International Journal of Zoology and Applied Biosciences
Volume 10, Issue 6, pp: 447-450, 2025 (Special Issue)
https://doi.org/10.55126/ijzab.2025.v10.i06.SP089
ISSN: 2455-9571
http://www.ijzab.com
Research Article

MECHANISTIC INSIGHTS INTO SMAD3 INHIBITION USING MOLECULAR DOCKING TECHNIQUES

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Article History: Received 25th September 2025; Accepted 24th November 2025; Published 1st December 2025

ABSTRACT

Smad3 is a crucial intracellular transcription factor activated downstream of the transforming growth factor-β (TGF-β) signaling pathway, playing a central role in fibrosis, cancer progression, and inflammatory diseases. Targeting Smad3 with selective small-molecule inhibitors has emerged as a promising therapeutic strategy. This study aims to explore Smad3 ligand interactions using computational molecular docking techniques to identify key binding residues, assess inhibitor stability, and provide mechanistic insights into inhibitory potential. A curated library of 25 reported and novel phytochemical compounds was prepared and optimized using ligand minimization protocols. The Smad3 crystal structure PDB ID was refined through energy minimization, followed by grid box generation around the MH2 functional domain. Docking was performed using AutoDock Vina, and ligand interactions were analyzed through hydrogen bonding, hydrophobic mapping, and binding energy scoring. Among the screened ligands, Compound S15 exhibited the highest binding affinity (−9.4 kcal/mol), forming stable hydrogen bonds with Ser360, Tyr226, and Pro270. Other compounds showed moderate binding profiles ranging from 6.1 to 8.2 kcal/mol. The interaction analysis indicates that competitive inhibition of the MH2 domain may prevent downstream Smad3 phosphorylation, dimerization, and nuclear translocation. Overall, this study provides computational evidence supporting the development of potent Smad3 inhibitors and offers structural rationale for future in vitro and in vivo studies.

Keywords: Smad3, TGF-β signaling, Molecular docking, In silico drug discovery, Protein ligand interaction.

INTRODUCTION

The transforming growth factor-β (TGF-β) pathway regulates cellular proliferation, differentiation, apoptosis, and extracellular matrix (ECM) deposition. Dysregulation of this pathway is strongly associated with fibrosis, tumor invasiveness, epithelial–mesenchymal transition (EMT), and chronic inflammatory disorders. Smad3, a receptor-regulated Smad (R-Smad), is one of the principal downstream mediators that becomes phosphorylated upon TGF-β receptor activation and translocates to the nucleus to regulate gene expression. Because of its central role in pathological ECM accumulation and tissue remodeling, Smad3 represents a promising therapeutic target in pulmonary fibrosis, hepatic fibrosis, renal fibrosis, and cancer metastasis. Small-molecule inhibitors capable of modulating Smad3 activity have gained increasing interest in the past decade. However, experimental screening remains time-consuming and costly, underscoring the importance of computational approaches such as molecular docking to predict binding mechanisms and identify potential inhibitors. This study employs structure-based molecular docking to decode the interactions between Smad3 and a library of bioactive compounds. The goal is to analyze protein–ligand binding mechanisms, pinpoint essential residues, and evaluate candidate molecules for future experimental validation.

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Transforming growth factor-β (TGF-β) signaling is a central regulator of diverse cellular functions, including proliferation, differentiation, immune modulation, and extracellular matrix deposition. Dysregulation of this pathway is strongly associated with cancer progression, fibrosis, and chronic inflammatory diseases. TGF-β ligands initiate signaling through type I and type II serine/threonine kinase receptors, which activate intracellular Smad proteins key transcriptional regulators in the canonical pathway (Heldin & Moustakas, 2016). The canonical signaling cascade depends primarily on receptor-regulated Smads (R-Smads), particularly Smad2 and Smad3, which form complexes with Smad4 to regulate gene transcription (Massagué, 2012). Both Smad-dependent and Smad-independent pathways contribute to the overall signaling output, enabling TGF-β to play context-specific roles in health and disease (Derynck & Zhang, 2003).

Among the R-Smads**,** Smad3 is particularly important because of its direct DNA-binding ability and its involvement in fibrosis, tumor metastasis, and immune suppression. Extensive research has demonstrated that aberrant Smad3 activation drives pathological fibrotic responses in multiple organs, positioning it as a therapeutic target (Meng *et al*., 2016). Recent studies highlight the regulatory complexity of Smad3, including transcriptional feedback, post-translational modifications, and interactions with long non-coding RNAs, which further fine-tune its activity (Xu *et al*., 2012; Tang *et al*., 2018). Additionally, structural studies have elucidated the mechanism of Smad3–Smad4 interaction, providing template information for inhibitor design (Wu *et al*., 2002). Given the prominent role of Smad3 in fibrosis and cancer, targeting the TGF-β/Smad axis has emerged as a promising therapeutic strategy.

Several investigations have explored small-molecule inhibitors that disrupt Smad3 phosphorylation, nuclear translocation, or transcriptional activity (Huang *et al*., 2020). Computational approaches, especially molecular docking and molecular dynamics simulations, have accelerated the discovery of these inhibitors by elucidating Smad3 conformational states and assessing ligand-binding mechanisms (Fang *et al*., 2014; Qi *et al*., 2018). These in silico studies also highlight Smad proteins' potential druggability despite being transcription factors, traditionally considered difficult to target (Zhao & Li, 2019). Molecular docking remains one of the most widely used approaches for predicting ligand–protein interactions because of its ability to estimate binding affinities and pose orientations (Morris & Lim-Wilby, 2008). Tools such as AutoDock Vina have further improved the speed and accuracy of docking calculations and have been widely adopted in structure-based drug discovery (Trott & Olson, 2010). Databases like PubChem facilitate rapid retrieval of diverse chemical scaffolds that can be evaluated as potential Smad3 inhibitors (Kim *et al*., 2016). Recent computational efforts have designed transcription-factor-targeting ligands with improved selectivity, showcasing the growing feasibility of Smad-directed drug design (Fernández & Caballero, 2020).

MATERIALS AND METHODS

The three-dimensional crystal structure of the Smad3 MH2 domain (PDB ID: 1MK2) was retrieved from the Protein Data Bank and prepared using UCSF Chimera by removing water molecules, heteroatoms, and crystallographic ligands. Polar hydrogens were added, and the structure was energy-minimized using the AMBER ff14SB force field. A library of 25 natural and synthetic compounds reported for anti-fibrotic or anti-inflammatory activity was compiled from PubChem and optimized through energy minimization with the MMFF94 force field, file conversion using OpenBabel, and protonation at physiological pH (7.4). The active site of the MH2 domain was identified through CASTp 3.0 pocket analysis and literature-reported inhibitory hotspots, with the docking grid centered around the catalytic pocket coordinates. Molecular docking was performed using AutoDock Vina with a grid dimension of 40 × 40 × 40 Å, exhaustiveness of 8, and the Vina empirical scoring function. The best-binding pose for each ligand was selected and analyzed using Discovery Studio Visualizer, PLIP interaction profiler, and PyMOL, assessing hydrogen bonds, hydrophobic contacts, π-π stacking, and salt bridges. Docking results revealed binding energies ranging from −6.1 to −9.4 kcal/mol, with top inhibitors such as S15 showing three hydrogen bonds with key residues Ser360, Tyr226, and Pro270, indicating strong affinity and potential inhibitory activity. Other important interacting residues included Lys378, stabilizing the ligand electrostatically, and Phe263 and Tyr203, contributing through π-π interactions. Mechanistically, ligand binding to the MH2 domain is predicted to block phosphorylation, disrupt Smad3–Smad4 dimerization, and prevent nuclear translocation, thereby inhibiting TGF-β-dependent gene transcription and reducing extracellular matrix accumulation. These findings suggest that high-affinity compounds like S15 could serve as promising anti-fibrotic or anti-cancer therapeutic leads.

Table 1. Docking scores of top Smad3 inhibitors.

Compound Code	Binding Energy (kcal/mol)	H-bonds	Key Residues
S15	−9.4	3	Ser360, Tyr226, Pro270
S11	−8.7	2	Lys378, Val219
S07	−8.3	2	Gln242, Phe263
S04	−7.9	1	Tyr203
S01	−7.2	1	Thr274

RESULTS AND DISCUSSION

Binding energy scores ranged between 6.1 and 9.4 kcal/mol. The top inhibitors demonstrated favorable interactions with the MH2 domain, suggesting strong affinity and potential inhibitory activity. Analysis of the docked complexes identified several key interacting residues within the Smad3 MH2 domain, including Ser360, Tyr226, and Pro270, which are critical for MH2 activity; Lys378, which stabilizes the ligand through electrostatic interactions; and Phe263 and Tyr203, which contribute via π-π stacking. These residues are integral to receptor-activated phosphorylation pathways, representing major inhibitory hotspots. Mechanistically, ligand binding to the MH2 domain is predicted to block phosphorylation, disrupt Smad3 dimerization with Smad4, and prevent nuclear translocation, thereby inhibiting transcription of TGF-β-dependent genes. Consequently, this interference reduces extracellular matrix accumulation and may slow fibrotic progression. High-affinity compounds, such as S15, therefore emerge as promising candidates for anti-fibrotic or anti-cancer therapeutic development. The broader biological implications of modulating Smad3 signaling are also evident from cancer studies, where TGF-β exerts dual tumor-suppressive and tumor-promoting roles depending on disease stage (Ono & Gelmann, 2000). Post-translational modifications, including phosphorylation by various kinases, further modulate Smad3 activity, linking it to therapeutic resistance (Wrighton *et al*., 2009; Rodríguez-García *et al*., 2011). Systems biology studies reinforce the importance of understanding Smad3 regulatory networks to design selective and effective inhibitors (Sánchez-Sánchez & Hernández-Lemus, 2021). Collectively, these findings highlight the critical need for structural and computational investigations into Smad3, providing a foundation for the development of targeted therapeutics.

CONCLUSION

This study provides mechanistic insights into Smad3 inhibition using molecular docking techniques. Among the screened compounds, S15 exhibited the strongest affinity for the MH2 domain, forming stable interactions with critical regulatory residues. These findings highlight the significance of structure-based drug discovery in designing selective Smad3 inhibitors. Further molecular dynamics simulations, ADMET analysis, and experimental validation are recommended to confirm inhibitory potential and assess therapeutic applicability.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Madras University for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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