Molecular Docking of Ipalbidine into Human Cyclooxygenase-2 Protein Crystal Structures

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Abstract. Cyclooxygenase-2 (COX-2) is an enzyme responsible for the production of the prostaglandins, lipid molecules related to pain, inflammation, etc. Ipalbidine is a compound extracted from the crushed seeds isolated from Ipomea Alba L. (Moon Flower), which is a herbaceous vine usually found in tropical and subtropical areas in the world. The strength of the binding between a biomolecule and a ligand (drug or inhibitor) can be expressed in terms of binding affinity. When performing molecular docking, the binding affinity of a ligand bound to a receptor, in this case Ipalbidine bound to COX-2 structures, can be acquired. The seven currently available human COX-2 structures from the Protein Data Bank have been downloaded and prepared for molecular docking. Four programs have been used for the study: Autodock Tools, Autodock Vina, Visual Molecular Dynamics, and Chimera. After preparation, the binding affinities of the original bound ligands within the respective COX-2 structures were calculated. Next, the binding affinities of Ipalbidine and the seven crystal structures of COX-2 were acquired using molecular docking. The results show that Ipalbidine has a slightly lower binding affinity to the COX-2 structures compared to the original bound ligands, except for one. However, the rigid COX-2 crystal structures used in this study are optimized to accommodate the original bound ligands, not Ipalbidine. In other words, the docking results for Ipalbidine are expected to systematically underestimate binding affinities.

1. Introduction
Enzymes are protein molecules responsible for accelerating and accelerating chemical reactions in the human body. These enzymes are also responsible for converting substrates to products [1]. A specific example of this is the prostaglandin-endoperoxide synthase which is responsible for production of prostaglandins, prostacyclin, and thromboxanes, which are members of the lipid molecules [2].

There has been a development regarding this enzyme. Flower, Simmons and colleagues were able to stumble upon a discovery of prostaglandin-endoperoxide synthase [3]. The discovery was that they were able to find an isozyme, an identical enzyme with a different structure, prostaglandin-endoperoxide synthase. This was when prostaglandin-endoperoxide synthase 2 was detected. It was also decided to name the original enzyme Cyclooxygenase-1 (COX-1) and the newly discovered enzyme Cyclooxygenase-2 (COX-2) [3].

Functioning both as dioxygenase and peroxidase, COX-2 is an empiric enzyme that is essential in a number of pathophysiological processes such as inflammation, angiogenesis and tumorigenesis [3]. COX-2 is not only an efficient enzyme that oxygenates fatty acids and endocannabinoid ester substrates, but also a tissue-specific enzyme that is stimulated by cytokines and growth hormones [4].
It has been found that this enzyme is present in pituitary tumors, pituitary carcinomas, plays a role in tumor progression and bridges the synthesis of prostaglandin that is involved in tumor invasiveness and angiogenesis\[4\]. COX-2 also takes part in the development of cerebral ischemic damage and is present in human brain after ischemic stroke. Moreover, this enzyme is expressed in high levels during inflammation. Drugs that selectively inhibit COX-2 (Coxibs) are one of the most common choices of non-steroidal anti-inflammatory drugs (NSAIDs). Ipalbidine is a non-addictive analgesic that has inhibitory effects on leukocyte respiratory bursts\[4\]. This bioactive compound may be extracted from crushed seeds isolated from Ipomea Alba L. (Moon Flower) is a herbaceous vine commonly found in the tropical and subtropical regions of the world \[5\].

Playing an integral role in the development of drugs, computer-aided drug design through molecular docking is a cost-effective and simple tool to determine how enzymes interact with small molecules or ligands\[6\]. Ligand binding is the primary step in both the enzyme response and inhibition. The interactions between these proteins and molecules must be fully understood in the context of a sound drug design strategy\[7\]. In addition, molecular docking uses a scoring function that is used to calculate the binding affinity of the protein ligand complex. This score function provides an estimate of the affinity of the compound to the desired protein \[8\].

Cells communicate only with a ligand that can be any drug or inhibitor that can be detected by the target cell. The strength of the binding relationship between a biomolecule and a ligand (a drug or inhibitor) is called binding affinity. It is also the most important type of quantity associated with the drug and its target receptor. It has also been studied that binding affinity affects the potency of a drug. The potency of a drug is a measure of the concentration of the drug in order to have an effect\[9\]. It has been observed that ligands and receptors must complement each other in terms of size and shape. Inappropriate shape of a particular binding site causes a ligand not to bind to a receptor\[9\]. The binding strength of a ligand to a protein molecule is governed by a free energy change in the binding process. Drugs with a binding affinity between-6.2 kcal / mol and-13.7 kcal / mol are considered to have a high binding affinity\[10\]. Unfortunately, structural drug designs face challenges in predicting reliably the binding affinity of a compound that binds to the target protein; simplified approximations are often used \[11\].

The objective of this study is to determine whether the Ipalbidine structure will be capable of supporting the seven currently available human COX-2 structures that have been downloaded from the RCSB Protein Data Bank (RCSB PDB)\[12\]. In addition, the study also aims to calculate the binding affinity of Ipalbidine and the seven crystal structures of COX-2 and to determine whether Ipalbidine can be a potential substitute for COX-2 inhibitors. Since it has been stated that there are two isozymes, COX-1 and COX-2, this study focuses only on binding to the COX-2 receptor. Since studies show that there are differences in chromosomal organization with COX-2 for humans and rodents \[3\], this study focuses only on human COX-2.

2. Methodology

The ligands bound to the protein and the ID name of the COX-2 crystal structures they were bound to are Salicylate (SAL) from 5FlA; Meclofenamic acid (JMS) from 51KQ; Mefenamic acid (ID8) from 5IKR; Tolfenamic acid (TLF) from 5IKT; Flufenamic acid (FLF) from 5IKV; Rofecoxib (RCX) from 5Kir; and 5Fl9 is without any bound ligand. The Fenamic Acids and SAL are different types of NSAIDs while RCX is a type of coxib \[13\].

Various programs are used to perform molecular docking and to compare the structures of the COX-2 crystal. The various programs used for different purposes are Autodock Tools (ADT) Autodock Vina, Visual Molecular Dynamics (VMD) and Chimera. The 7 different COX-2 crystal structures were downloaded from the RCSB PDB for preparation and the Ipalbidine structure was downloaded from PubChem. Afterwards, the 7 COX-2 structures were opened individually in Chimera, as shown in Figure 1.

Initially, VMD was to be used in the preparation and isolation of 7 different protein structures. Although VMD can locate the different molecules in the structure, there is no feature to select and
save the current selection made. Figure 2 shows the feature of Chimera to be able to make selections and save them. Since chain B is a copy of chain A, only one chain is needed for docking. In addition, other unwanted molecules except the bound ligand were removed. The 7 isolated COX-2 crystal structures with the original bound ligand were opened in VMD simultaneously. In VMD, the 7 crystal structures were aligned by using an extension of VMD called Multiseq. Multiseq can structurally align all the currently opened files in VMD as shown in Figure 2.

![Figure 1. Program Chimera used Skir structure](image1)

![Figure 2. Aligned crystal structures](image2)

After alignment, Chimera was used to separate the bound ligands from the 7 COX-2 crystal structures and to save them separately. After the separation of the COX-2 structures and their bound ligands, the COX-2 structures were opened in ADT to create the search box for docking. In order to center the search box to the binding site of the COX-2 crystal structure, the bound ligand of the crystal structure was also opened in ADT and it was selected as the place where to center the grid box. Since this ligand is already in the binding site of the COX-2 crystal structure, the location of the binding site of the ligand defines the coordinates of the grid box so that it covers the binding site of the COX-2 crystal structure. The acquired coordinates have been saved to be used as docking inputs in Autodock Vina.
3. Result and Discussion
By using Autodock Vina's score-only function, the binding affinities of each of the original bound ligands within their respective COX-2 crystal structure were calculated. The binding affinities and conformation of Ipalbidine within the 7 COX-2 structures were determined using the scoring function. The search space box has been set to (20 A, 20 A, 20 A) in (x, y, z) space. Figure 3 shows the binding site of the 5FlA COX-2 Crystal Structure (white surface) with its original bound ligand, SAL (blue), and the docked Ipalbidine (red). The binding affinity of SAL with the 5FlA crystal Structure is -5.1 kcal/mol. On the other hand, Ipalbidine has a binding affinity of -6.9 with the binding site of the 5FlA crystal structure.

![Figure 3](image3.png)

**Figure 3.** Binding site of the 5FlA COX-2 Crystal Structure (white surface) with its original bound ligand, SAL (blue), and the docked Ipalbidine (red).

Figure 4 shows the binding site of the 51KQ crystal structure (white) with the original bound ligand JMS (blue) and the docked Ipalbidine (red). The binding affinity of JMS with the crystal structure is -8.4 kcal/mol. The docked Ipalbidine has a binding affinity of -7.5 kcal/mol with the crystal structure.

![Figure 4](image4.png)

**Figure 4.** binding site of the 51KQ crystal structure (white) with the original bound ligand JMS (blue) and the docked Ipalbidine (red)

Figure 5 shows the binding site of the 51KR crystal structure (white) with the original bound ligand ID8 (blue) and the docked Ipalbidine (red). The binding affinity of ID8 with the crystal structure is -8.4 kcal/mol and Ipalbidine has a binding affinity of -7.6 kcal/mol.
Figure 5. binding site of the 5IKR crystal structure (white) with the original bound ligand ID8 (blue) and the docked Ipalbidine (red).

Figure 6 shows the binding site of the 5IKT crystal structure (white) with the original bound ligand TLF (blue), and the docked Ipalbidine (red). The binding affinity of TLF with the crystal structure is -8.3 and the binding affinity of Ipalbidine with the crystal structure is -7.9 kcal/mol.

Figure 6. 5IKT crystal structure (white) with the original bound ligand TLF (blue), and the docked Ipalbidine (red).

Figure 7 shows the binding site of the 5IKV crystal structure (white) with the original bound ligand FLF (blue) and the docked ligand Ipalbidine (red). The binding affinity of the bound FLF to the crystal structure is-8.3 kcal / mol. Lpalbidine, on the other hand, has a binding affinity of-7.8 kcal / mol.

Figure 7. binding site of the 5IKV crystal structure (white) with the original bound ligand FLF (blue) and the docked Ipalbidine (red).
Figure 8 shows the binding site of the 5KIR crystal structure (white) with the original bound RCX ligand (blue) and the docked Ipalbidine (red). The binding affinity of the RCX bound to the crystal structure is -9. It's 1 kcal / mol. And the binding affinity of Ipalbidine to the crystal structure is -8.3 kcal / mol.

![Figure 8](image1.png)

**Figure 8.** binding site of the 5KIR crystal structure (white) with the original bound ligand RCX (blue) and the docked Ipalbidine (red).

Figure 9 shows the binding site of the 5F19 crystal structure (white) and the Ipalbidine dome (red). Since the 5F19 is the COX-2 crystal structure without any bound ligand, only the binding affinity of Ipalbidine has been acquired. The binding affinity of Ipalbidine with the crystal structure is -8.7 kcal / mol.

![Figure 9](image2.png)

**Figure 9.** binding site of the 5F19 crystal structure (white) and the docked Ipalbidine (red).

As a result, it can be seen that Ipalbidine was able to take the orientation and space of the original SAL ligand within the 5FlA crystal structure (Figure 3). The binding affinity of Ipalbidine is also stronger than that of SAL. Figures 4 to 7 show that Ipalbidine has consistently been able to detect binding sites and conformations similar to those of the original ligands JMS, ID8, TLF and FLF. In addition, the binding affinity of Ipalbidine to 4 COX-2 crystal structures (5IKQ, 5IKR, 5IKT, 5IKV) is lower compared to 4 bound ligands of 4 crystal structures. In Figure 8, Ipalbidine was capable of entering the binding site and had a similar position to that of the RCX. Among all the original bound ligands, RCX has the strongest binding affinity of -9.1 kcal / mol. Finally, Figure 8 shows the docking of Ipalbidine at the binding site of 5F19. It can be observed that the binding site of 5F19 is slightly wider than the other crystal structures of COX-2. Figure 10 summarizes the binding affinity of the COX-2 crystals of the COX-2 crystal structures to Ipalbidine (green) and to the original ligand bound (red).
Figure 10. Summary of the binding affinity of the COX-2 crystal structures with Ipalbidine (green), and with the original ligand bound (red).

4. Conclusion
The ligand Ipalbidine was able to dock all 7 of the COX-2 crystal structures at the binding site. In addition, Ipalbidine was able to have similar positions compared to the 6 original bound ligands in the corresponding COX-2 crystal structures. Compared to the original bound ligands, except for SAL, Ipalbidine docked has a slightly lower binding affinity to COX-2. However, the rigid COX-2 crystal structures used in this study are optimized to accommodate the original bound ligands rather than Ipalbidine. In other words, the docking results for Ipalbidine are expected to systematically underestimate the binding affinities. The binding affinities and conformations of Ipalbidine to COX-2 acquired show that it may be a potential inhibitor of COX-2. In order to improve the binding of Ipalbidine to COX-2, it is recommended that different types of changes be made to Ipalbidine.

5. References
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