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Exploring the Potential of Molecular Modeling in Developing Anti-Inflammatory Agents from Nano CBD using ArgusLab

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Abstract: - The aim of this research was to identify the binding arrangements between compounds derived from nano lipid particles and cannabidiol, as well as the cyclooxygenase-2 (COX-2) enzyme. To achieve this, the ArgusLab 4.0.1 program was utilized as the docking engine, facilitating the exploration of potential binding conformations. This study was to investigate the binding mechanisms and conformations of compounds with the cyclooxygenase-2 (COX-2) enzyme. The research process began by creating a 2D structure of the compound using ChemSketch, which was then converted into a 3D structure through energy minimization using the Avogadro program. The structure of the COX-2 enzyme was obtained from the Brookhaven Protein Data Bank. The compounds and enzyme were subjected to ArgusLab 4.0.1 for docking experiment. The lowest energy binding with an appropriate structure comparing with NSAID SC-558 was selected for each run. The binding of these substances to COX-2 was analyzed using the ArgusLab program. The findings revealed that both Cannabidiol and Phosphatidylcholine (PC) exhibited selective COX-2 inhibitory activity, with Binding Energy values of -13.71 and -14.75 kilocalories/mol, respectively. These results were then used to explore the relationship between Binding Energy and IC50 values for both substances. The analysis showed a correlation coefficient (r) of 0.753135, indicating that the simulated binding interaction aligns with the inhibitory effect on COX-2. This simulation approach proves valuable in studying the mechanism of action of Cannabidiol and Phosphatidylcholine (PC).

Keywords: Anti-inflammatory, Molecular Modeling, cyclooxygenase-2, ArgusLab, Nano lipid particles , Cannabidiol and Phosphatidylcholine (PC).

I. INTRODUCTION

Inflammation is a fundamental physiological response that serves as a protective mechanism in the body. It plays a crucial role in defending against various threats, initiating the healing process, and restoring normal tissue function. (Nathan, & Ding, 2010). Understanding the intricate mechanisms of inflammation can provide insights into the development of therapeutic interventions for inflammatory disorders and contribute to overall health and well-being.

The inflammatory process can indeed be categorized into two main types: acute inflammation and chronic inflammation. Acute inflammation is characterized by a rapid onset, typically occurring within seconds or minutes after exposure to a stimulus. It is a short-lived response that generally lasts for about 2 to 3 days, but it usually does not exceed 1 week. Acute inflammation is a localized and controlled process that aims to eliminate the initial cause of injury or infection and initiate the healing process. It involves the dilation of blood vessels, increased permeability of blood vessels, migration of immune cells to the site of injury or infection, and release of inflammatory mediators (Serhan and Levy, 2018).

Inflammation and pain are closely interconnected processes in the body. Inflammation can often lead to the sensation of pain, and pain itself can trigger an inflammatory response. When tissue is injured or infected, the body initiates an inflammatory response as a protective mechanism. Inflammation involves the release of various chemical mediators, such as histamines, prostaglandins, and cytokines, which promote vasodilation, increase blood flow, and attract immune cells to the affected area. These mediators can also sensitize nerve endings, leading to the perception of pain. Inflammatory mediators can directly activate pain receptors (nociceptors) or lower their threshold for activation, making them more sensitive to stimuli (Ji and Xu, 2019).

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Inflammatory pain is a type of pain that arises from inflammation. It is typically described as a throbbing, aching, or burning sensation and is often accompanied by redness, swelling, and heat in the affected area. Inflammatory pain serves as a warning signal, alerting the body to the presence of tissue damage or infection. It helps to limit movement and protect the injured area, allowing for healing and recovery.

Chronic Inflammation and Chronic Pain: In some cases, inflammation can become chronic, lasting for an extended period. Chronic inflammation can lead to persistent pain, even after the initial cause of inflammation has been resolved. This can occur due to ongoing tissue damage, immune system dysfunction, or the development of sensitization in the pain pathways. Chronic inflammatory pain can significantly impact an individual's quality of life and may require specialized management and treatment approaches (Julius & Basbaum,2001) Conversely, pain itself can trigger an inflammatory response. Intense or prolonged pain can activate the release of inflammatory mediators, leading to localized inflammation. This can create a cycle where inflammation causes pain, and pain triggers further inflammation, resulting in a self-perpetuating loop (Woolf & Salter, 2000)

Inflammation can lead to pain through various mechanisms. During inflammation, immune cells release a variety of chemical mediators, such as histamines, prostaglandins, bradykinin, and cytokines. These mediators can sensitize nerve endings, particularly pain receptors called nociceptors. They can directly activate nociceptors or lower their threshold for activation, making them more sensitive to stimuli. This increased sensitivity of nociceptors contributes to the perception of pain (Schaible and Grubb,1993). Another protocol of inflammation leads to increased blood flow to the affected area, a process known as vasodilation. This increased blood flow can cause tissue swelling and put pressure on surrounding structures, including nerve endings. The compression of nerve endings can trigger pain signals and contribute to the sensation of pain (Julius & Basbaum, 2001)

Inflammation is often triggered by tissue injury, such as trauma or infection. This injury leads to the release of various chemical signals, including cytokines and growth factors. (Chen, Deng, Cui, et al, 2017). The activation of COX enzymes, particularly COX-2, plays a crucial role in the process of inflammation. When tissues in the body are injured or damaged, certain cells release chemical signals called cytokines and growth factors. These signals trigger the activation of Cox enzymes. Cox-1 is constitutively expressed in most tissues and is involved in maintaining normal physiological functions, such as protecting the stomach lining and promoting blood clotting. On the other hand, Cox-2 is an inducible enzyme that is primarily involved in the inflammatory response (Choi, Aid, & Bosetti, 2009).

The activation of Cox enzymes leads to the production of prostaglandins, which are hormone-like substances that regulate various physiological processes, including inflammation. Prostaglandins are involved in the dilation of blood vessels, increased blood flow to the site of injury, and the recruitment of immune cells to the affected area. (Chen, Deng, Cui, Fang, Zuo, Deng, Li, Y., & Zhao, 2017).

In the case of inflammation, the activation of Cox-2 leads to an increased production of prostaglandins, particularly prostaglandin E2 (PGE2). PGE2 promotes vasodilation, causing blood vessels to widen and allowing more blood to flow to the site of inflammation. This increased blood flow results in redness and warmth at the affected area. Moreover, PGE2 also sensitizes nerve endings to pain, contributing to the sensation of pain associated with inflammation. Additionally, prostaglandins can increase the permeability of blood vessels, allowing immune cells to migrate from the bloodstream into the tissues, where they can help fight off pathogens and promote tissue repair (Williams, Mann, & DuBois, 1999)

Anti-Inflammatory Properties, CBD extract has been shown to possess potent anti-inflammatory properties. This can be beneficial in addressing various inflammatory conditions, including infections. By reducing inflammation, CBD may help alleviate symptoms associated with infections and support the body's natural healing processes.

Using CBD extract, an herb with anti-inflammatory properties, as a potential replacement for antibiotics can offer several benefits. Some studies suggest that CBD extract may exhibit antibacterial properties. While more research is needed to fully understand its antibacterial mechanisms, preliminary findings indicate that CBD may have the ability to inhibit the growth of certain bacteria. This suggests that CBD could potentially be used as an alternative or adjunct to antibiotics in certain cases (Gildea, Ayariga, Ajayi, Xu, Villafane, & Samuel-Foo, 2022). Antibiotic resistance is a growing concern globally, as bacteria develop resistance to commonly used antibiotics. (Gildea, Ayariga, Xu, Villafane, Robertson, Samuel-Foo, & Ajayi, 2022). By exploring alternative options like CBD extract, which may have antibacterial properties, we can potentially reduce the reliance on antibiotics and mitigate the risk of antibiotic resistance. (Schofs, Sparo, & Sánchez Bruni, 2021).

Using molecular docking as a preliminary step in drug discovery and development, specifically for identifying selective COX-2 inhibitors from Nano CBD extracts, can offer several advantages over directly synthesizing and testing prototype substances. Molecular docking allows for a more efficient screening process compared to synthesizing and testing prototype substances. It involves virtually predicting the binding affinity and interactions between CBD compounds and the COX-2 enzyme. This computational approach significantly reduces the time and cost associated with chemical synthesis and experimental testing. Molecular docking provides insights into the binding modes and interactions between CBD compounds and the COX-2 enzyme. This information can guide the rational design of new compounds with improved selectivity and potency. By understanding the structural requirements for COX-2 inhibition, researchers can optimize the chemical structure of CBD derivatives to enhance their selectivity and efficacy. It's important to note that while molecular docking is a valuable tool in drug discovery, it is not a substitute for experimental validation. Synthesizing and testing prototype substances is still necessary to confirm the predicted binding affinities and selectivity of CBD compounds as COX-2 inhibitors. Molecular docking serves as a valuable initial step to guide and prioritize the experimental efforts in the drug discovery process.

This research aims to explore methods for simulating anti-inflammatory molecules, specifically focusing on the interaction between the structure of substances found in the herbal plant CBD and Phosphatidylcholine (PC) with the enzyme cyclooxygenase-2 (COX-2). The study employs a computer program utilizing the docking method, with the ArgusLab program serving as the primary tool for analyzing the molecular interactions. Additionally, the well-known selective COX-2 inhibitor, SC-558, is used as a reference for comparison. The objective is to elucidate the structural arrangement required for a substance to exhibit selective COX-2 inhibitory properties.

II. Literature Review

Inflammation and pain are closely interconnected processes in the body. In the article named “Inflammation in pathogenesis of chronic pain: Foe and friend", discusses the relationship between inflammation and chronic pain. It explains how acute pain, which involves immune cells and inflammatory mediators, can progress to chronic pain. The article highlights the importance of understanding inflammation in the development and management of chronic pain (Fang, Zhai, Zhu, M., He, Wang, & Zhang, 2023). The inflammatory response involves the activation of signaling pathways that regulate inflammatory mediators in both resident tissue cells and recruited inflammatory cells. These mediators can sensitize pain receptors, leading to the perception of pain (Chen, Deng, Cui & Zhao, 2017). Inflammation has been implicated in the pathophysiology of depression. Peripheral and central inflammation processes can affect brain function and structure, leading to depressive symptoms. Inflammatory markers have been found to be associated with altered neural circuitry and increased pain sensitivity in individuals with depression (Han, & Ham, 2021).

Several sources that discuss the release of chemical mediators by immune cells during inflammation. These mediators include histamines, prostaglandins, bradykinin, and cytokines. The article titled "The crucial roles of inflammatory mediators in inflammation: A review" provides an overview of the various chemical mediators involved in the inflammatory response. It mentions that vasoactive amines like histamine and serotonin, peptides like bradykinin, and eicosanoids like prostaglandins are released by inflammatory cells and injured tissue to contribute to and regulate the inflammatory response. (Abdulkhaleq, Assi, Abdullah, Zamri-Saad, Taufiq-Yap, & Hezmee, 2018). Another source titled "Role of Histamine in Modulating the Immune Response and Inflammation" specifically focuses on the role of histamine as a biogenic vasoactive amine in the immune response and inflammation. It mentions that histamine, along with other inflammatory mediators like cytokines, bradykinin, prostaglandins, and leukotrienes, can act as proinflammatory factors or regulatory components to establish homeostasis after injury (Branco, Yoshikawa, Pietrobon, & Sato,2018)

The crucial role of COX enzymes, particularly COX-2, in the process of inflammation. COX-2 is induced during inflammation and contributes to the production of prostaglandins, which are key mediators of inflammation and pain. The article titled "Cyclooxygenase-2: A Therapeutic Target in Inflammation and Pain" provides an in-depth review of the role of COX-2 in inflammation and pain. It discusses how COX-2 is induced during inflammation and contributes to the production of prostaglandins, which are important mediators of inflammation and pain (Zhang and An, 2007). Another source titled "Cyclooxygenase-2 and inflammation-mediated neurodegeneration: Opportunities for therapeutic intervention" focuses on the role of COX-2 in inflammation-mediated neurodegeneration. It discusses how COX-2 is upregulated in response to inflammatory stimuli and contributes to the production of pro-inflammatory mediators, leading to neuronal damage (Thomas, Murray, Flockhart, Pintar, Pollari, Fazil, Nesbitt, & Marshall, 2013). The article "Cyclooxygenase-2 and its role in inflammation" provides an

overview of the role of COX-2 in inflammation. It discusses how COX-2 is involved in the synthesis of prostaglandins, which are important mediators of inflammation and immune responses (Hagendoorn, Padera, Yock, Nielsen, et al , 2006). The review article titled "Cyclooxygenase-2: A pivotal mediator of inflammation and a target for anti-inflammatory drugs discusses the role of COX-2 as a pivotal mediator of inflammation. It highlights the importance of COX-2 in the production of prostaglandins and its involvement in various inflammatory conditions. (Hagendoorn, Padera, Yock, Nielsen, et al , 2006)

The role of COX-2 activation in increasing the production of prostaglandins, particularly prostaglandin E2 (PGE2), in the context of inflammation. The increased levels of PGE2 contribute to the inflammatory response by promoting vasodilation, edema, and immune cell recruitment. The article titled "Prostaglandin E2: An Essential Mediator of Inflammation" provides an overview of the role of prostaglandin E2 (PGE2) in inflammation. It discusses how COX-2 is upregulated during inflammation, leading to increased production of PGE2, which acts as a potent pro-inflammatory mediator. This paper also discusses the role of prostaglandin E2 (PGE2) in inflammation. It highlights how COX-2 activation leads to increased production of PGE2, which promotes vasodilation, edema, and recruitment of immune cells during the inflammatory process. (Ricciotti & FitzGerald, 2011). Another source titled "Prostaglandin E2: From Basic Science to New Therapeutic Approaches" focuses on the role of prostaglandin E2 (PGE2) in inflammation and its potential as a therapeutic target. It discusses how COX-2-derived PGE2 plays a crucial role in the initiation and maintenance of inflammation Golianu, Yeh, & Brooks, 2014).

Anyhow the potential of CBD extract to possess potent anti-inflammatory properties. CBD can modulate the immune response, reduce cytokine production, and interact with cannabinoid receptors to exert its anti-inflammatory effects. The article titled "Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress" discusses the anti-inflammatory properties of CBD extract. It highlights how CBD can modulate the immune response and reduce inflammation by interacting with cannabinoid receptors and other molecular targets (Booz, 2011). Another source titled "Cannabidiol in Inflammatory Bowel Diseases: A Brief Overview" focuses on the potential therapeutic effects of CBD in inflammatory bowel diseases. It discusses how CBD can reduce inflammation and improve symptoms by interacting with cannabinoid receptors and exerting anti-inflammatory effects (Nichols, & Kaplan, 2020). The review article titled "Cannabidiol as an Anti-Inflammatory Drug" provides an overview of the anti-inflammatory properties of CBD. It discusses how CBD can suppress inflammatory responses by inhibiting cytokine production, reducing immune cell activation, and modulating the endocannabinoid system (Nagarkatti, Pandey, Rieder, Hegde, & Nagarkatti,2009). The article "Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: Role for the adenosine A2A receptor" explores the anti-inflammatory effects of CBD in a murine model of acute lung injury. It demonstrates that CBD can reduce inflammation by activating the adenosine A2A receptor (Booz ,2011).

CBD extract, with its anti-inflammatory properties, may have the potential to enhance the activity of antibiotics against bacteria and reduce inflammation. The article titled "Cannabidiol, a Major Non-Psychotropic Cannabis Constituent Enhances the Activity of Antibiotics against Gram-Positive Bacteria" discusses the potential of CBD extract to enhance the activity of antibiotics against gram-positive bacteria. It suggests that CBD can be used as an adjuvant to improve the effectiveness of antibiotics (Viteles,1931). Another source titled "Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: Role for the adenosine A2A receptor" explores the anti-inflammatory effects of CBD. While it does not specifically focus on the replacement of antibiotics, it highlights the potential of CBD to reduce inflammation, which can be beneficial in various conditions (Booz, 2011). The article "Cannabidiol, a Major Non-Psychotropic Cannabis Constituent, Enhances the Activity of Bacitracin" investigates the synergistic effects of CBD and the antibiotic bacitracin against gram-positive bacteria. It suggests that CBD can enhance the antibacterial activity of bacitracin, potentially reducing the required dosage of antibiotics (Viteles,1931).

The advantages of using molecular docking as a preliminary step in drug discovery and development, specifically for identifying selective COX-2 inhibitors from Nano CBD extracts. Molecular docking allows for the screening of large compound libraries, prediction of binding affinity and selectivity, and optimization of potential drug candidates, saving time and resources compared to directly synthesizing and testing prototype substances. The article titled "Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery" provides an overview of molecular docking as a tool in drug discovery. It discusses how molecular docking can be used to predict the binding affinity and selectivity of potential drug candidates, including COX-2 inhibitors. (Luo, & Yobas, 2014). Another source titled "Molecular Docking: A Review of Recent Advances" focuses on the recent advances

in molecular docking techniques. It discusses how molecular docking can be used to screen large compound libraries and identify potential COX-2 inhibitors with high selectivity (Luo, & Yobas, 2014). In summary, utilizing molecular docking as a preliminary step in drug discovery and development, specifically for identifying selective COX-2 inhibitors from Nano CBD extracts, offers several advantages. It enables the efficient screening of a large number of compounds, prediction of binding affinity and selectivity, and exploration of structure-activity relationships. This approach can save time and resources, ultimately leading to the identification of potential drug candidates for further experimental validation.

The scope of this study is to explore methods for simulating anti-inflammatory molecules, specifically focusing on the interaction between the structure of substances found in the herbal plant Nano CBD and the enzyme cyclooxygenase-2 (COX-2).

In this study, we use these compounds to investigate.

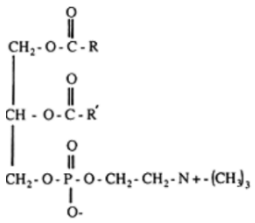
1) Cannabidiol (CBD) is a naturally occurring compound found in the cannabis plant. Its chemical structure consists of 21 carbon atoms, 30 hydrogen atoms, and 2 oxygen atoms. Here is the simplified chemical structure of cannabidiol:

H
|
H - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - C - O - H | | | | | | | | | | | | | | | | | | | | | | H H H H H H H H H H H H H H H H H H H H H H H H H H

This structure represents the arrangement of carbon, hydrogen, and oxygen atoms in a molecule of cannabidiol.

2) Phosphatidylcholine (PC):

Phosphatidylcholine (PC) is a type of phospholipid that is a major component of cell membranes. Its chemical structure consists of a glycerol backbone, two fatty acid chains, a phosphate group, and a choline molecule. Here is the simplified chemical structure of phosphatidylcholine:



In this structure, the glycerol backbone is represented by the central carbon atom (C) with three hydroxyl groups (OH) attached to it. The two fatty acid chains (R1 and R2) are attached to the first and second carbon atoms of the glycerol backbone, respectively. The phosphate group (PO4) is connected to the third carbon atom of the glycerol backbone, and the choline molecule (N(CH3)3+) is attached to the phosphate group. and location within the cell membrane.

Phosphatidylcholine (PC) is often used as a key component in the formulation of solid lipid nanoparticles (SLNs). SLNs are lipid-based nanoparticles that are used for drug delivery and other applications. PC is a type of phospholipid that is commonly found in cell membranes and has excellent biocompatibility and biodegradability (Mukherjee, Ray, & Thakur, 2009)

In the context of SLNs, PC is typically used as a surfactant or emulsifier to stabilize the nanoparticles and prevent their aggregation. The presence of PC in the lipid matrix of SLNs helps to improve their stability, enhance drug encapsulation efficiency, and control drug release. Phosphatidylcholine (PC) can be incorporated into the lipid matrix of SLNs during the preparation process. It forms a lipid bilayer structure around the core of the SLNs, providing stability and preventing drug leakage. The PC molecules on the surface of the SLNs also help to reduce the surface tension and prevent particle aggregation (Mishra, Bansal, Verma, Yadav, Thakur, Sudhakar, & Rosenholm, 2018).

Overall, phosphatidylcholine plays a crucial role in the formulation and stability of solid lipid nanoparticles. Its presence helps to improve the performance and biocompatibility of SLNs, making them effective carriers for drug delivery applications.

III. METHODOLOGY

3.1 Research Tools

To conduct molecular docking of nano CBD and Phosphatidylcholine (PC), in this research, we need to prepare the following

3.1.1 Software: Choose a molecular docking software that is suitable for your needs. Ensure that the software supports the specific file formats of nano CBD and PC. Some commonly used software for molecular docking include

- Computer CPU Intel Core i7-4500U (1.80 GHz, 4MB L3 Cache, up to 3.00 GHz), Graphic cards: nVidia GeForce GT 740M (2 GB GDDR3), Ram: 4 GB DDR3 , Hardisk: 4 GB DDR3, Widescreen : 14 inch WXGA (1366x76S8) LED
- Software :

3.1.1.1 ChemSketch : ChemSketch is a popular software tool for drawing chemical structures and is widely used in the scientific community. (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/)

3.1.1.2 Discovery Studio Visualiser (http://accelrys.com/products/discovery-studio/visualization-download.php)

3.1.1.3 Avogadro: Avogadro is a popular open-source software tool for molecular modeling and visualization (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/)

3.1.1.4ArgusLab (http://www.arguslab.com/arguslab.com/ArgusLab.html)

3.1.2 Protein Structure: Obtain the three-dimensional structure of the target protein or receptor with which you want to dock CBD and Phosphatidylcholine (PC). This can be obtained from protein structure databases like the Protein Data Bank (PDB).

3.1.3 Ligand Structure: Obtain the three-dimensional structure of CBD and Phosphatidylcholine (PC). Then generate it using molecular modeling software.

3.1.4 Ligand Preparation: Prepare the CBD and Phosphatidylcholine (PC) structure for docking by optimizing its geometry, adding hydrogen atoms, and assigning partial charges. This can be done using molecular modeling software available in the docking software itself.

3.1.5 Receptor Preparation: Prepare the protein structure for docking by removing any water molecules, ions, or other ligands that are not relevant to the docking study.

3.1.6 Grid Generation: Define the docking search space around the active site of the protein where CBD and Phosphatidylcholine (PC) will be docked.

3.1.7 Docking Parameters: Set the appropriate docking parameters, such as the search algorithm, scoring function, and number of docking poses to generate.

3.1.8 Docking Execution: Run the molecular docking simulation using the prepared ligand and receptor structures, along with the defined docking parameters. The software will generate multiple docking poses and rank them based on their predicted binding affinity.

3.1.9 Analysis and Visualization: Analyze the docking results to identify the most favorable binding poses and interactions between the molecular structures of CBD and Phosphatidylcholine (PC) and the target protein.

3.2 Research Process

To prepare the docking study with ArgusLab, we need to collect the molecular structures of CBD (Figure 1)and Phosphatidylcholine (PC) (Figure 2)

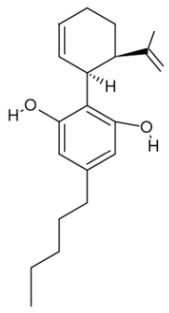


Figure 1 Molecular structures of CBD

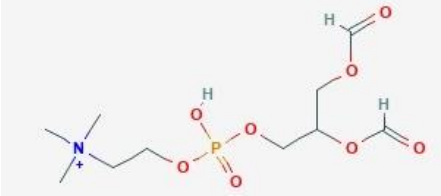


Figure 2 Molecular structures of Phosphatidylcholine (PC)

3.2.1 Input the molecular structures of CBD and Phosphatidylcholine (PC) and generate the 2D structures with ChemSketch, a chemical drawing software.

3.2.2 Obtaining the 3D structures of the molecular structures of CBD and Phosphatidylcholine (PC), ensuring they are in the appropriate file format (such as PDB or MOL2), and checking for any errors or inconsistencies in the structures. The 3D structures of the molecular structures of CBD and Phosphatidylcholine (PC) will be illustrated as attached (Figure 3 and Figure 4)

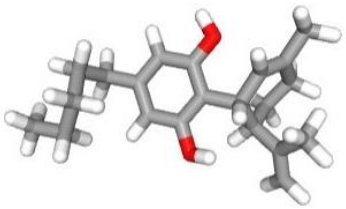


Figure 3 3D structures of the molecular structures of Cannabidiol (CBD)

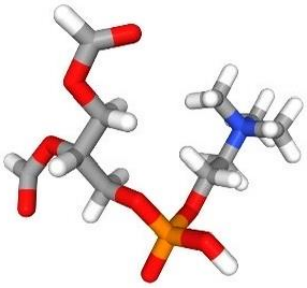


Figure 4 3D structures of the molecular structures of Phosphatidylcholine (PC)

3.2.3 Search the 3D structure of a selective COX-2 inhibitor by downloading from protein Data Bank (http://www.rcsb.org/pdb/). The Selective COX-2 inhibitors typically have a core structure that consists of a central aromatic ring system, often a sulfonamide or a sulfone group, and various substituents attached to the ring system. These substituents can include alkyl or aryl groups, which contribute to the selectivity of the inhibitor towards the COX-2 enzyme.

The 3D structure of a selective COX-2 inhibitor is characterized by the arrangement of these functional groups and their interactions with the active site of the COX-2 enzyme. The inhibitor is designed to fit into the active site of the enzyme and block its activity, specifically inhibiting the production of prostaglandins associated with inflammation.

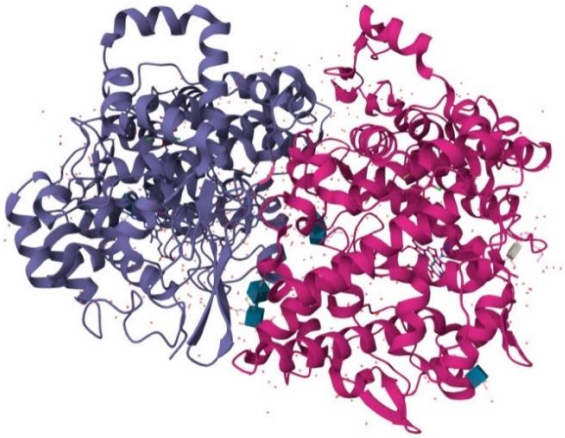


Figure 5 3D structures of selective COX-2 inhibitor

3.2.4 Bring the three-dimensional structures of cannabidiol, phosphatidylcholine (PC), and a selective COX-2 inhibitor into the ArgusLab program for docking.
3.2.4.1 Prepare the 3D structures of cannabidiol, phosphatidylcholine (PC), and the selective COX-2 inhibitor which obtained from reliable chemical databases or generated using molecular modeling software as PDB format file.
3.2.4.2 Import the structures in ArgusLab, go to the "File" menu and select "Open" or use the corresponding toolbar button to import the 3D structures of cannabidiol, phosphatidylcholine (PC), and the selective COX-2 inhibitor. Navigate to the location where the files are saved and select them for import. Then double click at selective COX-2 inhibitor - >Residues ->Misc -> S-558
3.2.4.3 Right click S-558 and make a BindingSite Group for this Group and press set up a Dock calculation and select each the 3D structures of cannabidiol, phosphatidylcholine (PC), and the selective COX-2 inhibitor and then calculate site and start
3.2.4.4 Double click at Calculations and ArgusDock to observe Conformations of Ligands including Binding energy of each Docking rank results. The lower the value of the Binding Energy, the more desirable it is. However, it is important to take into account other factors, such as the bonding of amino acids, in conjunction with the Binding Energy value.
3.2.4.5 Record the docking results as .agl .abd .pdb file

3.3 Research Analysis and Visualization

Interpreting the data analysis of molecular docking results obtained from Argus lab 4.0 involves several steps. Here's a general guide to help you interpret the data:
3.3.1 Start by examining the binding energy values for each docked complex. Lower binding energy values indicate stronger interactions between the ligand and the receptor, suggesting a more favorable binding affinity. Compare the binding energies of different docked poses to identify the most energetically favorable ones.
The ligand obtained at each rank was assessed for its similarity to the binding of S-558 in the cyclooxygenase-2 (cox-2) enzyme, in order to determine whether the ligand exhibited properties of a selective cox-2 inhibitor. This comparison was performed using the Arguslab program's viewing capabilities.
Save the image of each Ligand rank as a .pdb file. Then, open each file in the Discovery Studio 4.0 program to observe the interaction between the substance and the amino acid group in the enzyme. Next, obtain the three-dimensional structure of the enzyme, specifically cyclooxygenase-2 (Cox-2). Finally, overlay Cox-2 with each rank of ligand to analyze their interactions.
3.3.2 Utilize the visualization tools in Argus lab 4.0 to analyze the docked complexes. Pay attention to the orientation, conformation, and overall fit of the ligand within the binding site. Look for any steric clashes or favorable interactions, such as hydrogen bonds or hydrophobic contacts. Visualizing the complexes can provide insights into the quality of the docking results.
3.3.3 Examine the binding site of the receptor and observe how the ligand interacts with it. Identify specific amino acids or residues involved in the binding. Look for critical interactions, such as hydrogen bonds, salt bridges, or hydrophobic interactions. Analyzing the binding site can help understand the key molecular interactions driving ligand-receptor binding.
In this research, we align the ligands with the enzymes to determine whether the arm of the ligand extends into the side pocket of the selective COX-2 inhibitor.
To determine if a ligand has the potential to be a good selective COX-2 inhibitor, it is important to examine how closely its binding overlaps with the three-dimensional structure of the Selective COX-2 inhibitor. If the ligand, at any rank, exhibits binding that closely aligns with the structure of the Selective COX-2 inhibitor and shares the same direction of binding, along with a low energy value, it is considered to possess the necessary qualities to be a promising selective COX-2 inhibitor.
3.3.4 Evaluate the ranking and scoring of the docked complexes. Argus lab 4.0 typically provides a ranking of the docked poses based on their binding energies. Consider the top-ranked poses as they are more likely to represent the most favorable binding configurations. However, it's important to note that ranking alone may not always guarantee accuracy, so further analysis is recommended.
Based on the criteria mentioned above, Ligands that possess structures similar enough to function as selective COX-2 inhibitors were chosen.
3.3.5 Validate the docking results by comparing them with experimental data or known binding modes if available. If the docking results align with experimental findings or known binding orientations, it adds confidence to the

reliability of the predictions. Additionally, comparing the docking results with other computational methods or alternative docking software can provide a broader perspective on the reliability of the results**.**

Analyze the findings and present the results of the study by creating a graph that illustrates the correlation between the Ligand's Binding Energy and the substance's IC50. Additionally, examine if there is a linear relationship between the two variables.

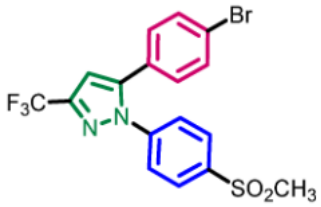


Figure 6 SC-558 2D Structure

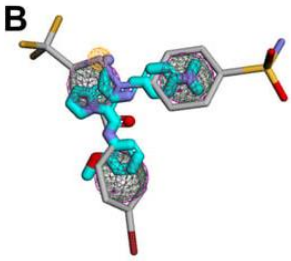


Figure 7 SC-558 3D Structure

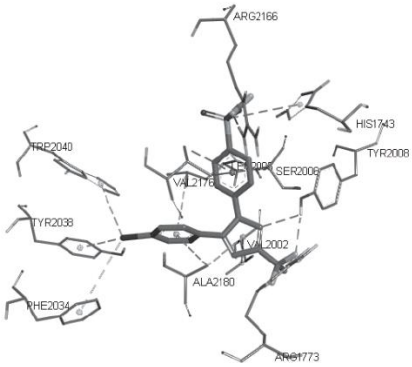


Figure 8 llustrate the positioning of SC-558 in proximity to VAL2176 and ARG2166, which represent the side pockets of the enzyme Cyclooxygenase-2 (COX-2), showcasing its structural arrangement.

IV. Results and Discussion

The docking result between plant substances (ligand) or a specific substance in CBD and lecithin, a type of fat, which is a mixture of unsaturated fatty acids, phosphates, and nitrogenous bases, was used to create a nano-type drug delivery system. This system, referred to as 1CX2, utilized phosphatidylcholine (PC), a chemical name for lecithin. The study findings were reported in terms of Binding Energy values in comparison to IC50 (Median Inhibitory Concentration) values.

Table 1: Results of docking between plant substances (Ligand) or substances in Cannabidiol (CBD), Phosphatidylcholine (PC) and Cyclooxygenase-2 enzyme (COX-2)

	Binding Energy (Kilocalories: Mole)	IC50 COX-2 (µM, N=3)
Cannabidiol (CBD)	-12.34	5±5
Phosphatidylcholine (PC)	-14.34	9±3

After docking between plant substances (Ligand) or substances in Cannabidiol (CBD), Phosphatidylcholine (PC) and Cyclooxygenase-2 enzyme (COX-2), Binding energy are -12.34 and -14.34 respectively.

From table 1, From the results of the study in Table 1, a graph was created to find the relationship between the negative values of Binding Energy and IC_{50} those of the two substances. It was found that there was a linear

relationship between the two values. The correlation coefficient (r) is equal to 0.753135, which is a good and appropriate value. This shows that the binding of a substance to an enzyme is related to its structure. This is explained in Figure 8 substances with low Binding Energy values correspond to low IC_{50} values.

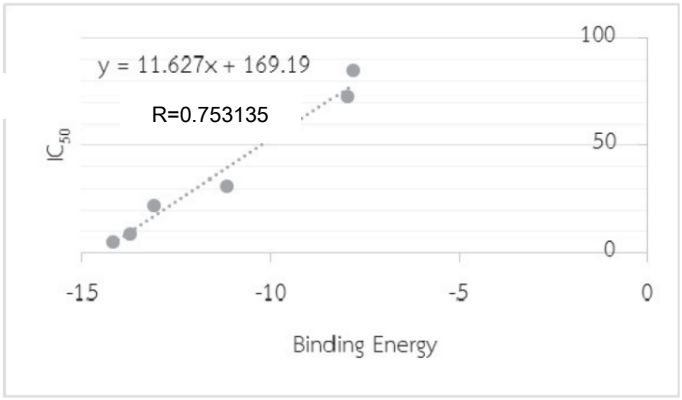


Figure 8 The relationship value of Binding Energy and IC50

Based on the results of the above analysis, it is evident that cannabidiol (CBD) and Phosphatidylcholine (PC) are two substances that exhibit the effect of being a Selective COX-2 inhibitor. The binding of their structure was found to overlap with the position of SC-558 in the plane and is positioned close to VAL2176 and ARG2166, which are side pockets of the cyclooxygenase-2 (COX-2) enzyme. This explains why the synthesized Nanoparticle Cannabidol (Nano CBD) formed by these substances can effectively prevent the substrate from binding with the enzyme, as depicted in Figure

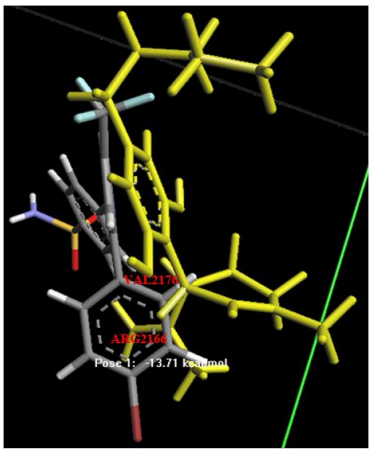


Figure 9 SC-558 (gray) binds to the binding site of the enzyme Cyclooxygenase-2 (COX-2).
Binding of SC-558 and Cannabidiol (Yellow)

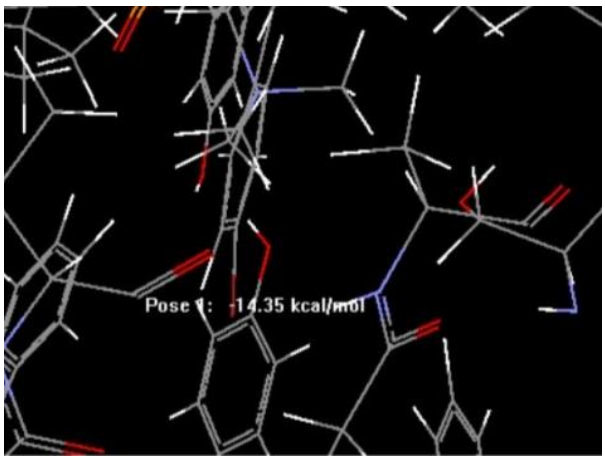


Figure 10 SC-558 (blue) binds to the binding site of the enzyme Cyclooxygenase-2 (COX-2).
Binding of SC-558 and Phosphatidylcholine (PC) (Red)

In a study by Hongmei Cao and colleagues, they discovered a substance derived from medicinal plants that can inhibit cyclooxygenase, an enzyme involved in inflammation treatment (Hongmei Cao, 2010). The experiment revealed that this inhibitor, with an IC50 value of around 10 micromolar, includes Cannabidiol and Phosphatidylcholine (PC), specifically targeting COX-2. The negative Binding Energy value indicates a strong binding between Cannabidiol, Phosphatidylcholine (PC), and the enzymes associated with their structure.

V. Discussion

Using the ArgusLab program, a simulation was conducted to examine how anti-inflammatory compounds derived from Cannabidiol and Phosphatidylcholine (PC) bind to the COX-2 enzyme. Both substances were found to act as selective COX-2 inhibitors, with IC50 values of 5±5 and 9±3, respectively. By analyzing Table 1 alongside the results, it becomes evident that the polar regions of all six compounds are positioned near VAL2176 and ARG2166, which are the side pockets of the COX-2 enzyme. This indicates that Cannabidiol and Phosphatidylcholine (PC) have polar components inserted into the side pocket and can align with SC-558 in the same plane, demonstrating the correct positioning as expected.

VI. Conclusion

This study focused on investigating the molecular structure of a compound through enzyme inhibitor molecular simulations. Specifically, the researchers examined the interaction between Cyclooxygenase-2 (COX-2) and Cannabidiol and Phosphatidylcholine (PC) when combined to form Cannabidiol Lipid Nanoparticles. The binding of these substances to COX-2 was analyzed using the ArgusLab program. The findings revealed that both Cannabidiol and Phosphatidylcholine (PC) exhibited selective COX-2 inhibitory activity, with Binding Energy values of -13.71 and -14.75 kilocalories/mol, respectively. These results were then used to explore the relationship between Binding Energy and IC_{50} values for both substances. The analysis showed a correlation coefficient (r) of 0.753135, indicating that the simulated binding interaction aligns with the inhibitory effect on COX-2. This simulation approach proves valuable in studying the mechanism of action of Cannabidiol and Phosphatidylcholine (PC).

VII. Further Research

Computer-based prediction methods can be utilized to screen a vast array of new substances, enabling the identification of compounds with desired effects. This approach can be extended to various herbal substances. Additionally, computers can aid in forecasting the activity of drugs prior to their synthesis or actual testing for anti-inflammatory effects. This suggests a potential avenue for future research, wherein a novel and modified structure could be introduced. Subsequently, laboratory synthesis and testing would be conducted to verify the enzyme inhibiting effect once again.

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