

In Silico analysis, prediction and ranking of Drug binding affinity of Cyclooxygenase-2(COX2) and Nuclear Factor KappaB (NFkB) using Curcumin and its analogues in Cancer Treatment

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Abstract. This present study investigates the binding affinities of curcumin and its derivatives with Nuclear Factor Kappa B(NF-kB) and Cyclooxygenase-2(COX-2), the most targeted molecules in oncological therapies. Employing *insilico* techniques, particularly molecular docking through Swiss Dock, ten conformers of curcumin were evaluated for their interactions with these proteins. As per the docking analysis, Bisdemethoxycurcumin exhibited the most favorable binding energies, signifying the strongest affinity for both NF-kB(-2218.61 kcal/mol) and COX-2(-1156.81kcal/mol). Moreover, Tetrahydrocurcumin and Demethoxycurcumin showed -promising binding affinities. The above observation simply that the curcumin derivatives, especially Bisdemethoxycurcumin, exhibit considerable potential as therapeutic agents for the inhibition of vital pathways associated with malignant transformation. The present research lead thread to future investigations into the creation of curcumin-based inhibitors aimed at NF-kB and COX-2 in cancer treatment.

Keywords. Curcumin, NF-kB, COX-2, Cancer, *InSilico*, Molecular Docking, Drug Discovery

1. Introduction

Do not add any page numbers. *In silico* drug discovery has become increasingly significant in recent years, owing to its ability to minimize the expenses, time taken for, and labor compared to conventional approaches. Through the application of *in silico* methods, scientists can now effectively forecast the binding affinity of huge number of drug candidates to their respective target proteins, facilitating a more streamlined and efficient selection process for potential therapeutic agents. The conventional drug discovery methods frequently rely on animal experimentation, both *in vivo* and *in vitro* experiments, which, although effective, can be time-intensive and financially burdensome. Conversely, *in silico* strategies enable the rapid evaluation of extensive compound libraries, yielding critical information

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regarding drug-target interactions prior to experimental confirmation.

Turmeric (*Curcuma longa*) resinoid (oleoresin), potently anti-inflammatory and anti-cancer, is rich in the polyphenol, curcumin, which has been documented to be effective in reducing the inflammation. It affects the activity of a number of signal transduction pathways, such as Nuclear Factor Kappa B (NF- κ B) and Cyclooxygenase-2 (COX-2), which are critical to the progression of cancer and inflammation. Current research provides evidence for curcumin's ability to down regulate several pathways involved in tumor growth and cancer-cell mediated apoptosis.

Nevertheless, despite the promise of curcumin in cancer treatment, its clinical application is hampered by its low bioavailability and quick metabolism. To address these issues, a range of curcumin analogues have been synthesized that possess similar or greater therapeutic benefits along with better bioavailability. The present study is aimed at the *insilico* study of curcumin and its analogues with the goal of determining their binding affinities for NF- κ B and COX-2, two important molecular targets in cancer treatment.

NF- κ B belongs to a family of transcription factors that exert influence on the genes important in the inflammatory response, immune response, and cell proliferation. Over expression of NF- κ B is common in cancer cells where it leads to unregulated growth and resistance to cell death. COX-2 which is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins is also over expressed in various cancer types where it exacerbates inflammation and helps in the growth of tumors. Targeting these proteins may significantly decrease cancer cell growth and formation of secondary tumors and therefore would be effective drug targets.

The aim of the current work is to find the best stylus establishing structural interplay with NF- κ B and COX-2 and use them as template molecules for the synthesis of compounds targeting cancer. Swiss Dock molecular modelling tool was employed to evaluate the five chosen curcumin analogues on their interactions with NF- κ B and COX-2. Out of the ten curcumin analogues, the docking results of these analogues were compared in order to determine the most reasonable competitive inhibitors to be developed further.

1.1 Curcumin as a Therapeutic agent

Curcumin is a polyphenol obtained from the turmeric plant, which has generated a considerable research interest related to its anti-inflammatory, antioxidant and anti-cancer properties. Curcumin has been verified to work on numerous molecular targets which may include transcription factors and cytokines, as well as enzymes such as Nuclear Factor Kappa B (NF- κ B) and Cyclooxygenase-2 (COX-2)[1-5]. Studies have shown that curcumin has the capability to inhibit tumor development, provoke apoptosis, and reduce metastasis in a variety of cancer models [6]. Due to its ability to down regulate pro-inflammatory factors and transcription factors such as NF- κ B, it has gained interest in cancer research [7]. Nevertheless, the low bioavailability of curcumin has led to the synthesis of non-curcumin compounds with better therapeutic efficacy[8].

1.2. Role of NF- κ B in Cancer

NF- κ B (Nuclear Factor kappa B) modulates a variety of immunological circuits and inflammatory processes necessary for cell growth and survival. Several studies implicate NF- κ B subunit pathways in the pathogenesis and progression of a wide range of human

malignancies, including colorectal and breast cancer [7]. Targeting the NF- κ B signaling will cause both apoptosis and increased cancer cells sensitivity to anti-cancer treatments. Other investigators focus on the development of curcumin and its derivatives as inhibitors of NF- κ B activation and subsequently prevent tumor formation and enhance cancer interventions .

1.3 COX-2 and its Role in Cancer Progression

Colon and breast cancer are among the type of cancers demonstrating COX-2 over expression, which is an enzyme responsible for the synthesis of Prostaglandins, that is known to trigger inflammation [9]. Tumor Angiogenesis, Immune evasion, and apoptosis resistance are positively correlated with the over expression of COX-2. The first selective COX-2 inhibitor to be marketed was celecoxib and the drug resulted in a reduction in the size of tumors and an improvement in the survival of those with cancer [10] Recently a new perspective has been adopted in clinical trials where in the combinatorial therapy or COX-2 inhibitors were administered along with standard treatments of cancer in order to enhance the efficiency cancer treatment [11]

1.4 Molecular Docking and *In silico* Drug Discovery

Molecular docking is a recently developed *in silico* process that focuses on the interactions between a target protein and a small molecule, in this case a ligand. This is worth useful in the drug discovery process, as it allows researchers to screen large libraries of compounds cost-effectively and quickly [12]. There are several software applications available for molecular docking studies, and Swiss Dock is among them. It enhances the ability to estimate the free energy of proteins and ligands in a binding state . A few Docking studies have shown that curcumin and its analogues have a promising therapeutic potential to target malignancy through effective binding to receptors like NF- κ B and COX-2[13,14].

1.5 Curcumin Analogues in Cancer Therapy

Curcumin and its analogues have proven to be potential in treating cancer but have limited its usage due to their low bioavailability. As per literature, Indian curcumins, Dimethoxycurcumin, Bisdemethoxycurcumin, and Tetrahydrocurcumin are some of the potent molecules which can be considered for therapeutic targets[15,16]. Additionally, they improve on curcumin's stability and structural integrity which resist the compound from breakdown by our body's serum proteins and enzymes therefore improving its pharmacokinetic [17].

1.6 Current and Future Perspectives

Research now focuses more on optimizing curcumin analogues for better bioavailability and therapeutic efficacy. Curcumin and its analogues are highly demanding in cancer treatment as they target multiple signaling pathways including NF- κ B and COX-2, highlighting their potential as multi-targeted agents [8]. Even then, to validate these findings in clinical settings and to explore curcumin and its analogues in combinatorial cancer therapies demands further studies [10]

2. Materials and Methods

2.1 Proteins and Ligands

The target proteins selected for the in silico docking studies were Nuclear Factor Kappa B (NF- κ B) and Cyclooxygenase-2 (COX-2). To begin with, the crystal structures of NF- κ B (PDB ID: 1SVC) and COX-2 (PDB ID: 1OQ5) were downloaded from the Protein Data Bank (PDB). These proteins were reported to have well established roles in inflammation, tumor progression, and thus known as key molecular targets in cancer treatment. *For the molecular docking studies, ten conformers of curcumin and its analogues were selected. The molecular ligands are listed below:*

Swiss Dock web server, which is based on the EADock DSS algorithm were used for the molecular docking which effectively demonstrated protein-ligand docking and predicted the binding interactions between the ligand and the active site of the proteins.

Initially the following steps were followed for molecular docking

1. Protein Preparation: The PDB structures of NF- κ B and COX-2 were downloaded, and cleaned to optimize for docking by removing any non-essential ligands, water molecules, and cofactors

2. Ligand Preparation: Pubchem database is then utilized to obtain the 3D structures of the curcumin analogues and then converted to the '.mol2 format' suitable for further Docking analysis.

3. Docking Process: Both the curcumin analogues or ligands and protein targets were uploaded to Swiss Dock and after that simulations were performed. The results obtained were ranked based on the drug binding affinity, represented as Full Fitness energy scores (kcal/mol). The docked poses with the lowest binding energies were selected for further analysis

3. Results and Discussion

The binding affinities between the ligands and proteins were evaluated based on their Full Fitness energy values. The docking results provided insight into the binding strength and stability of the ligand-protein complexes (Fig. 1 – 6).

For NF- κ B, the ligands with the highest binding affinity were:

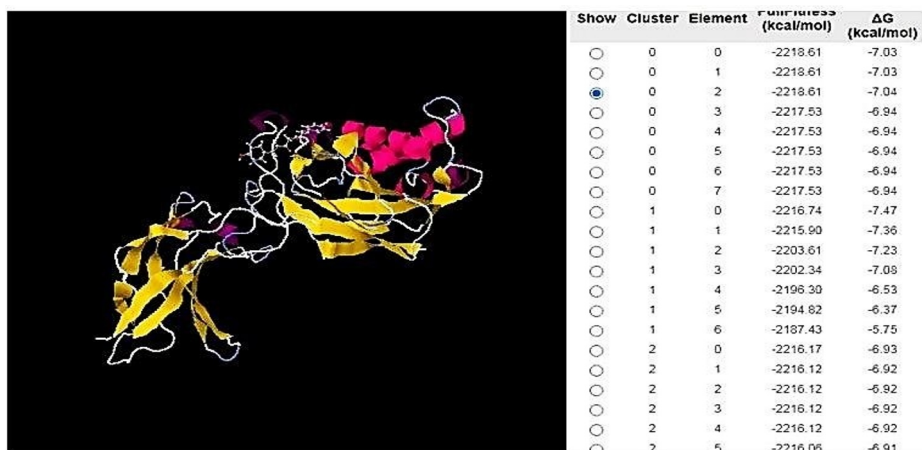


Fig. 1. Conformer Bisdemethoxycurcumin PUBCHEM ID-5315472(-2218.61 kcal/mol)

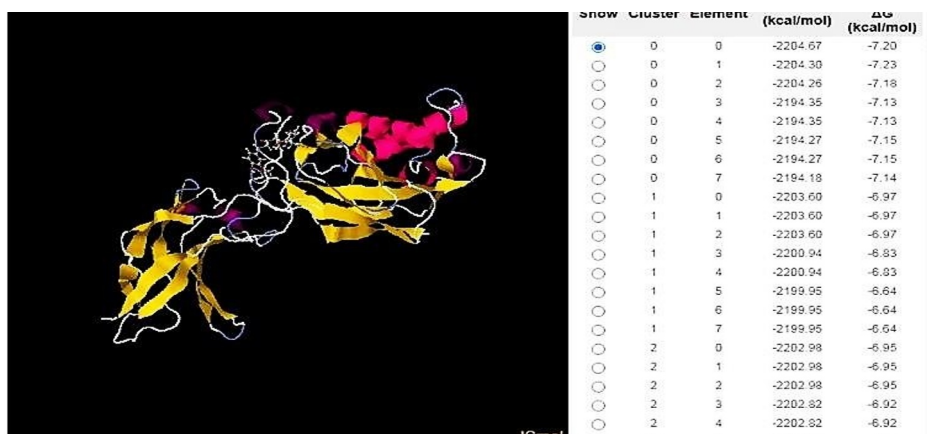


Fig. 2. Conformer Tetrahydrocurcumin PUBCHEM ID-124072(-2204.67 kcal/mol)

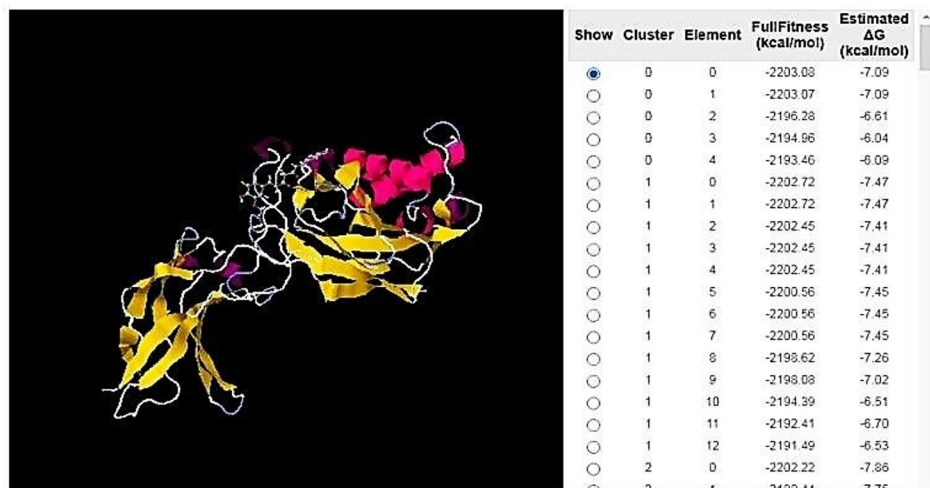


Fig. 3. Conformer Demethoxycurcumin PUBCHEM ID-546924 (-2203.08 kcal/mol)

For COX-2, the most potent ligands were:

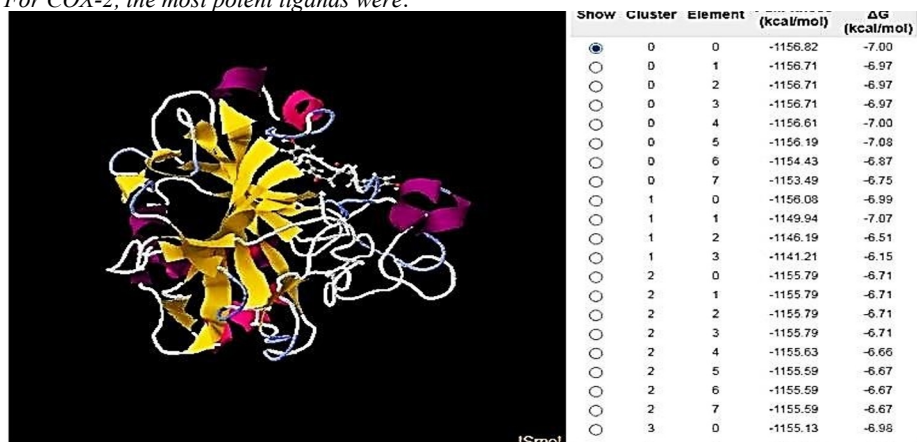


Fig. 4. Conformer Bisdemethoxycurcumin PUBCHEM ID-5315472 (-1156.82 kcal/mol)



Fig. 5. Conformer Tetrahydrocurcumin PUBCHEM ID-124072 (-1147.67 kcal/mol)

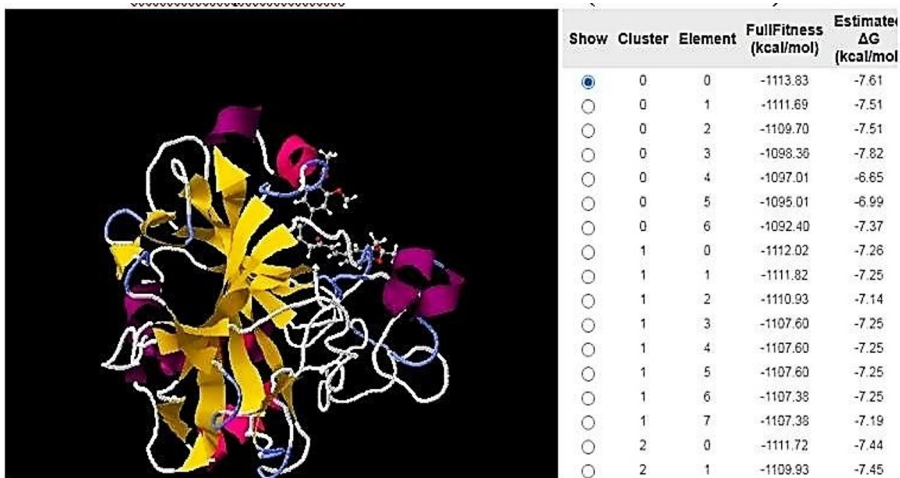


Fig. 6. Conformer Demethoxycurcumin PUBCHEM ID-546924 (-1143.48 kcal/mol)

Curcumin and its analogues with NF-kB (PDB ID:1SVC) and COX-2(PDB ID:1OQ5) when studied by molecular docking revealed important insights in binding affinities. Ten curcumin conformers including Demethoxycurcumin Bisacurone, Bisdemethoxycurcumin, Dimethoxycurcumin and Tetrahydrocurcumin were tested.

The docking results showed that Bisdemethoxycurcumin exhibited the strongest binding affinity with both NF-kB and COX-2, as reflected by its lowest FullFitness scores (-2218.61 kcal/mol for NF-kB and -1156.82kcal/mol for COX-2). This suggests that Bisdemethoxycurcumin may effectively inhibit these molecular targets, which are crucial in cancer-related pathways such as inflammation, tumor proliferation, and metastasis.

Tetrahydrocurcumin and Demethoxycurcumin also demonstrated strong interactions with both NF-kB and COX-2, with binding affinities close to that of Bisdemethoxycurcumin. Specifically, Tetrahydrocurcumin showed a binding energy of -2204.67 kcal/mol with NF-kB and -1147.67 kcal/mol with COX-2, while Demethoxycurcumin had values of -2203.08kcal/mol and -1143.48kcal/mol, respectively.

Table 1. Molecular Docking of NF-KB(1SVC) with 10 differentConformers and Ranking of Conformers Based on Binding Affinity

Rank	Conformer	ID	Binding Affinity FullFitness (kcal/mol) of NF-KB	Relative Stability (based on binding affinity)
1	DEMETHOXYCURCUMIN	546924	-2203.08	Highly stable
2	BISACURONE	14287397	-2182.37	Moderately stable
3	BISDEMETHOXYCURCUMIN	5315472	-2218.61	Highly stable
4	DIMETHOXYCURCUMIN	9952605	-2165.57	Moderately stable
5	TETRAHYDROCURCUMIN	124072	-2204.67	Highly stable
6	DIACETYLCURCUMIN	6441419	-2171.19	Moderately stable
7	CYCLOCURCUMIN	69879809	-2192.41	Moderately stable
8	OCTAHYDROCURCUMIN	11068834	-2191.37	Moderately stable
9	HEXAHYDROCURCUMIN	5318039	-2189.54	Moderately stable
10	CURCUMINGLUCURONIDE	71315012	-2117.75	Moderately stable

Table 2. Molecular Docking of COX2(1OQ5) with 10 different Conformers and Ranking of Conformers Based on Binding Affinity

Rank	Conformer	ID	Binding Affinity FullFitness (kcal/mol) of COX 2	Relative Stability (based on binding affinity)
1	DEMETHOXYCURCUMIN	546924	-1143.48	Highly stable
2	BISACURONE	14287397	-1124.30	Moderately stable
3	BISDEMETHOXYCURCUMIN	5315472	-1156.82	Highly stable
4	DIMETHOXYCURCUMIN	9952605	-1113.83	Moderately stable
5	TETRAHYDROCURCUMIN	124072	-1147.67	Highly stable
6	DIACETYLCURCUMIN	6441419	-1112.62	Moderately stable
7	CYCLOCURCUMIN	69879809	-1132.46	Moderately stable
8	OCTAHYDROCURCUMIN	11068834	-1134.68	Moderately stable
9	HEXAHYDROCURCUMIN	5318039	-1134.34	Moderately stable
10	CURCUMINGLUCURONIDE	71315012	-1053.32	Moderately stable

3.1 Analysis of Ligand- Protein Interactions

NF- κ B is a major player having a pivotal role in regulating genes that control cell survival, apoptosis, and inflammation. In majority of cancers, NF- κ B is most often constitutively active, contributing resistance to therapies owing to disease progression. As per the docking results, the high binding affinity of the curcumin analogues used in the study to effectively bind with NF- κ B is worth significant, as binding to NF- κ B with high affinity, Bisdemethoxycurcumin, Tetrahydrocurcumin, and Demethoxycurcumin may disrupt the molecular down stream pathway, inducing cancer cell apoptosis and reducing cancer cell proliferation.

Similarly, the strong binding affinities observed in Bisdemethoxycurcumin and the other curcumin analogues to COX-2 is also promising as COX-2 is another key enzyme having pivotal role in cancer, that is frequently over expressed in various types of cancers, promoting inflammation, angiogenesis, and tumor growth. The current research work based on molecular docking studies suggest the potential of curcumin analogues in inhibiting COX-2 activity, thereby suppressing cancer progression

3.2 Discussion of Therapeutic Potential

The study highlights the therapeutic potential of curcumin analogues, specifically Bisdemethoxycurcumin, in cancer treatment. The findings in the ability of these compounds

to effectively bind to both NF- κ B and COX-2 suggesting their dual role in targeting key oncogenic pathways. This dual inhibition is particularly important in cancers where both inflammation and abnormal cell signaling drive tumor growth and resistance to treatment.

The use of in silico methods, such as molecular docking, has proven valuable in identifying promising lead compounds that can be further developed for clinical use. The results from this study provide a foundation for future experimental validation, including in vitro and in vivo studies, to confirm the efficacy of these curcumin analogues in inhibiting cancer progression.

4. Conclusion

The present research work successfully employed in silico techniques to analyze the drug binding affinities of curcumin and its analogues with two key molecular targets in cancer therapy, the Nuclear Factor Kappa B (NF- κ B) and Cyclooxygenase-2 (COX-2). Among the ten analogues tested, Bisdemethoxycurcumin exhibited the strongest binding affinities for both NF- κ B (-2218.61 kcal/mol) and COX-2 (-1156.81 kcal/mol), suggesting its therapeutic potential as a powerful inhibitor of the above mentioned key oncogenic protein targets. Tetrahydrocurcumin and Demethoxycurcumin also demonstrated relatively favorable binding energies, making them viable candidates for further exploration. The findings suggest that the selected or ranked curcumin analogues can be leveraged as promising lead candidate molecules in therapeutics aimed at inhibiting inflammation and tumorigenesis pathways.

Declarations

Ethics approval and consent to participate: Approved by the Departmental council

Availability of data and materials: Included in the manuscript

Competing interests: There is no competing interest

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Authors' contributions: Concept, supervision and final draft (Auth: 2), Experimentation and first draft (Auth :1)

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