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CONTENTS

In-silico analysis of curcumin conjugates targeting the Wnt signaling pathway in Breast Cancer Stem Cells

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ABSTRACT: Breast cancer stem cells (BCSCs) play a pivotal role in tumor progression, metastasis, and therapeutic resistance. The β-Catenin, a key effector of the Wnt signaling pathway, is critically involved in maintaining the self-renewal and pluripotency of BCSCs, making it an attractive molecular target for cancer therapy. Contemporary medicines utilized for targeting breast cancer have not been effective because of poor prognosis and relapse of the disease with many side effects. Natural compounds like curcumin have shown promising anticancer properties with minimal side effects, but limitations in bioavailability and efficacy have prompted the development of suitably designed curcumin conjugates. This study explores the potential of curcumin conjugates as β-Catenin inhibitors through a computational approach. A series of ligand molecules, including curcumin, cinnamic acid and its conjugates along with a known binder of β-Catenin (R9Q), were subjected to molecular docking against the β-Catenin protein structure. Structural parameters of the protein and ligand interactions were analyzed to evaluate their potential to modulate -Catenin activity. The study aims to explore structurally designed conjugates with the potential to modulate β-Catenin signaling in breast cancer stem cells, laying the groundwork for future therapeutic strategies. The findings from *in-silico* study underscore the need for further *in vitro* and *in vivo* investigations to validate the efficacy and biological relevance of the designed conjugates as promising therapeutic agents against breast cancer stem cells.

Keywords: β-Catenin, Breast Cancer Stem Cells, curcumin conjugates, molecular docking, Wnt signaling pathway

Cancer, characterized by the uncontrolled proliferation of cells, remains a leading cause of mortality worldwide. Globally, cancer causes nearly 10 million deaths annually, with a disproportionate impact on low-resource regions. Among common types, breast cancer remains a major concern due to its high prevalence and mortality. Breast cancer is the most commonly diagnosed cancer and a leading cause of cancer-related death among women globally. According to WHO (World Health Organization), approximately 2.3 million new cases were reported, with around 685,000 deaths globally in 2020 (Arnold *et al.,* 2022). The incidence continues to rise worldwide, with projections estimating 2.64 million cases and 1.7 million deaths annually by 2030. Developed countries show higher incidence rates (66.4/100,000) in comparison of developing nations (27.3/100,000). Globally, breast cancer incidence has increased by 57.8% over the past 30 years (Shang and Xu, 2022). Breast cancer accounts for the highest disability-adjusted life years (DALYs) lost among all cancer types in women (Yan *et al.,* 2022). Conventional treatments such as chemotherapy and radiotherapy have not proven fully effective in eradicating malignant cells, leading to frequent disease recurrence and resistance (Kaur *et al.,* 2023). Despite advancements in therapeutic strategies, breast cancer continues to be a major cause of death in women due to its high incidence, recurrence, and resistance to conventional therapies. A critical factor contributing to the aggressiveness and therapeutic resistance of breast cancer is the presence of a distinct subpopulation of cells known as Cancer Stem Cells (CSCs). These cells possess the unique ability to self-renew and differentiate, playing a pivotal role in tumor initiation, progression, and relapse. CSCs are also known as tumor-initiating cells (TICs) or tumor-propagating cells due to their capacity to recreate the heterogeneity of the original tumor and regenerate tumors in immunocompromised models (Albini *et al.,* 2015). Breast cancer stem cells (BCSCs) have emerged as a major therapeutic target, with their identification and characterization offering insights into mechanisms of drug resistance and recurrence (Zheng *et al.,* 2021). Targeting signaling pathways

such as Wnt has been recognized as a promising strategy to eliminate BCSCs. The Wnt signaling pathway is frequently deregulated in breast cancer and plays a vital role in maintaining stemness, modulating the tumor immune microenvironment, and contributing to therapeutic resistance (Xu *et al.,* 2020). Wnt signaling exhibits a dual, context-dependent role in breast cancer, promoting tumor progression, EMT (Epithelial- Mesenchymal Transition), and cancer stemness particularly in claudin-low TNBC (Triple negative Breast Cancer), while also inducing squamous differentiation and growth arrest in specific models (Zhan *et al.,* 2017). Limitations of current chemotherapeutic strategies in effectively targeting cancer stem cells have prompted the exploration of alternative therapeutic approaches for improved disease management. Recent years have witnessed growing interest in natural products for their ability to combat cancer with minimal or no toxicity **(**Huang *et al.,* 2021). Among these, turmeric—commonly known as "Indian saffron"—has long held a significant place in traditional Indian medicine. This golden-yellow spice is derived from the rhizomatous herb *Curcuma longa* and contains curcumin as its primary bioactive constituent, credited for its extensive medicinal potential (Prasad and Aggarwal, 2011). The multifunctional nature of curcumin attributes its interaction with numerous molecular targets, contributing to its pharmacological activity in a range of clinical conditions such as cancer, cardiovascular disorders, ulcerative colitis, tropical pancreatitis, and autoimmune diseases (Kumar *et al.,* 2015; Wiggers *et al.,* 2017).

Curcumin, the principal polyphenolic compound derived from *Curcuma longa*, demonstrates potent inhibitory effects on the Wnt/β-Catenin signaling pathway in breast cancer stem cells, contributing to its anti-proliferative and pro-apoptotic properties. It downregulates key regulatory proteins such as disheveled (Dvl), β-Catenin, cyclin D1, and slug, thereby inhibiting β-Catenin nuclear translocation and suppressing transcriptional activation of oncogenic targets like c-Myc and cyclin D1 (Arzi *et al.,* 2022). In hormone receptor-negative models, curcumin downregulates Dvl and activates GSK3β, inhibiting β-Catenin translocation and thereby reducing proliferation and metastatic potential (Fatima *et al.,* 2024).

Curcumin exhibits potent anticancer effects in breast cancer models, yet several limitations impede its clinical translation. Its poor bioavailability, resulting from low absorption and rapid metabolism, significantly restricts its therapeutic impact (Anand *et al.,* 2007). Additionally, curcumin is hydrophobic and chemically unstable in aqueous and physiological environments, which compromises its stability and effectiveness. This study aims to improve the bioavailability and therapeutic efficacy of curcumin by modifying its free phenolic hydroxyl group through conjugation with cinnamic acid, which may exert synergistic effects. Previous reports have shown that curcumin bioconjugates with various ligands exhibit enhanced antibacterial and antiproliferative properties compared to native curcumin (Abd El Hack *et al.,* 2021; Dubey *et al.,* 2008). Cinnamic acid, a naturally occurring phenylpropanoid, possesses anticancer activity and is known to modulate oncogenic signaling pathways, including Wnt/β-Catenin signaling, which is critically involved in the regulation of breast cancer stem cells (BCSCs) proliferation and maintenance (Olayiwola and Gollahon, 2024). Conjugation with cinnamic acid is hypothesized to augment curcumin's ability to disrupt Wnt-driven stemness and tumor progression. The present work focuses on molecular docking to investigate the interaction of curcumin–cinnamic acid conjugates with key components of the Wnt/β-Catenin signaling pathway associated with BCSCs regulation.

MATERIALS AND METHODS

Protein Structure Preparation

The key active site residues of β-Catenin were identified using the UniProt Knowledgebase (UniProtKB) under the UniProt ID: P35222. The crystal structure of β-Catenin (PDB ID: 7AFW) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org). The selection of this specific PDB entry was based on multiple quality parameters, including high structural resolution, acceptable R-value (a measure of the model's agreement with experimental data), and comprehensive sequence

coverage, ensuring accurate representation of the protein's functional domains Protein preparation of β-Catenin was done via chimera1.17.3 (Butt *et al.,* 2020). The processed structure was then saved in PDB format for use in further molecular docking depicted in Fig 1.

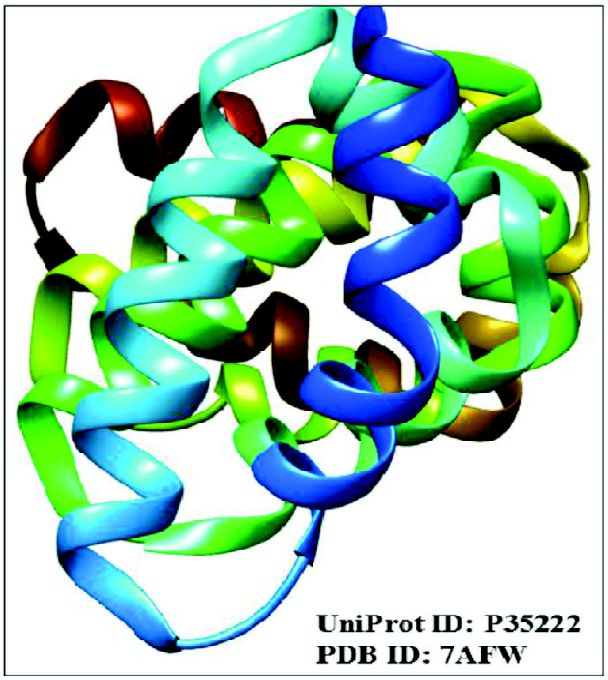

Fig 1: The 3D structure of β-Catenin protein

Ligand Structure Preparation

The preparation of ligand structures is a critical step in ensuring the generation of a single, energetically stable structure with correct spatial orientation and stereochemistry. Cinnamic acid was selected as a suitable ligand for conjugation with curcumin based on its reported anticancer properties and its potential synergistic effect in enhancing curcumin's efficacy against breast cancer stem cells (Meirelles *et al.,* 2023). Ligand structure preparation plays a pivotal role in generating energetically stable molecules with accurate three-dimensional geometry and stereochemistry, thereby enhancing the probability of identifying biologically active compounds during virtual screening. Both curcumin and cinnamic acid have been widely studied for their chemopreventive and therapeutic roles in cancer, primarily through the induction of apoptosis and inhibition of cell proliferation. The curcumin-cinnamic acid conjugate might enhance antiproliferative effects and improved bioavailability due to the combined bioactivity of both constituents. The conjugate structure was designed using ChemDraw Ultra 8.0, and its three-dimensional geometry was energy-minimized using Chem3D with the MM2 force field to ensure a stable conformation for *in silico* docking studies, as depicted in Fig 2.

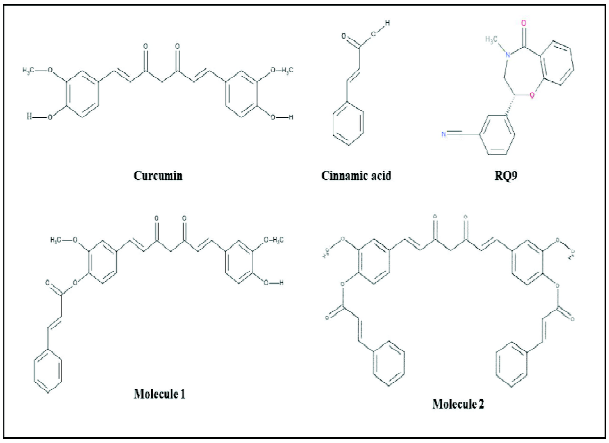

Fig 2: 2D structure of selected Ligand molecules with Curcumin, Cinnamic acid and KB

Molecular Docking

Molecular docking of protein and ligands was performed using AutoDockVina integrated within the PyRx platform (Li *et al.,* 2023). During the docking process, ligands were treated as flexible molecules, while the protein structure was considered rigid. The configuration file for the docking grid was generated with appropriate dimensions, and the protein and ligand structures were converted to '.pdbqt' format for compatibility with AutoDockVina. The molecular docking of curcumin and its conjugates was conducted to evaluate their binding interactions with β-Catenin (PDB ID: 7AFW), a key regulator in the Wnt/β-Catenin signaling pathway. Curcumin and cinnamic acid were sourced from the ZINC15 database in ready-to-dock formats (https://zinc15.docking.org/). The SDF files of curcumin (ZINC000100067274) and cinnamic acid (ZINC16051516) were converted to PDB format using Open Babel 2.4.1 (Yoshikawa and Hutchison, 2019). The curcumin–cinnamic acid conjugate was sketched in ChemDraw (CDX format) and further

converted to PDB using Open Babel for downstream processing. Subsequently, the prepared PDB file was imported into PyRx, where the receptor protein and ligands were designated as macromolecule and ligand, respectively. Ligands were energy-minimized, converted into PDBQT format, and subjected to docking by generating a grid encompassing the entire binding pocket. Docking simulations were carried out using PyRx, and ligand conformers were assessed based on their binding

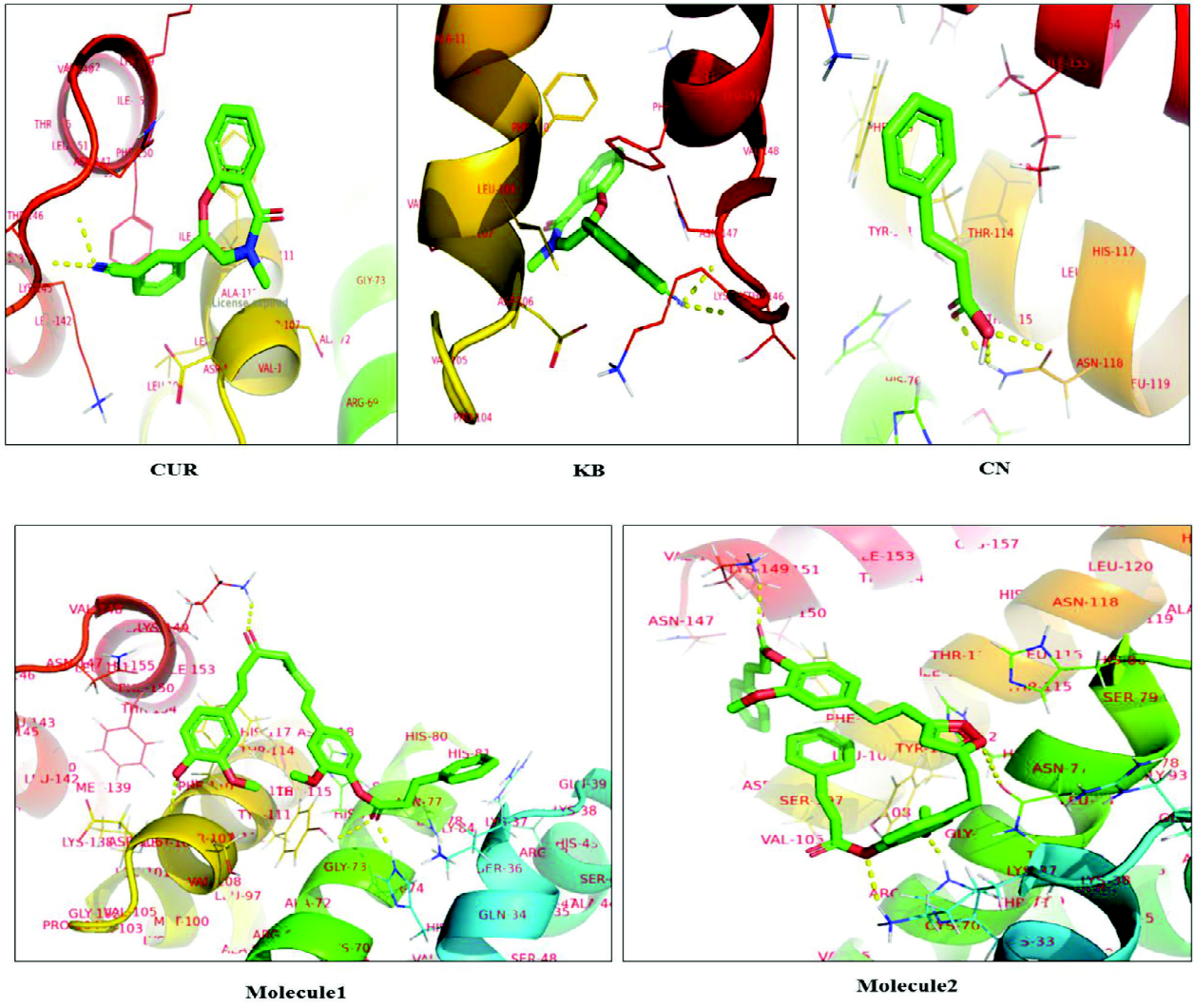

Fig 3: 3D representation of docked conformation of molecules 1, 2, Curcumin, Cinnamic acid and KB with β-Catenin using PyMol

Table 1: Comparative docking simulation result of Curcumin, its Molecule 1,2, Cinnamic acid and Known binder with β-Catenin

Name of the ligand	Residues	Binding Free Energy (kcal/mol)
Curcumin	Asn147, Phe110, Asp106, Tyr111, Asn77, Arg42, Ser107, Lys37, His76	-6.5
Molecule 1	Lys37, Try111, His76, Ser107, Asp106, Phe110, Lys149, His33	-6.2
Molecule 2	Asn147, Phe110, Lys37, Tyr111, Asn77, His33, Lys149	-6.0
Cinnamic acid	Ile153, Phe110, Thr114, His80, His76, Lys149, Asn118	-5.6
R9Q (Known Binder)	Phe110, Ser107, Phe150, Leu109, Asp106, Lys145, Thr146, Asnq147	-7.8

affinities and acceptable RMSD values (≤ 2 Å). Protein–ligand interactions were visualized in PyMol 2.0.

RESULTS AND DISCUSSION

Recognition of Target Protein

PDB ID: 7AFW was selected from a total of 46 β-Catenin crystal structures based on its highest resolution of 1.81 Å. The target protein used for docking studies, β-Catenin, possesses a total molecular weight of 18.45 kDa and consists of 1,314 atoms. The structure includes 167 deposited residues, out of which 161 residues were successfully modeled for analysis. The structure comprises a single unique protein chain, ensuring a consistent binding pocket for ligand interaction studies. This structural integrity supports reliable molecular docking and interaction profiling with curcumin and its designed conjugates.

Molecular Docking

Molecular docking was performed to evaluate the binding affinity and interaction profiles of curcumin, cinnamic acid, and their conjugate with β-Catenin, a key effector protein in the Wnt signaling pathway, which plays a pivotal role in stem cell regulation and cancer progression. The primary objective was to understand the molecular basis of ligand binding and to assess the potential of these compounds as inhibitors of β-Catenin activity. Docking studies were performed using AutoDockVina integrated in PyRx, with β-Catenin (PDB ID: 7AFW) as the target protein. The ligands were ranked based on their binding free energies, and key molecular interactions were analyzed to determine their suitability as potential therapeutic agents. The binding affinity and interacting residues of curcumin, its conjugate (Molecule 1 and Molecule 2), cinnamic acid, and the known binder R9Q with the target protein were evaluated through molecular docking (Table1 and Fig3).

The known binder R9Q exhibited the highest binding affinity with a binding free energy of -7.8 kcal/mol, interacting with key residues including Phe110, Ser107, and Asp106. Curcumin showed a notable binding affinity of -6.5 kcal/mol, engaging residues such as Asn147, Phe110, and Tyr111. Molecule 1(CUR+CN) and Molecule 2 (CN+CUR+CN) demonstrated slightly lower binding affinities of -6.2 kcal/mol and -6.0 kcal/mol, respectively, yet maintained interactions with crucial residues like Phe110, Lys37, and Tyr111, indicating comparable binding profiles to curcumin. In contrast, cinnamic acid displayed the least binding affinity (-5.6 kcal/mol), interacting with residues such as His76, Lys149, and Asn118. The binding affinity of the curcumin–cinnamic acid conjugate and its stable interaction network with β-Catenin suggests its potential to inhibit Wnt/β-Catenin signaling, as a potential lead compound for further development of cancer therapeutics targeting stemness and proliferation.

CONCLUSION

The molecular docking analysis highlights the potential of curcumin and its conjugates to interact effectively with β-Catenin, a key regulator of the Wnt signaling pathway and cancer stem cell maintenance. Curcumin showed strong interaction with key residues, while its conjugates maintained comparable binding energies and preserved crucial contacts within the β-Catenin binding site, suggesting their potential as effective modulators. Although cinnamic acid alone displayed lower binding affinity, its integration into conjugates contributed to enhanced binding characteristics. Notably, these conjugates retained interactions with core active site residues, including Phe110, Tyr111, and Lys37, indicating stable ligand-protein complexes. The incorporation of cinnamic acid, despite its relatively moderate individual binding, appears to contribute synergistically within the conjugate framework, aiming to enhance the overall binding efficacy and therapeutic potential of the curcumin conjugates. The known binder R9Q displayed the strongest interaction (-7.8 kcal/mol), validating the docking approach and providing a benchmark for comparison. Overall, these computational findings support the therapeutic promise of curcumin conjugates as potential inhibitors of β-Catenin in Wnt signaling. However, extensive *in vitro* and *in vivo* validation is essential to confirm their bioactivity,

pharmacological safety, and overall efficacy. Computational studies offer a compelling foundation, but further biological evaluation is crucial to establish these conjugates as viable candidates for natural, targeted cancer chemotherapy.

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