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Computational Evaluation of Vitamin D3 Binding to KRAS and TGF-β1 in Colorectal Cancer-Associated Signalling Pathways

Jauharotus Shobahah[1*], Sri Puji Astuti Wahyuningsih[2], Mochammad Aqilah Herdiansyah[2], M. Ainun Najib Aly[3]

[1]Department of Biotechnology, Faculty of Food Security, Universitas Negeri Surabaya, Surabaya, Indonesia
[2]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Hang Tuah University, Surabaya, Indonesia
[*]e-mail: jauharotusshobahah@unesa.ac.id

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Abstract: Vitamin D3 has been widely investigated for its anticancer properties, yet the structural basis of its interaction with key oncogenic signaling proteins remains incompletely understood. This study aimed to evaluate the molecular interactions between vitamin D3 and KRAS and TGF-β1 using molecular docking and molecular dynamics simulations. Molecular docking analysis was performed using AutoDock Vina, followed by molecular dynamics simulation using CABS-flex to evaluate structural stability using root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg). Comparative benchmarking was performed against the reference inhibitors Sotorasib (KRAS) and Galunisertib (TGF-β pathway). Docking analysis revealed that vitamin D3 binds to KRAS (PDB ID: 4OBE) with a binding affinity of –7.8 kcal/mol, compared to –8.6 kcal/mol for Sotorasib. The interaction was localized within the nucleotide-binding pocket adjacent to the Switch I and Switch II regions, which are critical for conformational regulation. For TGF-β1 (PDB ID: 3KFD), vitamin D3 demonstrated a binding affinity of –8.2 kcal/mol, slightly exceeding that of Galunisertib (–8.1 kcal/mol), with interaction occurring at the receptor-binding interface. Molecular dynamics simulation showed stable complex formation, with RMSD values of 2.79 Å for the KRAS complex and 1.535 Å for the TGF-β1 complex, indicating acceptable structural stability. Residue fluctuation analysis further supported moderate flexibility without global destabilization. These findings suggest that vitamin D3 may function as a multi-target signaling modulator interacting with both intracellular and extracellular regulators of colorectal cancer pathways, providing a structural basis for further experimental investigation.

Keywords: Epithelial–Mesenchymal Transition (EMT); KRAS; Molecular Docking; TGF-β1; Vitamin D3.

Introduction

Colorectal cancer (CRC) remains one of the most prevalent malignancies worldwide and represents a major contributor to cancer-related mortality [1,2]. The molecular pathogenesis of CRC involves progressive accumulation of genetic alterations and dysregulation of critical signaling networks that govern cellular proliferation, survival, differentiation, and microenvironmental interactions [3–7]. Among these networks, KRAS-mediated signaling and transforming growth factor-beta (TGF-β)–associated pathways play central yet mechanistically distinct roles in colorectal tumor development [8,9].

KRAS encodes a small GTPase that functions as a molecular switch cycling between an active GTP-bound state and an inactive GDP-bound state. Through this nucleotide-dependent conformational regulation, KRAS controls downstream signaling cascades including the MAPK/ERK pathway, which is frequently hyperactivated in colorectal cancer [10,11]. While activating mutations in KRAS are common in CRC, structural characterization of the wild-type protein remains essential for understanding conformational landscapes and ligand accessibility within defined GDP-bound or GTP-bound states. The GDP-bound conformation represents a structurally stable form frequently utilized for computational and inhibitor-binding studies due to its well-defined crystallographic features [12–14].

In parallel, TGF-β1 is a multifunctional cytokine that orchestrates diverse biological processes including cell cycle regulation, extracellular matrix remodeling, and immune modulation. TGF-β1 is secreted as a dimeric ligand that initiates signaling through binding to TGF-β receptors, subsequently activating canonical SMAD-dependent and non-canonical pathways [15–17]. In colorectal cancer, TGF-β signaling exhibits context-dependent behavior: it contributes to growth suppression in early stages yet may promote epithelial–mesenchymal transition and tumor progression in advanced disease. Structural characterization of the TGF-β1 ligand provides insight into molecular interfaces involved in receptor recognition and extracellular signaling modulation [18–20].

The coexistence of KRAS-driven oncogenic signaling and TGF-β–associated regulatory mechanisms reflects the complex signaling environment that shapes colorectal tumor progression. Although these pathways operate at different levels—KRAS primarily as an intracellular molecular switch and TGF-β1 as an extracellular ligand—their combined influence contributes to cellular plasticity, survival adaptation, and tumor microenvironment interactions [20–23].

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Vitamin D3 (cholecalciferol) has traditionally been recognized for its role in calcium metabolism; however, increasing interest has emerged regarding its broader biological effects on cellular signaling processes [24,25]. While many reported effects are mediated through the vitamin D receptor (VDR), the potential for structural interaction between vitamin D3 and non-classical signaling proteins remains incompletely characterized. Computational molecular docking provides a systematic framework to evaluate potential ligand–protein interactions by assessing predicted binding affinity, residue-level engagement, and spatial localization within defined structural domains [26,27].

Increasing evidence suggests that oncogenic KRAS signaling and TGF-β–mediated pathways do not operate independently in colorectal cancer but rather exhibit complex signaling crosstalk that contributes to tumor progression. KRAS-driven activation of downstream pathways such as MAPK and PI3K can influence cellular responses to TGF-β signaling, while persistent TGF-β activity may further enhance epithelial–mesenchymal transition (EMT), invasion, and metastatic potential [28–30]. The interaction between these intracellular oncogenic regulators and extracellular signaling mediators creates a dynamic regulatory network that supports tumor cell plasticity and survival.

Although numerous studies have investigated the individual roles of KRAS signaling and TGF-β pathways in colorectal cancer progression, the structural interaction of small bioactive molecules with components of both pathways simultaneously remains insufficiently explored. In particular, most studies on vitamin D3 focus primarily on vitamin D receptor (VDR)-mediated genomic mechanisms, while potential direct interactions between vitamin D3 and oncogenic signaling proteins such as KRAS and TGF-β1 have rarely been evaluated at the structural level. Therefore, investigating whether vitamin D3 can structurally interact with both intracellular and extracellular regulators of colorectal cancer signaling may provide new insights into its potential role as a multi-target signaling modulator.

Considering the pleiotropic biological activities of vitamin D3 and its reported influence on multiple cancer-related signaling cascades, investigating its potential structural interaction with key regulators such as KRAS and TGF-β1 may provide new mechanistic insights into its anticancer properties.

Given the pivotal roles of KRAS and TGF-β1 in colorectal cancer–associated signalling, this study aims to investigate the structural interaction profiles of vitamin D3 with human wild-type KRAS in its GDP-bound conformation and with the TGF-β1 ligand using molecular docking analysis. By characterizing predicted binding energies and interaction patterns within structurally defined regions, this work seeks to provide a computational basis for understanding potential molecular associations between vitamin D3 and key components of colorectal cancer signaling. Importantly, the present study focuses on structural interaction profiling and does not presume direct functional inhibition or pathway modulation without further experimental validation.

Research Methods

This study employed a computational structure-based approach combining molecular docking and molecular dynamics simulations to evaluate the interaction between vitamin D3 and two proteins involved in colorectal cancer signaling pathways. The study design aimed to investigate potential ligand–protein binding affinity, interaction patterns, and structural stability of the resulting complexes.

Ligand Preparation

Vitamin D3 (cholecalciferol; PubChem CID: 5280795) was used as the primary ligand in this study. The two-dimensional structure was constructed in ChemDraw Version 18 (PerkinElmer Informatics Inc., USA) and converted to a three-dimensional structure using Chem3D. Geometry optimization was performed employing the MM2 force field to obtain a stable low-energy conformation, following common procedures used in molecular docking preparation [31,32]. The optimized structure was saved in PDB format and subsequently converted into PDBQT format using PyRx 0.8 prior to docking simulations.

For comparative benchmarking, the reference inhibitors Sotorasib and Galunisertib were retrieved from the PubChem database in SDF format and subjected to identical geometry optimization procedures to ensure consistency in computational preparation. All ligands were protonated appropriately and converted into PDBQT format under the same parameter settings.

Protein Structure Retrieval and Preparation

The crystallographic structures of the target proteins were obtained from the Protein Data Bank (PDB). The structure of human KRAS in its GDP-bound conformation (PDB ID: 4OBE) and human TGF-β1 ligand (PDB ID: 3KFD) were selected for docking analysis. The crystal structure of human KRAS (PDB ID: 4OBE) was selected because it represents the GDP-bound conformation, providing a structurally stable model frequently used in computational docking studies. The structure of TGF-β1 (PDB ID: 3KFD) was selected due to its well-characterized ligand conformation and availability of the receptor-binding interface necessary for interaction analysis [33–35]. Protein preprocessing was performed using BIOVIA Discovery Studio 2019 (Dassault Systèmes BIOVIA, USA). Co-crystallized ligands and crystallographic water molecules were removed to avoid interference during docking. Non-essential heteroatoms were removed, and polar hydrogen atoms were added to ensure accurate representation of hydrogen-bonding interactions. Kollman charges were assigned prior to conversion into PDBQT format using PyRx 0.8.

Docking Protocol and Comparative Benchmarking

Molecular docking simulations were performed using PyRx 0.8, integrated with AutoDock Vina, a widely used algorithm for structure-based virtual screening and ligand–protein interaction analysis [32,36]. Grid box parameters were defined based on functionally relevant regions of each protein. For KRAS (4OBE), the docking grid was centered

on the nucleotide-binding pocket encompassing the GDP-binding site as well as adjacent Switch I and Switch II regions that are critical for conformational regulation. For TGF-β1 (3KFD), the grid box encompassed the receptor-binding interface region involved in downstream signaling activation.

To strengthen biological relevance, comparative benchmarking was conducted using Sotorasib as a reference inhibitor for KRAS and Galunisertib as a reference inhibitor for the TGF-β signaling pathway. All docking simulations were performed using identical grid coordinates, exhaustiveness parameters (set to 8), and scoring functions to ensure methodological consistency across ligands. Binding affinity values (kcal/mol) for vitamin D3 were directly compared with those of the reference inhibitors under the same computational conditions. The most favorable binding poses were selected based on lowest predicted binding energy and spatial plausibility within the predefined functional domains.

Interaction Analysis and Structural Visualization

The selected docking conformations were analyzed using BIOVIA Discovery Studio 2019 to characterize molecular interactions at the amino acid residue level. Two-dimensional interaction maps were generated to identify hydrogen bonds, hydrophobic interactions, van der Waals forces, and π-alkyl contacts. Three-dimensional visualization was performed to evaluate ligand positioning within the KRAS nucleotide-binding pocket and the TGF-β1 receptor-interaction surface. Structural interpretation focused on identifying interactions occurring within catalytically or conformationally critical residues that may influence protein functionality.

Molecular Dynamics Simulation

To evaluate structural stability and conformational flexibility of the ligand–protein complexes, molecular dynamics simulations were conducted using the CABS-flex 2.0 web server. The docked complexes of vitamin D3 with KRAS and TGF-β1 were submitted in PDB format and simulated under default parameters. Molecular dynamics stability was evaluated using root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) parameters. RMSD values were used to assess overall structural deviation during simulation, RMSF to measure residue-level flexibility, and radius of gyration to evaluate global structural compactness of the protein–ligand complexes. Root Mean Square Deviation (RMSD) was calculated to assess overall structural deviation during simulation, while Root Mean Square Fluctuation (RMSF) analysis was used to evaluate residue-level flexibility. The radius of gyration (Rg) was determined to assess the compactness of each complex and structural integrity during dynamic simulation. Representative centroid structures from dominant clusters were extracted for further structural comparison.

Data Analysis

Binding affinity values obtained from molecular docking simulations were tabulated and compared for vitamin D3 and the reference inhibitors. Binding affinity values obtained from AutoDock Vina were interpreted based on predicted docking scores, where more negative values indicate stronger predicted binding interactions. Docking poses were selected based on the lowest binding energy and spatial plausibility within functionally relevant regions of the target proteins [37,38]. Structural interaction patterns and molecular dynamics parameters (RMSD, RMSF, and Rg) were analyzed descriptively to interpret stability, flexibility, and potential functional modulation of the target proteins.

Results and Discussion

Molecular Docking Analysis

Vitamin D3 has been increasingly recognized as a pleiotropic secosteroid hormone with regulatory functions extending beyond calcium metabolism. In oncology, its biological effects have been associated with anti-proliferative, pro-differentiative, and anti-inflammatory activities [39,40]. While these effects are classically attributed to vitamin D receptor (VDR)-mediated transcriptional regulation, emerging evidence suggests that non-genomic mechanisms involving rapid modulation of intracellular signaling cascades may also contribute to its anticancer properties [41–44]. Molecular docking and molecular dynamics simulations provide a structural platform to explore whether vitamin D3 can directly engage key oncogenic regulators [45–47] such as KRAS and TGF-β1 at the protein level.

Docking analysis revealed that vitamin D3 binds to KRAS (PDB ID: 4OBE) with a predicted binding affinity of –7.8 kcal/mol, whereas the reference inhibitor Sotorasib exhibited a stronger affinity of –8.6 kcal/mol under identical parameters (Table 1). Although vitamin D3 does not surpass Sotorasib in binding strength, its spatial localization within the nucleotide-binding pocket adjacent to the Switch I and Switch II regions is structurally significant. For TGF-β1 (PDB ID: 3KFD), vitamin D3 demonstrated a binding affinity of –8.2 kcal/mol, slightly stronger than Galunisertib (–8.1 kcal/mol). Notably, vitamin D3 was predicted to occupy the receptor-binding interface of the TGF-β1 ligand. This region is crucial for interaction with TGF-β receptors I and II, which subsequently initiate SMAD phosphorylation and transcriptional regulation [48,49].

Table 1. Molecular docking-based binding affinity (kcal/mol) of vitamin D3 and known inhibitors against KRAS and TGF-β for benchmarking purposes.

Ligand	Receptor	Binding Affinity
Vitamin D3	KRAS	-7.8
	TGF-β	-8.2
Sotorasib	KRAS	-8.6
Galunisertib	TGF-β	-8.1

Interaction Profile Analysis

The interaction profile of vitamin D3 with KRAS involved residues such as Phe141, Glu143, Gln150, Asp154, Thr158, and Arg161, as well as hydrophobic contacts with Pro140 and Ile142. Several of these residues lie within conformationally sensitive regions that contribute to stabilization of active or inactive states [50,50–52]. The

Switch I (residues 30–38) and Switch II (residues 59–76) domains are highly dynamic segments that undergo conformational rearrangement upon GDP/GTP exchange. These regions are essential for the recruitment of effector proteins, including RAF kinases and PI3K.

Unlike Sotorasib, which is designed to covalently stabilize the inactive conformation of mutant KRAS [53,54], vitamin D3 appears to interact primarily through hydrophobic and van der Waals contacts. This mode of interaction suggests that vitamin D3 may not function as a mutation-specific inhibitor but rather as a conformational modulator. Subtle alterations in Switch II flexibility can influence nucleotide exchange kinetics and effector binding affinity, thereby attenuating downstream MAPK and PI3K signaling without completely abolishing KRAS activity [55–57].

For the TGF-β1 complex, the docking analysis revealed interactions with residues including Trp52, Leu64, Gln67, Cys62, Thr72, and Tyr50, as well as hydrophobic contacts involving Pro49 and Ile51. These residues are positioned within the surface topology responsible for receptor recognition and dimer interface stabilization. Although an unfavorable donor–donor interaction was observed at Thr74, the overall binding conformation remained energetically favorable.

The dual role of TGF-β signaling in cancer progression adds mechanistic importance to this finding. In early-stage tumors, TGF-β exerts tumor-suppressive effects through growth inhibition and apoptosis induction. However, in advanced malignancy, persistent TGF-β signaling promotes epithelial–mesenchymal transition (EMT), invasion, immune evasion, and metastasis. Structural perturbation at the ligand–receptor interface may alter receptor engagement efficiency or downstream SMAD complex formation, potentially modulating the balance between suppressive and pro-metastatic signaling [18,58,59].

Table 2. Amino acid residues and interaction types involved in KRAS and TGF-β as predicted by molecular docking.

Ligand	Receptor	Type of Interaction	Amino Acids Involved in Interaction
Vitamin D3	KRAS	vdw	Gln150(A), Gly151(A), Glu143(A), Arg161(A), Phe141(A), Asp154(A), Arg161(A), Thr158(A), Asp153(B), Asp154(B), Ile147(B), Phe141(B)
		HI	Pro140(A), Ile142(A), Tyr157(B)
	TGF-β	vdw	Trp52(D), Leu64(D), Gln67(D), Phe60(K), Cys62(K), Ala63(K), Thr72(K), Ser33(K), Val34(K), Thr35(K), His43(K), Asn44(K)
		HI	Pro49(D), Ile51(D), Tyr50(D), Phe31(K), Ile42(K)
		PHI	Val61(K)
		Unfavorable donor-donor	Thr74(K)
Sotorasib	KRAS	vdw	Glu162(A), Lys165(A), Glu143(A), Phe141(A), Pro140(B), Thr158(B), Asp153(B)
		HI	Pro140(A), Ile142(A), Ile142(B), Tyrr157(B)
		PHI	Gln150(A), Asp154(B), Arg161(B)
		Pi-sigma	Thr158(A)
		Unfavorable bump	Unk1(N)
Galunisertib	TGF-β	vdw	Trp52(C), Ile51(C), Gln67(C), Asn14(C), Thr35(L), Ser66(L), His43(L), Asn44(L), Phe31(L), Ser33(L), Thr74(L), Pro64(L)
		HI	Pro49(C), Ile42(L)
		PHI	Ser65(L)
		Pi-sigma	Thr72(L)
		Pi-pi T-shaped	Tyr50(C)

Molecular Dynamics Stability

Molecular dynamics simulations were conducted to evaluate the structural stability and flexibility of the ligand–protein complexes. The vitamin D3–KRAS complex exhibited an RMSD of 2.79 Å, indicating acceptable structural stability throughout the simulation. The RMSF value of 1.696 Å suggests moderate residue-level flexibility, which is consistent with the intrinsically dynamic nature of the KRAS Switch regions (Table 3).

Residue fluctuation analysis revealed that most fluctuations occurred within surface loops and flexible

regions, whereas residues on the binding interface showed relatively restrained mobility (Figure 1A). This observation indicates that vitamin D3 binding does not induce major conformational destabilization within the nucleotide-binding pocket. The radius of gyration remained approximately constant at 1.000 during the simulation, suggesting preservation of the global structural compactness of the KRAS protein (Figure 2A). The absence of significant deviation in Rg values further supports that ligand binding does not induce large-scale unfolding or structural disruption. For the vitamin D3–TGF-β1 complex, molecular dynamics simulation demonstrated an RMSD value of 1.535 Å, indicating high conformational stability (Table 3). The RMSF value of 1.896 Å suggests moderate flexibility primarily localized at peripheral residues, while residues located within the receptor-binding interface remained relatively stable (Figure 1B). Similar to the KRAS complex, the radius of gyration remained constant at approximately 1.000 during the simulation period (Figure 2B), indicating maintained structural compactness of the TGF-β1 protein. Compared with KRAS, the TGF-β1 complex exhibited slightly greater structural stability, suggesting greater structural compatibility of vitamin D3 at the extracellular ligand interface.

Table 3. Molecular dynamics simulation parameters of vitamin D3 interaction with KRAS and TGF-β proteins.

Plot	∑ RMSF value	RMSD value	∑ Radius of gyration
Vitamin D3-KRAS	1.696 Å	2.79 Å	1.000
Vitamin D3-TGF-β	1.896 Å	1.535 Å	1.000

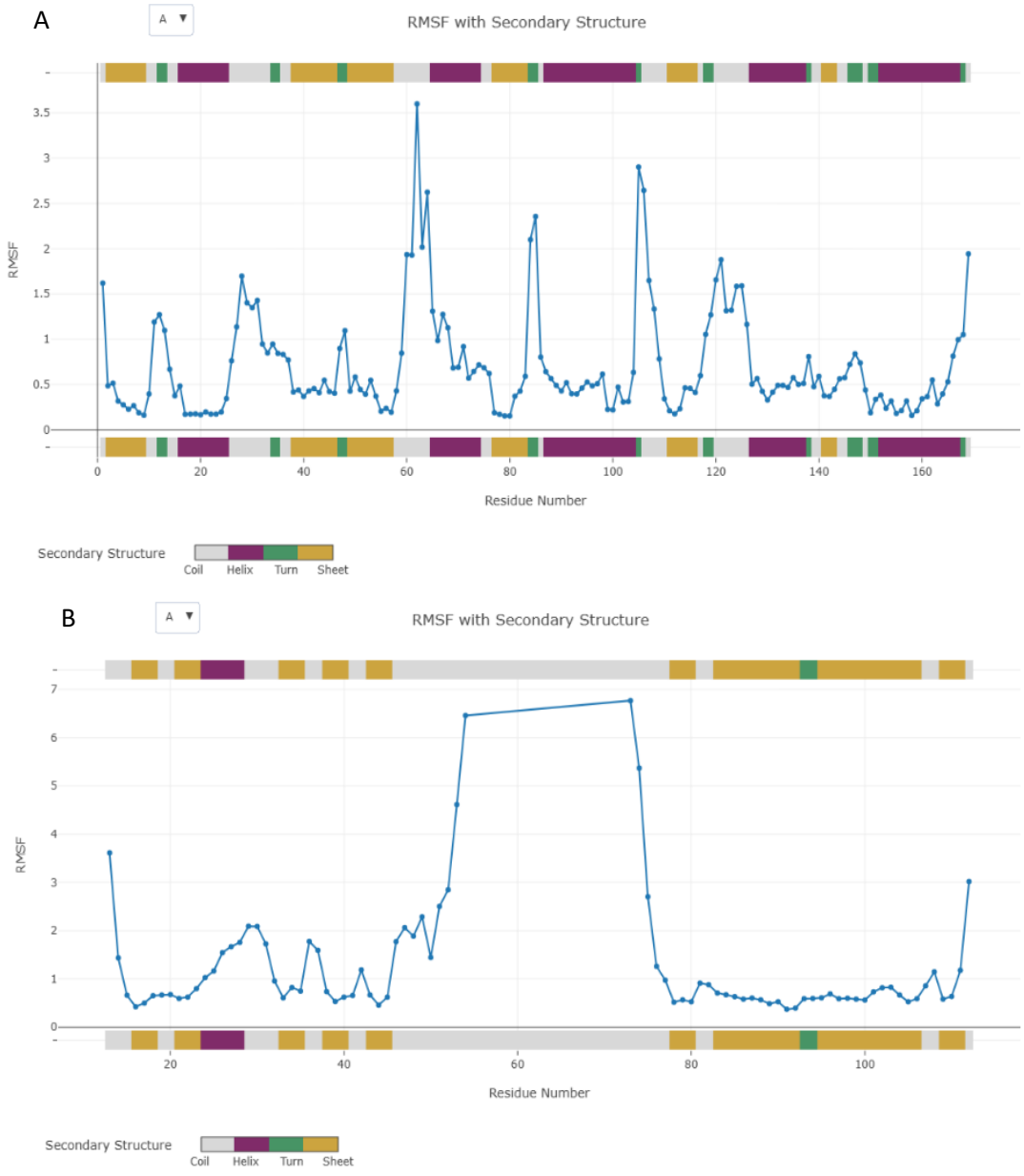

Figure 1. RMSF plot of protein interacted with vitamin D3 and highlighted key residue. A. KRAS. B. TGF-β.

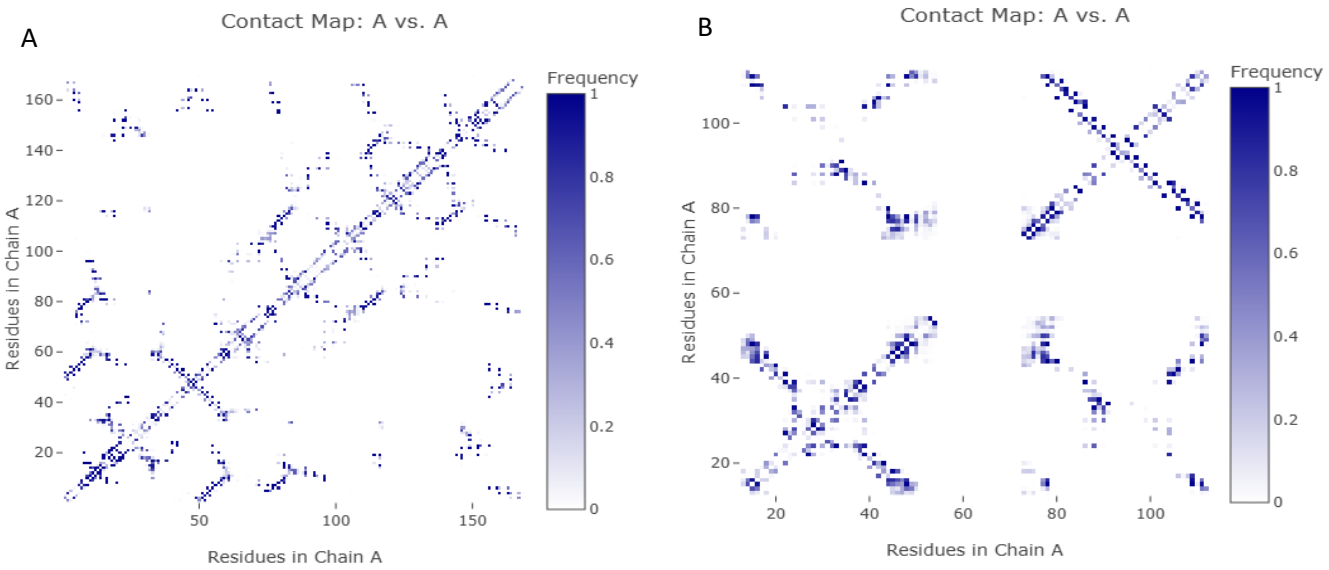

Figure 2. Radius of gyration of protein interacted with vitamin D3 and highlighted key residue. A. KRAS. B. TGF-β.

Visualization of Docking Interactions

Visual inspection of the two-dimensional and three-dimensional docking representations further corroborated the predicted binding orientation of vitamin D3 within the KRAS pocket (Figure 3A). The 2D interaction map (Figure 4A) showed that the ligand was stabilized predominantly through van der Waals and hydrophobic contacts, with limited polar hydrogen interactions. In contrast, the reference inhibitor Sotorasib exhibited additional π–sigma interactions, particularly involving Thr158, resulting in a more complex interaction network within the binding cleft. Notably, both vitamin D3 and Sotorasib shared contacts with residues such as Phe141, Glu143, and Pro140, suggesting partial overlap in binding topology (Figure 5A and 6A).

The predominance of hydrophobic and van der Waals interactions in the vitamin D3–KRAS complex is consistent with the lipophilic nature of the secosteroid backbone. Although vitamin D3 lacks the covalent stabilization mechanism characteristic of Sotorasib, the absence of steric clashes or unfavorable bumps indicates a geometrically compatible binding pose within the KRAS pocket. Such interaction characteristics support the interpretation that vitamin D3 may influence local conformational flexibility rather than acting as a mutation-specific inhibitor.

Visualization analysis of the vitamin D3–TGF-β1 complex revealed a well-oriented binding pose located at the receptor-interaction surface (Figure 3B). The 2D interaction diagram (Figure 4B) indicated a combination of hydrophobic contacts and polar interactions involving residues such as Trp52, Ile51, and Gln67, which contribute to receptor recognition topology. Compared with the reference inhibitor Galunisertib, vitamin D3 displayed a slightly different interaction pattern, relying primarily on hydrophobic stabilization, whereas Galunisertib formed additional π–π T-shaped interactions with Tyr50 (Figure 5B and 6B).

An unfavorable donor–donor interaction was observed at Thr74 in the vitamin D3–TGF-β1 complex. However, this interaction did not significantly compromise overall binding affinity (–8.2 kcal/mol). In structural modeling, localized unfavorable contacts may occur due to geometric constraints but can be compensated by surrounding stabilizing interactions. Overall, the visualization and interaction profile analyses support the structural plausibility of vitamin D3 binding at the TGF-β1 receptor-binding interface, where hydrophobic stabilization and selective polar contacts may contribute to ligand–receptor engagement without inducing major structural distortion.

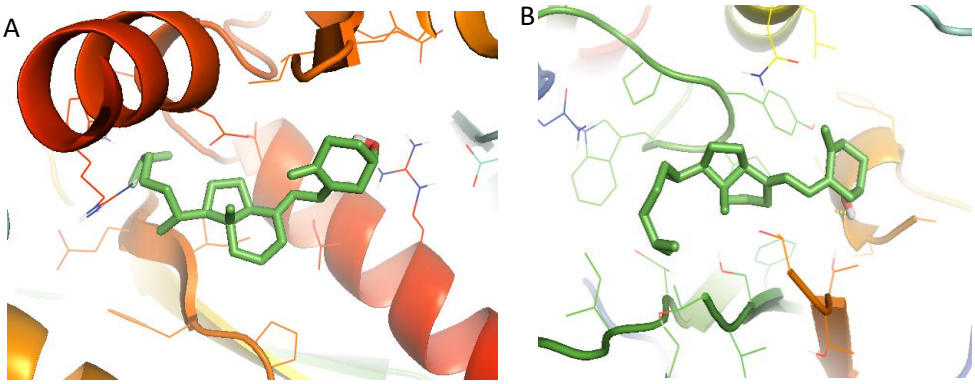

Figure 3. Three-dimensional molecular interaction binding of protein interacted with vitamin D3. A. KRAS. B. TGF-β.

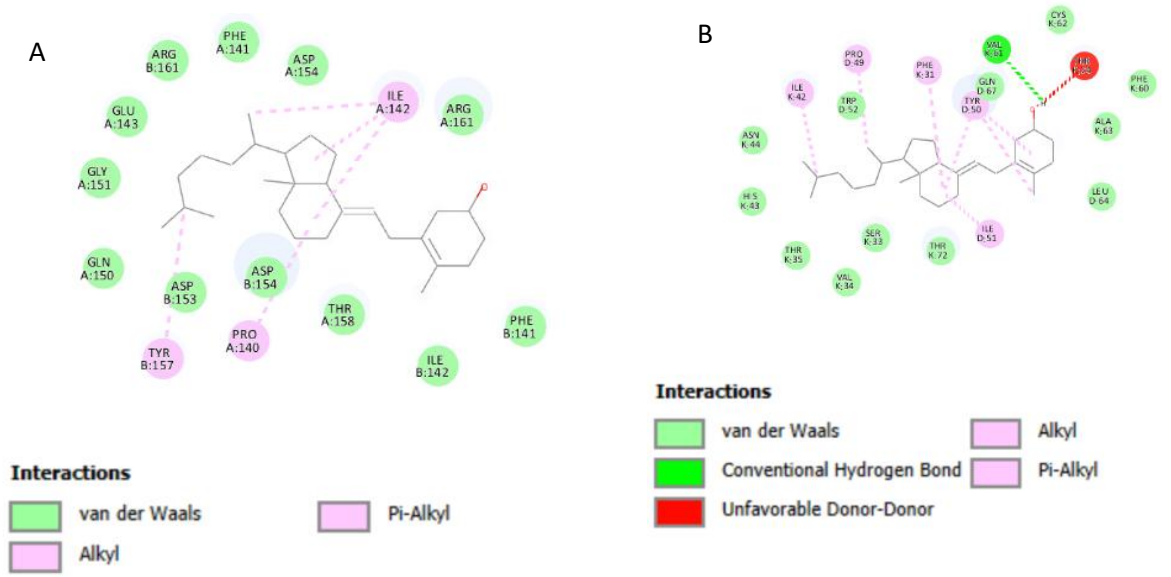

Figure 4. Two-dimensional visualization of the interaction of the receptor–compound complex interacted with vitamin D3. A. KRAS. B. TGF-β.

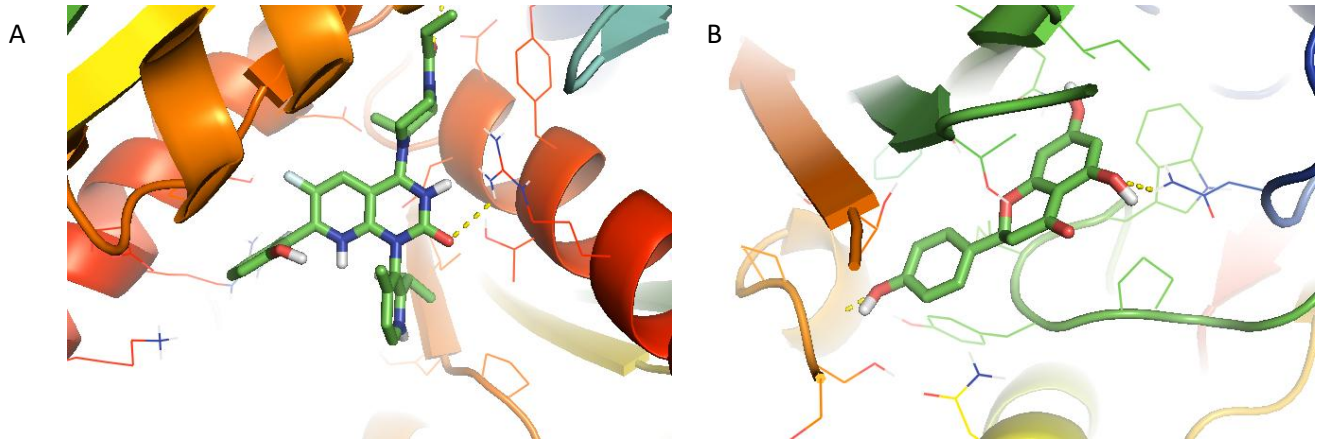

Figure 5. Three-dimensional molecular interaction binding. A. KRAS interacted with Sotorasib. B. TGF-β interacted with Galunisertib.

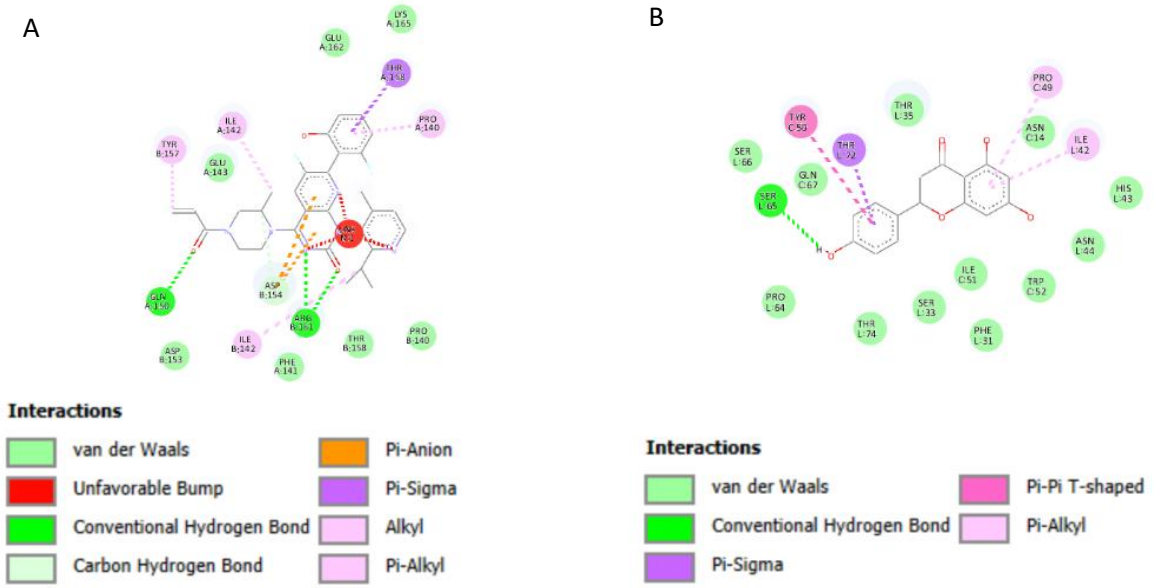

Figure 6. Two-dimensional visualization of the interaction of the receptor–compound complex. A. KRAS interacted with Sotorasib. B. TGF-β interacted with Galunisertib.

Integrated Mechanistic Perspective

When integrating docking energy, residue-level interaction, and dynamic stability data, a pattern emerges in which vitamin D3 demonstrates structurally meaningful engagement with both intracellular (KRAS) and extracellular (TGF-β1) signaling regulators. The interaction with KRAS appears to involve modulation of conformationally flexible Switch domains, potentially influencing effector binding and signal propagation. Meanwhile, interaction with TGF-β1 occurs at the receptor-binding surface, suggesting possible alteration of ligand–receptor complex assembly and downstream SMAD activation.

This dual-target structural engagement aligns with the broader biological profile of vitamin D3 as a pleiotropic regulator. Rather than acting as a high-affinity, single-target inhibitor, vitamin D3 may exert coordinated attenuation across interconnected signalling pathways. Such multi-pathway modulation could be particularly advantageous in cancer systems characterized by pathway redundancy and compensatory signalling mechanisms [25,60–62].

Importantly, while the binding affinity of vitamin D3 does not dramatically exceed that of specialized inhibitors such as Sotorasib, its dynamically stable interaction—especially with TGF-β1—supports the plausibility of functional modulation. These findings provide structural rationale for further experimental validation to determine whether vitamin D3 influences KRAS-driven MAPK signalling or TGF-β-mediated EMT processes in cellular models.

Clinical and Translational Relevance

The structural findings observed in this study provide a mechanistic framework that may help contextualize the reported anticancer effects of vitamin D3 from a signalling perspective. Although vitamin D3 is not classified as a targeted oncologic agent, its ability to interact with both KRAS and TGF-β1 suggests a potential role as a signalling modulator within interconnected oncogenic pathways.

KRAS-driven malignancies are often characterized by persistent activation of downstream MAPK and PI3K pathways, contributing to uncontrolled proliferation and resistance to therapy [63–66]. Direct pharmacological inhibition of KRAS remains challenging due to high nucleotide affinity and limited druggable pockets [67–70]. The observation that vitamin D3 can engage residues within conformationally sensitive Switch regions raises the possibility that it may influence signaling amplitude rather than completely suppress pathway activation. Such partial modulation could be biologically relevant in contexts where pathway hyperactivation contributes to tumor progression but complete inhibition is either unachievable or associated with toxicity.

Similarly, aberrant TGF-β signaling plays a critical role in tumor progression, particularly in advanced stages characterized by epithelial–mesenchymal transition (EMT), immune suppression, and metastatic dissemination [17,59,71,72]. Structural interaction of vitamin D3 at the TGF-β1 receptor-binding interface suggests a potential influence on ligand–receptor engagement dynamics. Even modest perturbation of this interface may alter downstream SMAD signaling intensity, thereby affecting EMT-related transcriptional programs.

From a translational standpoint, these findings support the concept that vitamin D3 may function as an adjuvant signaling regulator rather than a standalone cytotoxic agent [43,60,62]. Multi-target modulation across KRAS and TGF-β pathways could theoretically complement conventional therapies by attenuating pathway redundancy and compensatory signaling. Moreover, the relatively stable molecular dynamics profile observed in this study strengthens the structural plausibility of such interactions.

Nevertheless, it is important to emphasize that in silico predictions do not directly equate to clinical efficacy. Experimental validation using biochemical binding assays, pathway activity analysis, and functional cellular models would be required to confirm whether the predicted structural interactions translate into measurable biological effects. Future studies integrating in vitro and in vivo models may clarify whether vitamin D3-mediated pathway modulation can enhance therapeutic responsiveness or reduce metastatic potential.

Overall, the present structural analysis offers a preliminary translational hypothesis: vitamin D3 may exert coordinated modulation of oncogenic signaling networks through direct interaction with key regulatory proteins, providing a mechanistic basis for further experimental and clinical investigation.

Conclusion

This study demonstrates that vitamin D3 is capable of forming energetically favorable and structurally stable complexes with KRAS and TGF-β1, two key regulators of oncogenic signaling pathways. Molecular docking analysis revealed binding affinities of –7.8 kcal/mol for the KRAS complex and –8.2 kcal/mol for the TGF-β1 complex. Although the interaction with KRAS was weaker than that of the reference inhibitor Sotorasib, vitamin D3 was localized within the nucleotide-binding region adjacent to the Switch domains, suggesting potential modulation of conformational dynamics. In contrast, binding to TGF-β1 exhibited comparable affinity to Galunisertib and occurred at the receptor-binding interface, indicating possible influence on ligand–receptor engagement. Molecular dynamics simulations further supported the structural stability of these complexes, with RMSD values of 2.79 Å for KRAS and 1.535 Å for TGF-β1, indicating acceptable conformational equilibrium under simulated conditions. Collectively, these findings suggest that vitamin D3 may act as a multi-target signaling modulator rather than a high-affinity mutation-specific inhibitor. The structural insights obtained in this study provide a mechanistic basis for further experimental validation to determine whether vitamin D3 can attenuate KRAS-driven signaling and TGF-β-mediated tumor progression in biological systems. Despite these promising structural findings, the present study has limitations because the results are based solely on computational predictions. Experimental validation through biochemical binding assays, cellular signaling analysis, and in vitro or in vivo models is required to confirm the biological relevance of these interactions. Future studies integrating experimental approaches may clarify whether vitamin D3-mediated modulation of KRAS and TGF-β signaling pathways can

contribute to improved therapeutic strategies in colorectal cancer.

Author's Contribution

J. Shobahah: Conceived, designed the experiements, and drafted the manuscript. S.P.A. Wahyuningsih: Conceived, designed the experiements. M.A. Herdiansyah: performed the experiments; M. A.N. Aly: participated in the data analysis. All authors have read and approved the final version of the manuscript.

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