular surface (white). The binding affinity of each ligand are, -7.8 kcal/mol for unmodified ipalbidine (green), -7.9 kcal/mol for inverted chirality (red), and -7.2 kcal/mol for modified double bond to a single bond (blue).

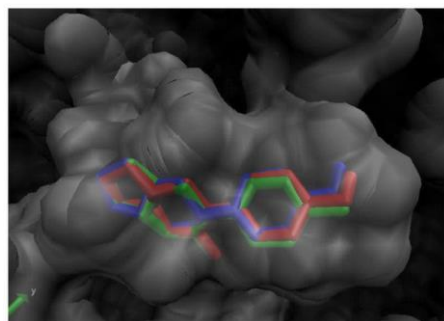


Figure 8. 5IKV binding site's molecular surface

Figure 9 shows 5KIR binding site's molecular surface (white). The binding affinity of each ligand are, -8.3 kcal/mol for unmodified ipalbidine (green), -8.6 kcal/mol for inverted chirality (red), and 8.2 kcal/mol for modified double bond to a single bond (blue).

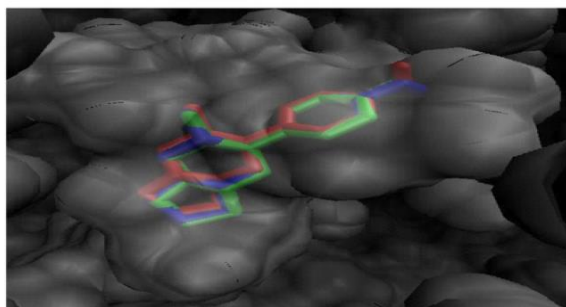


Figure 9. 5KIR binding site 's molecular surface

Thus overall, the unmodified ipalbidine (green line) was used as a baseline to compare the binding affinities of the modified ipalbidine. Focusing on the modification where a carbon double bond was replaced with a single bond to allow more hydrogen to bond with the ligand (blue line), the effects on the ligand 's binding affinity is somewhat random where it becomes less (in magnitude) for 5Fla, 5Fl9, and 5KIR; and more (in magnitude) for 5IKQ and 5IKT. This is possibly due to the hydrophobicity of the inner linings of the binding site which repels the ligand and reduces the binding affinity. For the cases in which the binding affinity increases by modify ing the double bond to a single bond thereby increasing the hydrogen atoms on the ligand. According to El-Azab et al. [14], the amino acids lining the binding site are largely hydrophobic with exception to residues (amino acid) Arg, Tyr, and Ser which are indeed present in the binding site of Cox-2. Focusing on the inverted chirality (red line), across the seven cox-2 protein structures, the inverted chirality shows higher binding affinity . This is supported by Arias S et al. [15] where a drug in which the left- handed or the right- handed chirality will have more efficacy or a different effect altogether. In the case of ipalbidine, the data shows that the inverted chirality has greater binding affinity which implies a more stable bond and more spontaneity for the bonding to occur. A reminder that changes in chirality are constant only because of the chirality of the nitrogen atom. The ligand's flexibility will rotate the ligand during the docking process in order to optimize the binding affinity by making changes in the chirality of the oxygen atom and the carbon ring null.

4. Conclusion

The ligand ipalbidine was modified using the Avogadro program to change its chirality and to change its carbon double bond to a single bond to increase the amount of hydrogen bonded to the ligand. The modified ligands were successfully docked into seven protein structures of COX-2 and were able to bind to the same locations as unmodified ipalbidine. From the obtained binding affinities, it was found that changing the double bond to a single bond and thus increasing the hydrogen atoms on the ligand caused a weakened bond. The increased number of hydrogen atoms may have caused a weaker binding due to the hydrophobic liner of theCox-2 binding site. For the modification of the chirality. The reversal of the chirality of the nitrogen atom showed more negative binding affinities across the board. This means better efficacy and bond strength. However, the change in the chirality of a drug may change the selectivity or effect of the drug as a whole, and therefore clinical trials may be required to see possible side-effects that may arise.

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