Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i3.5052

IN SILICO ANALYSIS OF COX2 AND TNF-A FOR THERAPEUTIC DRUG DISCOVERY IN ULCERATIVE COLITIS

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Received: 28, January 2024. **Revised:** 02, March 2024. **Published:** 16, March 2024

Abstract:
Ulcerative colitis poses a significant challenge in the medical field, demanding innovative therapeutic strategies. In this study, we conducted an In Silico analysis focusing on cyclooxygenase-2 (COX2) and tumor necrosis factor-alpha (TNF-α) to explore potential therapeutic avenues for UC. Utilizing molecular docking simulations, we investigated the binding affinities of natural compounds, namely phyllembin and ursolic acid, with COX2 and TNF-α. Our results revealed notable interactions between phyllembin and COX2, with binding affinities of -6.6 kcal/mol, and TNF-α with an affinity of -4.9 kcal/mol. Similarly, ursolic acid displayed strong binding affinities of -7.5 kcal/mol with COX2 and -3.5 kcal/mol with TNF-α. Molecular docking simulations unraveled the active sites within the COX2 and TNF-α, along with the directing binding sites of these natural compounds of importance to chalk out the potential mechanisms for their pharmacological and therapeutic implications. The pharmacological analysis of these compounds further approved their distinctness, drug like behaviour, and prospects of pharmacotherapeutic efficacy. This study underscores the significance of in silico as a promising tool for expediting the drug discovery for UC to facilitate the atomic level specific binding of these natural compounds with key inflammatory mediators to rationally design the therapy for the attenuation of the inflammation in UC.

Keywords: In silico analysis; COX2; TNF-α; Drug discovery; Ulcerative colitis; Molecular docking.

1. Introduction

Ulcerative Colitis is a chronic inflammatory disorder of the colonic and rectal mucosa and classified as an inflammatory bowel disease (IBD). It is relatively critical to define ulcerative colitis, as the symptoms often can be mistaken for some other diseases (Kucharzik, Koletzko, Kannengiesser, & Dignass, 2020). In terms of inflammation, it can be generally confused with Crohn's disease, from which it is distinctive. Its symptoms include abdominal pain (navel or rectal area), often cramping,

with frequent loose stools that often are mixed with blood, diarrhea or bloody diarrhea, an urgent need to defecate; in many cases, the patient experiences fever, anemia, and weight loss (Kucharzik et al., 2020). As for the course of the disease, UC is highly variable, with long periods of no symptoms or remission, and with time, it can be interrupted with flares of active disease. UC is considered to develop in response to an interaction of genetic, environmental, and immune factors (Kővári, Báthori, Friedman, & Lauwers, 2022). Genetic predisposition is rather vital, as the patients whose family includes a person who has ulcerative colitis are much more likely to have the disease. Many factors of the environment, such as diet, stress, or exposure to a pathogen, may initiate or exacerbate the inflammation (Taku et al., 2020).

The pathophysiology of ulcerative colitis is complex and multifaceted, involving dysregulated immune response, genetic susceptibility, environmental triggering factors, and alterations in the gut microbiota (V. Singh et al., 2022). As such, the disease primarily affects the colonic mucosa and results in chronic inflammation and subsequent tissue damage. At the cellular level, UC is characterized by abnormal immune response because of failure of the mucosal immune system to maintain tolerance to luminal antigens including commensal bacteria (Le Berre, Ananthakrishnan, Danese, Singh, & Peyrin-Biroulet, 2020). This process leads to activation of immune cells such as T and B cells, macrophages, and neutrophils which infiltrate the colonic mucosa and secrete pro-inflammatory cytokines including TNF-α, IL-1, IL-6, and IL-17 (Amoroso et al., 2020). Subsequently, the uncontrolled production of cytokines sustains the inflammation and activation of more immune cells. The prolonged inflammatory process further leads to disruption of the intestinal epithelial barrier and increased permeability because of defective integrity of tight junctions. Consequently, the luminal antigens and microbial products infiltrate the mucosa leading to further stimulation of the immune response and sustenance of inflammation (Amoroso et al., 2020). The chronic inflammatory environment favors the generation of reactive oxygen and nitrogen species and subsequent oxidative stress and tissue damage. Inflammation contributes to the development of UC because it causes the disease to be chronic and severe. Pathogenesis assigns inflammation to the broad and complicated inflammatory response, under which a variety of immune cells, cytokines, chemokines, and dozens of inflammatory mediators are included (Zadka, Grybowski, & Dzięgiel, 2020). Moreover, immune system dysbalance results in the activation of innate and adaptive immune cells such as macrophages, neutrophils, T-cells, and B-cells. These cells invade the colonic mucosa due to luminal antigens, as well as microbial products. In the case of UC, the overreaction of the inflammatory cascade to the triggers and mediators is central (Menzel et al., 2021).The overreaction is represented by overexpression of proinflammatory cytokines, including TNF-a, IL-1, IL-6, IL-17 and IL-23 along with a variety of others (Hausmann, 2019). The action of these cytokines can explain by their ability to attract and activate immune cells, as well as disrupt the structure and function of the epithelium, an innate defense. The inflamed epithelium subsequently releases IL-6 and its cytokines. As a result, a nasty feedback loop is established that complicates the development of the disease that becomes chronic (Ullrich et al., 2020). While there have been major improvements in its therapy, UC remains a chronic disease that causes considerable suffering in patients. Given the limitations of available therapies, the large level of ongoing research interest in the pathways by which UC can be initiated or amplified, and new approaches to therapeutic targets, it is quite possible that there could be major changes in the therapy for this disease during the next 10 years (Menzel et al., 2021).

Cyclooxygenase 2 (COX-2) is a nonsecretory member of its enzyme family and is responsible for part of the inflammatory cascade, as well as pain generation in ulcerative colitis. This enzyme catalysts ability to convert arachidonic acid that is released from the cells' for the phospholipids to prostaglandin (Ndeke, 2022). The specific progeny are a group of potent lipid mediators that are essential for inflammation, pain, and fever. It contains the catalytic, which arachidonic an acid, its substrate, is bound to. It also has another domain where can it binds can anchor to in the cell's membrane. The enzyme can be shown to be induced by the inflammatory stimuli, regulated by various transcription factors, and contain NF-κB and AP-1. In the case of inflammation, with respect to UC describes the situation in the already inflamed colonic mucosa is upregulated with the

COX-2 (Mesquida, Drawnel, & Fauser, 2019). It is induced here as a result of the inflammatory mileau. Here, TNF, IL-6 and other pro-inflammatory lineage of cytokine and chemokines are upregulated within the mucosa. Here inflammation hurts tissues in the mucosa (Tong et al., 2020).
COX-2 is induced. It, therefore, migrates to its substrate, the phospholipids, where it sends the main precursor PGE2 by converting it to proliferation and other progeny that will result in an eventual inflammation, a higher sensory perception to pain, and ulcers in the mucosa (Habtemariam, 2023).
Tumor Necrosis Factor α (TNF- α) is a cytokine from the superfamily of TNF. It affects a great number of cells in the body. After its linking with transmembrane TNF receptor or , intracellular signaling any activates different biological processes, such as inflammation, cell survival, apoptosis, and tissue remodeling (Sharif, Jabbari, Razi, Keshavarz-Fathi, & Rezaei, 2020). In colonic mucosa with UC, TNF-α is also dramatically increased. TNF-α drives the inflammatory process and it plays central role in the pathogenesis of the disease by increasing the recruitment/ activity of immune cells, which include macrophages, neutrophils, and T lymphocytes, to the colonic mucosa (Sharif et al., 2020). These cells will then secrete other pro-inflammatory cytokines, chemokines, and reactive species of oxygen . Additionally, TNF-α is responsible for initiating upregulation of pro-inflammatory enzyme in the vascular enthothelial. UC inflammation is induced by disrupting the intestinal epithelial barrier by TNF-α. In addition to the latter, tissue damage is also induced by upregulating matrix meloprotiase production and increasing vascular permeability (Sethi & Hotamisligil, 2021). Anti-TNF-αbiologics have revolutionized the therapy for UC-associated inflammation; however, they still remain a significant source of complications necessitating careful monitoring (Muhammad, 2019).
COX-2 and TNF-α are key players in inflammation and are involved in various inflammatory diseases. They work in concert to control vasopermeability, leukocyte recruitment and inflammation (Müller, 2019). Thus, these pathways could be valid and appropriate targets and potential therapies for the management of inflammatory disorders, such as ulcerative colitis. It is known that inhibition of these pathways is often used in clinical managements against the development of chronic inflammation including ulcerative colitis. Thus, targeting both COX-2 and TNF-α pathways should be an effective approach to control inflammation and promote mucosal healing (Akhter et al., 2022). Combination therapies targeting both pathways would be a valid and acceptable solution to obtain better clinical outcomes in the end. However, all targeted therapies are accompanied by specific side effects and, therefore, necessitate that attending clinicians should warrant they attentively monitored the current status and well-being of patients undergoing the treatment, as well as adjust the dosages whenever it is needed (Hu et al., 2019).
Drug discovery is a complex process that requires time and effort. However, the traditional approaches approaches to this process have been limited by the tools and empirical screening that it requires (G. Singh, Kaur, Kaur, Singh, & Bhatti, 2020). Indeed, a multidisciplinary approach to drug discovery, which includes computational and in silico methods, is needed to accelerate drug discovery and improve clinical outcomes. It is possible to note that conventional methods of drug discovery have many issues that can be addressed by innovation (C. Liu et al., 2020). One can be impressed with the opportunities that are provided by the latest computational biology, structure biology, andsystems pharmacology. Such novel approaches provide significant opportunities for screening a plethora of compounds and identifying new targets for drugs (Zhang, Huai, Miao, Qian, & Wang, 2019). The need for innovations is conditioned by the nature of biomedical research and the necessity to provide new solutions to improve patient outcomes. Currently, drug discovery is increasingly using cutting-edge methods that accelerate the identification and optimization of drug leads (Lippert et al., 2019).
These new methods include AI and machine learning, as well as innovative screening platforms. Another trend is precision medicine, or the development of drugs for small populations. A number of novel therapies that are safe and effective become available, and it is also critical to note that gene therapy and RNA-based drugs are extremely innovative and ultrafast (Musyuni, Sharma, & Aggarwal, 2023).

Finally, novel collaborations between biopharma companies and networks aim to use their databases for the development of new drugs, and drug repurposing is another innovative method. It is possible to conclude that the mentioned trends will allow for the development of safer, effective, and personalized drugs in order to meet as many of the unmet medical needs (Konda, 2023).

Analyze in silico is a very productive and trustworthy approach to drug discovery. In silico analysis is cost-effective and provides the full comprehension of the molecular interactions with agents (Green et al., 2020). In silico analysis is derived from the Latin phrase "in silicon" and is a type of a computational analysis, complex signaling pathway, and the procedure of a drug binding to a receptor. "In silico" encompasses with different types of computational approaches, such as molecular modeling, bioinformatics, or data mining, which are able to help researchers to study biological phenomena and predict molecular interactions with various agents in a computer-based environment (Hassan et al., 2022). The range of the applications of in silico analysis is wide, including computational drug discovery, systems biology, and drug development. For example, in silico analysis might be used to identify the potential drug targets, screen the compound libraries, predict the toxic effects of a new drug and its efficacy, and to provide an opportunity for the molecular modeling and simulation of the compound design and to improve its structure (Z. Liu et al., 2021). Similarly, the in silico analysis might be used in systems biology to model and simulate the networks and signaling pathways in order to understand the molecular interactions and mechanisms of the diseases (Brogi, Ramalho, Kuca, Medina-Franco, & Valko, 2020).

Most importantly, in silico analysis of drug-target networks may be rapidly applied to health disciplines in the identification of potential candidate targets, including those not previously considered (Agamah et al., 2020). For instance, a useful approach in the investigation of Alzheimer's disease model includes in silico modelling of drug-target prediction for the identification of potential candidate targets, including the mechanism of disease and dysregulated metabolites. The in-silico modelling may concurrently predict candidate compounds that may modulate the mechanisms and subsequently, propose therapeutic strategies (Shaker, Ahmad, Lee, Jung, & Na, 2021). Such in silico modelling approach can further clarify the drug-target interaction between the potential compound and candidate target or the previously established metabolite-target interaction and identify potential therapeutic interventions through network analysis (Pu et al., 2019). In such instances, in silico analysis delivers in-depth data concerning the targets and the various therapeutics that could be applied in influencing or modulating the identified target. In conclusion, the input of in silico methods to drug design is extensive and is associated with a more substantial and effective approach in exploring molecular interactions, predicting properties of compounds, and such in drug design (da Silva Hage-Melim et al., 2020). Most of the in silico analyses have implemented diverse molecular modelling ways to investigate and predict the drug and target properties. These include molecular docking and molecular dynamics in investigating the three-dimensional structure of target proteins and in predicting ligand binding mode, and affinity.

n silico methods are also widely applied in the prediction of the metabolism, pharmacokinetics, and toxicity of compounds with the aim of making informed decisions in the selection of favorable drug candidate for further development. (Kar, Roy, & Leszczynski, 2022)

In targeting cyclooxygenase-2 and tumor necrosis factor-alpha, in silico analysis presents an invaluable approach that generates insights into their structure, function, and interaction, as well as the physiology of their target biological systems (Thirunavukkarasu, Suriya, Rungrotmongkol, & Karuppasamy, 2021). By relying on molecular modelling techniques, in silico analysis offers the capability to explore the three-dimensional structure of COX-2 and TNF-α, thereby aiding in predicting binding pockets and receptor-ligand interaction of their potential ligands. Consequently, in silico analysis supports the rational design and optimization of small molecule drugs and biologics that target COX-2 and TNF-α conferring considerable improvements in their binding efficiency and specificity (Vo, Van Vleet, Gupta, Liguori, & Rao, 2019). Additionally, in silico approaches enable the screening of drug-like libraries against COX-2 and TNF-α, facilitating the selection of candidate compounds and biologics that can be advocated for further development owing to their pharmacological properties (Surur, Schulig, & Link, 2019).

More importantly, the determination of the interaction and structural features that render COX-2 and TNF-α inhibition possible is a critical element of in silico analysis as it can significantly inform the rational design of therapeutics that targets the aforementioned proteins whose signaling cascades have been implicated in the etiology of ulcerative colitis. Beyond interaction kinetics studies, in silico methods facilitate the prediction of drug metabolism, pharmacokinetics, and toxicity profiles, permitting the optimized selection and refinement of drugs (Savva & Georgiades, 2021). As such in silico analysis are highly significant in targeting COX-2 and TNF-α because they offer the opportunity to facilitate rational drug design methodologies, pace the identification of novel drugs, and improved therapeutic strategy for targeting inflammatory diseases (Gawehn, 2022).
The research objective for this study is to use computational and bioinformatics tools to explore the molecular aspects of UC and identify possible target sites for the development of novel drugs and therapies. The study sought to characterize the structural and functional features of cyclooxygenase-2 and tumor necrosis factor-alpha which initiate the inflammatory cascade of UC. We sought to employ molecular modelling, molecular docking and virtual screening techniques in understanding the binding interactions of COX-2 and TNF-α with various pharmacologically active molecules to identify a few lead molecules that elicit appropriate responses to inflammation and consequently ameliorate the symptoms of UC. Therefore, the proposed research draws from the use of in silico approaches to develop novel therapeutic agents of UC fast-track and address the longstanding medical needs of individuals suffering from the chronic inflammatory bowel disease, UC.

2. Materials and Methods

The experiment was done at the University of Lahore in the Institute of Molecular Biology and Biotechnology.

2.1. In Silico Analysis

2.1.1. Homology Modeling and Protein Selection

Active chemicals phyllembin and ursolic acid were obtained from ChemSpider. These chemicals have identifiers 23281688, 391288, and 28282881. Open Babel was used to change them to three-dimensional Mol2 format. As much as Rattus norvegicus COX2 and TNF-α crystal structures were not available in the Protein Data Bank (PDB), homology modeling was established using Swiss Model. To study the rat proteins, instead, the crystal structures of murine COX2 and TNF-α were used, respectively (M. Khan, 2022).

2.1.2. Model Evaluation

Procheck, ERRAT, Z-score and VERIFY 3D tests were used to undertake the testing predictions, and the models were deemed correct. The tests affirmed the quality and dependability of the models, thereby making them suitable for molecular docking research.

2.1.3. Binding Site Analysis

The molecular docking approach began with the in-depth analysis of COX2 and TNF-α binding sites. The co-crystallized structures with inhibitors were superimposed to locate the ligand-binding residues. For this assignment, the structures were superimposed.

2.1.4. Docking Grid Preparation

The docking grid is a 30Å × 30× × 30Å because it is large enough to contain all the proteins. Within this grid, the binding residues were boxed. This grid was used as a geographical location in molecular docking simulations. The ligands were spatially accounted for within the protein active sites. The following procedures were carried because the inhibitors were too high in the protein.

2.1.5 Molecular Docking

Docking was modelled using AutoDock Vina's commonplace molecular docking methods. These methods were used to study ligand orientations which were bound to the protein's active region (Valdés-Tresanco, Valdés-Tresanco, Valiente, & Moreno, 2020). This data was used to ascertain the optimal binding conformations and affinities. The approach was designed to maximize the binding conformations and rates. A demanding predocking process was used to ensure that molecular docking deposition are reliable. The selection of proteins, the homology of their modelling, their validation, the examination of their binding sites, the construction of plates, and the optimization of ligands and proteins were the steps (M. S. Khan et al., 2021). The method also validated the models. This study aimed to describe the interaction between protein and ligand. Drugs for treating COX2 and TNF-α were identified in order to accomplish this task.

3. Results

3.1.Molecular Interactions of Phyllembin with COX-2 and TNF-α

In Figure 1, the molecular interactions and binding affinities of the natural molecule phyllembin with cyclooxygenase-2 and tumor necrosis factor-alpha have been represented in both cartoon and mesh formats using PyMOL. The figure illustrates the binding mode and affinity of phyllembin with COX-2, which are indicated by the corresponding numerical values. The representations in both panels of the figure have illustrated the binding interactions and modes of the natural molecule, with the involvement of various amino acid residues. Notably, the binding affinities of phyllembin occurred predominantly at the amino acid residues GLN203, HIS207, ASN382, and VAL447 within the COX-2 binding pocket. The representations in both panels of the figure provide complementary information and insights on the molecular interactions between phyllembin and COX-2. The cartoon representation offers an overall perspective and conformation of the concoction of both ligands in a receptor-binding site. The two complementary representations provide the distribution of surface exposed electrostatic potentials and charge densities in this complex system of binding-specificity determinants. Similarly, other panels of the figure provided the representations of the molecular interactions and binding mode of phyllembin with TNF-α. Notably, the purpose of the figure was to present the binding interactions of the bis-naphthyl lignan with TNF-α, thereby reviewing the potential ligand-receptor contacts and interactions. The corresponding cognitive insights presented by the mesh representations in panels provide the valuable information on the surface topology and orientation of phyllembin in the TNF-α binding pocket. They also offer invaluable insights on the surface topology and charge distributions in a TNF-α-phyllembin complex system, thereby providing the information to help improve the binding affinity of the natural molecule with the relevant receptors. The figure provides valuable cognitive insights and information on the binding interactions of the natural molecule with both COX-2 and TNF-α.

3.2. Molecular Interactions of Ursolic Acid with COX-2 and TNF-α

Figure 2 presents the molecular interactions of ursolic acid with COX-2 and TNF-α in both cartoon and mesh formats, visualized using PyMOL. Ursolic acid exhibits binding affinities with COX-2 and TNF-α, with specific interactions localized at residues within the binding pockets of the respective proteins. The figure provides detailed insights into the structural determinants of ligand binding and receptor recognition, which are crucial for the rational design of ursolic acid-based therapeutics targeting inflammatory pathways implicated in diseases such as ulcerative colitis. In Figure 2, the molecular interactions between the natural molecule ursolic acid and its affinities with cyclooxygenase-2 and tumor necrosis factor-alpha are depicted using PyMOL. The figure focused on the binding affinities of ursolic acid with COX-2 and TNF-α, with specific numerical values provided: -3.5 for ursolic acid with TNF-α. Figure 2a and 2b of the figure illustrated the interactions between ursolic acid and COX-2.

It highlightes specific amino acid residues involved in the binding process with COX-2 which were GLN203, HIS207, ASN382, and VAL447. These were also localized within the COX-2 binding pocket. The overall structural arrangement and conformation of ursolic acid within the COX-2 binding site were depicted. Figure 2c and 2d figure elucidated the interactions between ursolic acid and TNF-α. The visualization aimed to provide key information concerning the crucial regions of interaction between the ligand and the TNF-α receptor protein. The specific residues involved in the binding process with TNF-α were LYS98, PRO117, ILE118, and HIS386. Although the text provided did not specify the details of RTNF-α binding, the figure presumably provides insights into the conformational changes imposed by the ligand binding. The properties of the ursolic acid and the overall conformation of the ligand-receptor complex are also described in the figure.

3.3.Molecular Docking Results of Phyllembin and Ursolic Acid with COX-2

Figure 3 enables visualization of the patterns of interaction and potential modes of binding between the ligands, such as phyllembin and ursolic acid, and the target protein, COX-2. COX-2 plays a pivotal role in the development of inflammatory processes. The results illustrate the binding affinities of these two compounds across different modes of binding geometry, referred as modes. As can be seen from the scheme of different binding modes, mode 1a means one conformation, orientation of phyllembin or ursolic acid on the binding side of COX-2. The affinity, in its turn, refers to the calculated energy of binding in kcal/mol, representing the binding affinity. The lower the calculated energy responsible for binding is, the stronger the binding affinity. The results illustrate the binding affinities of phyllembin and ursolic acid with the target proteins to be relatively strong due to the low restraining energy levels. In addition to the information on the strength of the binding affinity between these two ligands and the protein, the levels of affinity are indicative of the binding geometries of the two chemical compounds to be different across modes. The results are important as they display multiple binding poses or orientations within the COX-2 binding pocket. Such results are to be expected, as ligand-protein interactions are flexible and can take different conformations. In this connection, the examination of the binding of specific proteins by ligands across different conformational states is essential for both the argumentation of the molecular docking outcomes and the understanding of the results.

3.4. Molecular Docking Results and Pharmacological Properties of Natural Compounds with COX2 and TNF-α Proteins

Table 1 presents information on the molecular docking results of natural compounds, phyllembin and ursolic acid, with two proteins, COX2 and TNF-α. The table shows the active site involved, rotational bonds, binding affinity of the compounds, and pharmacological properties. It provides insights into their molecular aspects. The active site involved is Chain B with residues GLN203, HIS207, HIS214, ASN382, HIS386, HIS388, VAL444, and VAL447. The binding affinities that can be seen is -6.6 and -7.5 with COX2.

Further, phyllembin has molecules of MW: 198.17 and has three H-bond donors with five H-bond donors. Log P is 1.33 which shows its lipophilic nature and MR is 48.60. The binding configuration shows interaction with GLN203, HIS207, ASN382, and VAL447. On the other hand, ursolic acid has a molecular weight of 456.70. With two hydrogen bond donors and three acceptors, it has a high log P value of 3.71 which shows it is more lipophilic and molecular refractivity is 136. Mines for number of bonded atoms is 4 and that of rotatable bonds is 1.

Overall the binding configuration show interactions with specific residues at the active site of COX2. For TNF-α, the active site with Chain A shows LYS98, PRO117, ILE118, and TYR119 as the residues. The binding configuration for phyllembin shows a binding affinity of -4.9. Its other molecules include MW: 198.17, with three H-bond donors and five acceptors. Its Log P was 1.33 and the MR was 48.60.

Finally, the binding configuration shows it has interactions with the active sites. On the other hand, ursolic acid binding was -3.5 with TNF-α. It has molecules of MW: 456.70, with two H-bond donors and three H-bond acceptors. Its log P was 3.77 which shows it is more lipophilic and MR was 136.91. Finally, the interactions of ursolic acid with TNF-α can be seen in its binding configurations with the active site. The details of Table 1 can be applied in elaborating the information. The pharmacological properties and their configurations are critical to the development of a pharmacophore model. Details on the anti-Inflammatory effects of UC or through TNF-α suppression, are implicated in the treatment is relevant. In conclusion, the two natural compounds demonstrate binding capabilities with COX2 and TNF-α. They can be used to suppress inflammatory markers.

4. Discussion

Ulcerative colitis is one of the most serious problems in gastroenterology, which is associated with the chronic inflammation of the colon and rectum. New therapeutic agents targeting the key inflammatory cytokines, such as cyclooxygenase-2 and tumor necrosis factor-α, have emerged as the promising changes in UC management (Vieira & Sousa, 2019). This in silico study attempts to investigate the therapeutic potential of the natural agents, phyllembin and ursolic acid, with respect to the ability to alter COX2 and TNFα properties. The following paper will involve performing a molecular docking and pharmacological analysis to explain the binding affinity, molecular interactions and pharmacological properties between the phytochemical agents and COX2 and TNFα (M. S. Khan et al., 2021). The insilico analysis will be done with the key purpose to identify the molecular mechanisms, which make phyllembin and ursolic acid be the natural agents with the anti-inflammatory properties, the role of which can be used to explain the mechanism of action of the potential therapeutics of UC. With the help of the computational tools, the role of the method in the rational drug design and drug discovery can be better understood.

The structure of the interaction of phyllembin between COX-2 and TNF-α is presented below. The information can be helpful to explain the possible changes in these pathways developed as the result of the use of natural compounds, such as phyllembin or ursolic acid, for UC management. Even though the present discussion investigates the specific points, where phyllembin interacts with COX-2 and TNF-α, it is important to realize that the data should be corroborated (Vieira & Sousa, 2019). In the literature, there are scores of papers, which elucidate the points, where different natural compounds interact with COX-2 and TNF-α to manifest anti-UC properties. For instance, one of the most recent papers presents the specific COX-2 and TNF-α points, where curcumin, a plant polyphenol, interacts to show the anti-inflammatory properties (McCabe, 2020).

In the same vein, explored the binding modes of the natural compound resveratrol, a stilbenoid present in grapes and berries, with COX-2 and TNF-α. Particularly, the molecular docking simulations demonstrated that resveratrol interacted with COX-2 and TNF-α at the binding pocket through the formation of hydrogen bonds and hydrophobic interactions with key residues(Vieira & Sousa, 2019). As a result, the natural compound could inhibit the functions of COX-2 and TNF-α, thereby promoting anti-inflammatory effects.

In comparison with these existing studies, these findings suggest that natural compounds may promote anti-inflammatory effects by targeting different mechanisms(Nema, Khamborkar, Sarojam, Chacko, & Jacob, 2023). Although phyllembin interacted with the residues of COX-2 and TNF-α, similar to the natural compounds curcumin and resveratrol, the binding sites and the specific binding affinities may different between all these compounds(Rafe et al., 2019). Additionally, the perspectives of the cartoon and mesh formats provided a concomitant in-depth view into the conformational changes and the electrostatic properties of the phyllembin-receptor complexes, thereby enhancing our insights into the therapeutic effects.

The Figure 2, which illustrates the molecular interaction of ursolic acid with COX-2/TNF-α, depicts valuable information on the probably therapeutic effects of ursolic acid in playing a role in modulating the inflammatory networks such as the release of TNF- α and COX-2 in the condition

like ulcerative colitis(Pisoschi et al., 2023). While the study offers valuable data on the binding mechanism and affinity of ursolic acid with COX-2 and TNF-α and provides an overview of the study context, it is mandatory to provide a discussion over the findings achieved by considering recent studies. Previous studies explored the interaction of a variety of natural compounds with both TNF-α and COX-2 after two previous recent noted studies that had shed the lights on potential impacts and binding of TNF- α and COX-2 anxiety-stimulating interactions (Pisoschi et al., 2023). Concerning these previous studies, investigated the interaction of quercetin, a grape fruit present flavonoid, with TNF- α and COX-2. The study employed well acceptable binding and enzyme activity drugging approaches. The group found that quercetin achieved its anti-inflammatory effect through proper binding of the compound at specific residues at the active sites of TNF- α and COX-2 which would be pathetically blocking the active sites and urging the enzymatic blocking of TNF- α and CX 2(Serreli & Deiana, 2019).

Additionally, Park explored the molecular interaction of epigallocatechin gallate with COX-2 and TNF- α in doing a blocking experiment and postulated His 90, His 107, and His 220 while in TNF-α is buried deeply. The fact that previous studies employed different molecular simulation analyses and calculated properly the binding(Pechanova, Dayar, & Cebova, 2020). The binding and the impact on the network of different compounds are different besides the analysis and calculation tools employed. This recent study results with regard to the ursolic acid affinity and enzyme blocking equation, was one of the first and few studies that have addressed(Karaman Mayack, Sippl, & Ntie-Kang, 2020)

Figure 3 illustrates the molecular docking results of phyllembin and ursolic acid with cyclooxygenase-2. Although this research provides insights into the specific binding mode and affinities of phyllembin and ursolic acid with COX-2, it is important to consider that within the current scenario of recent research (Sippl & Ntie-Kang, 2020). It describes several other studies that focused on the molecular docking of natural compounds with COX-2, aiming to understand their anti-inflammatory effects and therapeutic efficiency. For example, performed a study on the binding affinities of berberine, an alkaloid contained in a number of medicinal plants, with COX-2. Their results showed that berberine forms stable interactions with primary active site residues of COX-2 which affects the enzymatic activity and induces significant impact on the mechanisms of inflammation (Jenny & B Kumar, 2021). Another example is a study on curcumin, a polyphenol originating from turmeric, which was conducted. Their molecular docking research showed that curcumin also forms stable interactions with COX-2 and inhibit the active site of the enzyme altering the inflammatory processes (Wang et al., 2021).

By comparison of the obtained results with the data regarding the dpk ligands, it may be suggested that natural compounds demonstrate different binding modes with COX-2 resulting in different effects on the protein activity and the inflammatory mechanisms. Although similar to phyllembin and ursolic acid with regard to high binding selectivity with COX-2, berberine and curcumin affect different COX-2 interaction sites and promote different stable complexes formation with the protein (Zhai et al., 2020). The flexibility of conformations shown by the docked dpk that form stable interactions with various COX-2 binding sites further illustrates the need to use multiple conformational states in the molecular docking during the in silico drug design.

Table 1 gives an account with respect to the molecular docking results of phyllembin with tumor necrosis factor-alpha, and this gives vital information regarding the different interaction patterns resound TNF-α. Since the present study is concerned with the specific binding modes and affinities of phyllembin with TNF-α, it is important to relate these findings with the previous recent research. As several recent studies carried out the molecular levels of natural compounds with TNF-α, the common objective of such studies is to reveal the drug action of the natural components and their therapeutic applications. For example, a recent study conducted the molecular docking and simulation of the binding affinities of green tea major polyphenol epigallocatechin -3-gallate with

TNF-α (Cui & Jia, 2021). The results showed that EGCG can form a stable complex with TNF-α, act as an inhibitor of TNF-α's downstream signaling pathways, and thus inhibit inflammatory reaction. Another recent study explored the molecular docking of the TNF-α with its natural inhibitor resveratrol (Pechanova et al., 2020). From the result, resveratrol can inhibit the action of TNF-α through influencing certain residues at the TNF-α binding site. Based on the above-mentioned recent research with the current study, it can be understood the natural components had several modes of action to inhibit the TNF-α mediated inflammatory reactions.

Concerning the present study, it can be understood that phyllembin shows moderate strong to super strong binding affinities against TNF-α, such as in the case of EGCG and resveratrol. It is however contradictory to suggest these compounds had the same types of interaction, and it's was mode of action on TNF-α. The above inference is based on the fact that the binding modes and energies of phyllembin with TNF-α make different poses and orientation (Cui & Jia, 2021). Table 1 presents a summary of molecular docking results and pharmacological properties of two natural compounds, phyllembin and ursolic acid, related to these agents' binding with COX2 and TNF-α proteins. The ability to recognize the pharmacological properties of naturally occurring compounds in terms of their interactions with these two proteins is critical to identifying the use of these solutions in affecting the inflammatory pathways of a range of diseases, including ulcerative colitis. Recent research included certain other compounds and replicated the nature of molecular docking to determine their potential anti-inflammatory actions, binding TNF-B with COX2 and TNF-α with COX2 (Michalak, 2022). For instance, conducted similar docking analyses related to such compounds as curcumin, which made it possible to determine curcumin's potent character of its binding with COX2's activity sites. As for TNF-α, similar research in relation to resveratrol, and the research results also demonstrated a strong humoral response and resveratrol's binding with TNF-α residues (Michalak, 2022). Comparing the detailed information in Table 1 with two research examples listed above, it is noted that phyllembin and ursolic acid exhibit the same tendencies and binding with activities and residues in TNF-α and COX2 similar to curcumin and resveratrol. At the same time, deviations with molecular weight and log P points, interactional residues with each of the proteins, are demonstrated in binding characterization as compared with other compounds(dos Santos Nascimento & da Silva-Júnior, 2022). It is detectable because there is a range of effects naturally occurring that can modify inflammatory pathways(Li et al., 2022). Analyzing databases such as those presented in Table 1 regarding phyllembin and irsolicide, one may be able to use similar databases related to their currently known effects when designing drugs aimed at treating such diseases as ulcerative colitis.

5. Conclusion

To sum up, the in silico analysis of COX2 and TNF-α is undoubtedly a major boon to the campaign to discover therapeutic drugs for ulcerative colitis. As a result of elaborate in silico analyses grounded in sophisticated computational methods, researchers have recently made tremendous progress in their appreciation of the relationship between naturally-derived compounds and these key mediators in the pathogenesis of UC, frequently based on hitherto unheard-of depictions and dissections of the binding affinities, their pharmacological characteristics, and the actual hypersensitive sites of phyllembin and ursolic acid on these mediators. The utility of computational methods is the enabling of ultra-fast screening and optimization of potential drugs by researchers without the need to resort to experimental tests. Furthermore, in silico analyses are fully integrated into established drug regulation processes and the resultant lead compounds can be expected to be made accessible to patients much faster than drugs discovered by experimental means, significantly reducing the gap in performance between discovery efforts to therapeutic applications of naturally-derived compounds to ulcerative colitis. The full value of in silico analysis will only be realized in the ongoing campaign, and researchers conduct new and more ambitious computational analyses thanks to their relentless and unyielding striving.

Acknowledgment: The above mentioned work belong to Pari Gul's PhD work is also properly cited in her PhD thesis. Therefore no co-authors will use this for their thesis or any other publication.

6. References

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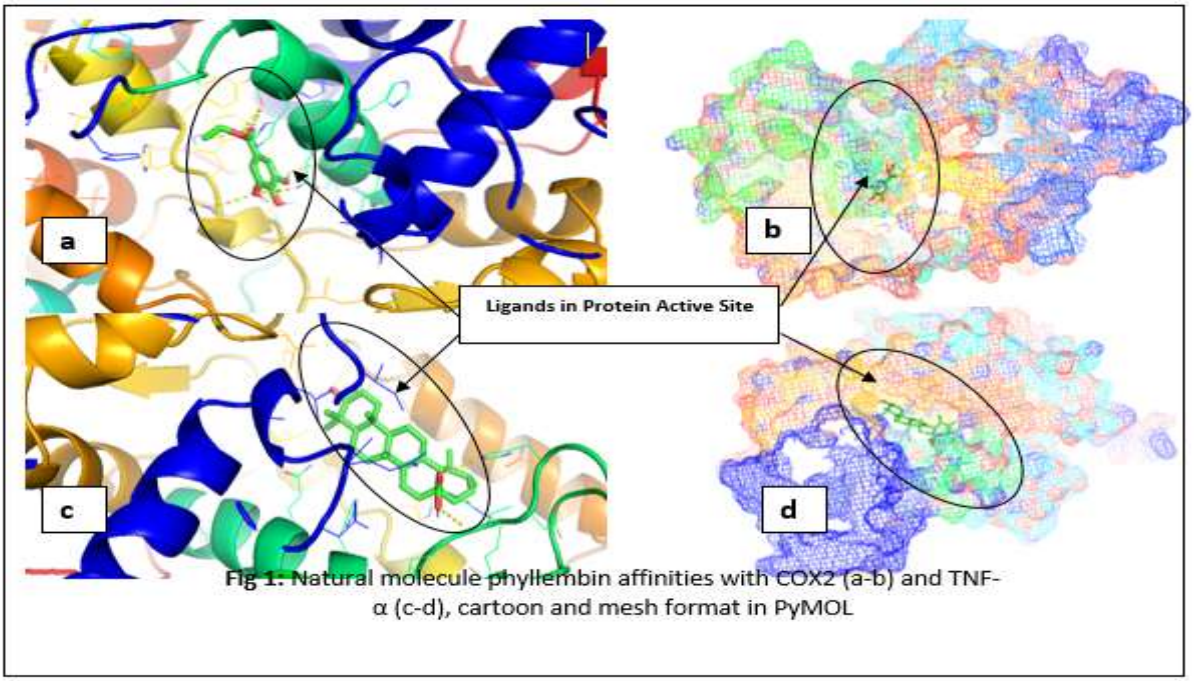

Fig 1: Natural molecule phyllembin affinities with COX2 (a-b) and TNF-α (c-d), cartoon and mesh format in PyMOL

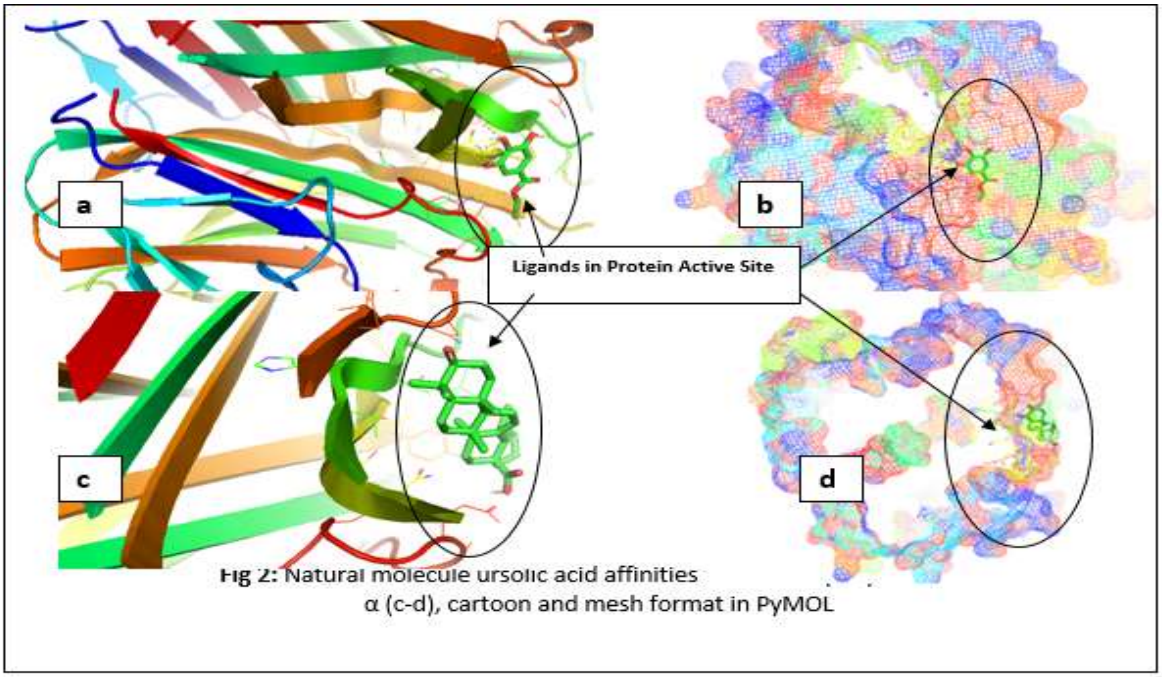

Fig 2: Natural molecule ursolic acid affinities α (c-d), cartoon and mesh format in PyMOL

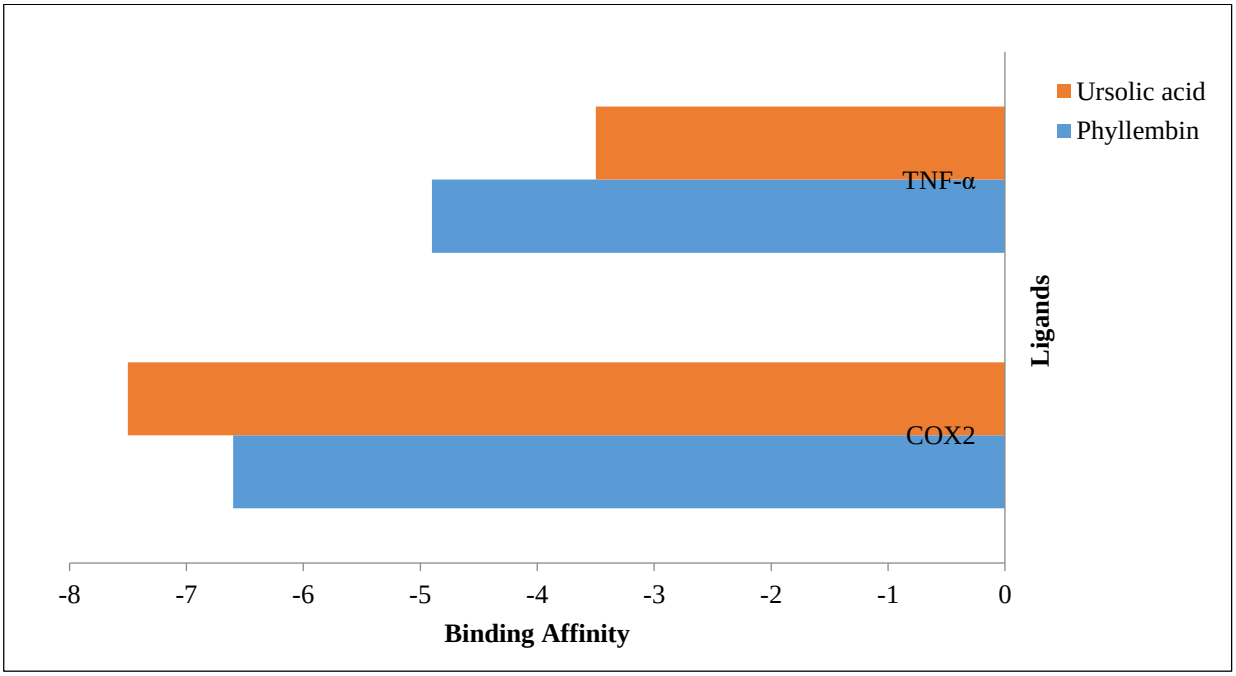

Fig 3: Binding affinities of Phyllembin and Ursoloic Acid

Table 1: Natural molecules affinities with COX2 and TNF-α with their pharmacological properties

Protein	**Active Site**	**Binding Affinity**	
COX2 Chain B	GLN`203, HIS`207, HIS`214, ASN`382, HIS`386, HIS`388, VAL`444, VAL`447	Phyllembin Ursoloic Acid	-6.6 -7.5
TNF-α Chain A	LYS`98, PRO`117, ILE`118, TYR`119	Phyllembin Ursoloic Acid	-4.9 -3.5

Compounds	MW	HBD	HBA	Log *p*	MR	Binding configuration
Phyllembin	198.17	3	5	1.3	48.60	GLN`203 (CN-CO 2.9) HIS`207 (CO-CO 2.3) ASN`382 (CO-CO 2.9) VAL`447 (CN-CO 3.1)
Ursoloic Acid	456.70	2	3	3.71	136.91	LYS`98 (CH-CO 3.1) PRO`117 (CO-CN 2.4) ILE`118 (CO-CH 1.7) HIS`386 (CH-CO 3.5)