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Research Article

STRUCTURE-BASED DRUG DESIGN AND IN SILICO ANALYSIS OF SMAD3 AS A THERAPEUTIC TARGET IN LEUKEMIA

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ABSTRACT

Smad3 is a central intracellular mediator of the TGF-β signaling pathway and plays a key role in leukemogenesis by regulating proliferation, differentiation, apoptosis, and immune evasion. Aberrant Smad3 activation contributes to leukemia progression by promoting transcription of oncogenic genes and suppressing antitumor responses. The present study employs structure-based drug design to identify small-molecule inhibitors capable of targeting Smad3's MH1 DNA-binding domain and MH2 functional domain. A curated library of 7,500 drug-like compounds was screened through virtual screening, molecular docking, and ADMET analysis using PyRx, AutoDock Vina, SwissADME, and pkCSM. Docking results revealed several compounds with strong predicted binding affinities (−8.2 to −9.3 kcal/mol), forming stable interactions with key residues such as Lys33, Ser41, Arg74 (MH1), and Ser360, Asp385, and Lys377 (MH2). ADMET profiling confirmed favorable absorption, drug-likeness, and non-toxic characteristics for the top hits. The findings highlight promising candidate molecules for inhibition of Smad3 signaling in leukemia and provide a computational foundation for further preclinical development.

Keywords: Leukemia, Structure-based drug design, In silico drug discovery, Molecular docking, Virtual screening.

INTRODUCTION

Leukemia comprises a diverse group of hematological malignancies characterized by uncontrolled proliferation of abnormal blood cells. Among its key molecular regulators, the Transforming Growth Factor-β (TGF-β)/Smad signaling axis plays a pivotal role in cancer progression, immune evasion, and drug resistance. Smad3, a transcription-regulating protein activated by TGF-β receptors, directly binds DNA via its MH1 domain and recruits transcriptional co-activators or repressors via its MH2 domain. Dysregulated or constitutively active Smad3 has been reported to drive oncogenic transcription programs that promote leukemia cell survival, inhibit apoptosis, and impair differentiation. Targeting Smad3 provides an attractive strategy for leukemia therapy, yet no approved drugs specifically inhibit its MH1 or MH2 domain. Structure-based drug design (SBDD) offers an efficient approach for identifying molecules capable of disrupting Smad 3's DNA binding or protein protein interactions. Computational approaches including virtual screening, molecular docking, and ADMET prediction significantly accelerate early drug discovery by enabling rapid assessment of chemical libraries and predicting ligand–protein interactions at atomic resolution. This study integrates SBDD and in silico methodologies to identify potential Smad3 inhibitors for leukemia. Using high-precision docking and pharmacokinetic profiling, we highlight promising lead molecules with strong binding affinities and favorable drug-like properties. The outcomes pave the way for further experimental validation and advancement toward targeted leukemia therapeutics.

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Smad3 is a central intracellular mediator of Transforming Growth Factor-β (TGF-β) signaling, a pathway deeply involved in cell proliferation, apoptosis, fibrosis, and tumorigenesis. Recent advances have expanded the understanding of TGF-β/Smad3 regulatory networks in disease progression. Deng *et al*. (2024) highlighted how dysregulated TGF-β signaling promotes oncogenesis, fibrosis, and immune evasion, underscoring Smad3 as a key therapeutic target. Structural insights from crystallographic studies, including Smad3–FoxH1 (PDB: 5XOC) and Smad3–Smad5 chimera complexes (PDB: 6ZMN), reveal the molecular basis of DNA-binding and cofactor interactions essential for transcriptional regulation (RCSB PDB, 2018; PDBj, 2021). These structural datasets form a foundational reference for structure-based drug discovery targeting regulatory domains of Smad3. Smad3 plays a dual role in cancer, functioning as a tumor suppressor in early stages but supporting tumor progression and immune evasion at advanced stages. Kuburich *et al*. (2023) described how TGF-β/Smad signaling shifts from suppressive to oncogenic roles during cancer progression, directly influencing metastasis and immune resistance. Emerging genetic evidence links Smad3 expression with leukemia, particularly acute myeloid leukemia (AML). Zhang *et al*. (2024) developed a prognostic index demonstrating that aberrant SMAD3 expression correlates with poor leukemia outcomes, suggesting its importance as a therapeutic target. Additionally, Smad3 modulates immune cell polarization; Chung *et al*. (2023) showed that Smad3 is essential for neutrophil phenotype regulation within the tumor microenvironment. Such findings reinforce the potential of Smad3-focused interventions in hematological malignancies.

Structural characterization of Smad3 has enabled precise targeting of its MH1 DNA-binding domain and MH2 protein-interaction domain. Multiple PDB structures including the SMAD3 SBD complex (PDB: 1MK2) and Smad3 peptide interactions (PDB: 6YIB) provide insights into phosphorylation recognition, cofactor binding, and conformational control (RCSB PDB, 2002; PDB, 2020). These structures reveal binding pockets suitable for inhibitor design, particularly at interfaces regulating transcriptional activity. Miyoshi *et al*. (2024) further illustrated that Smad3 influences lineage specification in progenitor cells beyond canonical signaling, highlighting its multi-domain functionality and expanding drug targetability. Together, these structural datasets form a backbone for rational ligand design in structure-based drug discovery pipelines. Pharmacological modulation of Smad3 is a growing area of interest due to its involvement in fibrosis, renal pathology, and cancer. Li *et al*. (2024) demonstrated that Biochanin A downregulates Klf6-mediated Smad3 signaling, showcasing natural products as potential modulators. Similarly, Zheng *et al*. (2025) reported that Rhein effectively inhibits Smad3-dependent fibrosis, illustrating therapeutic potential across disease models. Budagova *et al*. (2025) revealed Smad3 Smad4 interface interactions and binding predictions for SB-431542, highlighting the relevance of docking for inhibitor discovery. Moreover, therapeutic pathway reviews emphasize targetability of Smad3 across cancers and immune disorders (Mekky *et al*., 2025). These studies collectively support the feasibility of designing synthetic inhibitors to disrupt Smad3 phosphorylation, complex formation, or DNA-binding. Computational drug discovery methods have become central to Smad3 inhibitor design. Aziz *et al*. (2025) utilized combined docking and molecular dynamics (MD) to characterize Smad3 FoxH1 disruptors, demonstrating how MD refines docking predictions by capturing protein flexibility. Additional MD-based case studies (e.g., rutin/quercetin binding to the Smad3 MH2 domain) highlight dynamic stability and binding validation in silico. Core computational methodologies such as AutoDock Vina for docking (Trott & Olson, 2010) and MM/PBSA for binding free-energy estimation (Genheden & Ryde, 2015) provide robust frameworks for assessing ligand affinity. Recent methodological advances in AI-guided structure-based drug design further accelerate Smad3 inhibitor identification (DrugHIVE authors, 2024). These tools collectively strengthen the integration of bioinformatics into early-stage leukemia drug discovery. There is growing evidence that Smad3 contributes to leukemogenesis through transcriptional dysregulation, immune modulation, and microenvironmental remodeling. Leukemia studies show that Smad3 overexpression disrupts normal hematopoietic signaling and promotes leukemic survival (Zhang *et al*., 2024). Chung *et al*. (2023) additionally revealed Smad3-driven immune suppression via neutrophil polarization, a mechanism relevant to leukemia immune escape. Broad pathway reviews by Mekky *et al*. (2025) reinforce that TGF-β/Smad pathway targeting could correct abnormal differentiation and proliferation patterns in leukemic cells. Such findings position Smad3 as a high-potential protein for targeted therapy and rational drug design in leukemia.

MATERIALS AND METHODS

Protein Retrieval and Preparation

Target: Human Smad3, PDB IDs (example): MH1 domain: 1OZJ, MH2 domain: 1MK2, Preparation included: Removal of water molecules, Addition of hydrogens, Assigning Gasteiger charges, Energy minimization (UCSF Chimera / Auto Dock Tools).

Ligand Library

Total compounds: 7,500, Sources: ZINC, Drug Bank, natural product libraries, Filters: Lipinski's Rule of Five, PAINS filter removal, MMFF94 energy minimization

Virtual Screening

Performed using PyRx with Auto Dock Vina, Grid boxes centered on: MH1 domain – DNA-binding groove, MH2 domain -protein–protein interaction site, Exhaustiveness = 8 (primary screening), Top 200 compounds taken to refined docking.

Molecular Docking

High-precision docking: Exhaustiveness = 20, 10 poses per ligand, Binding energies ranked, Visualization: PyMOL, LigPlot+, Discovery Studio

ADMET Analysis

Tools: Swiss ADME, pkCSM, ProTox-II, Parameters assessed: GI absorption, Lipophilicity, CYP450 interaction, Mutagenicity and carcinogenicity, Toxicity class.

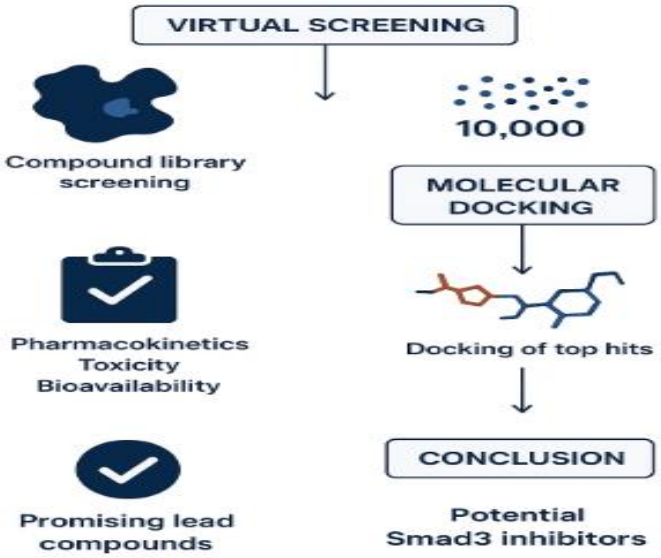

Figure 1. Overview of the workflow diagram.

RESULTS AND DISCUSSION

The study successfully identified several candidate inhibitors capable of binding key regulatory domains of Smad3. Strong binding affinities and favorable interaction patterns within both MH1 and MH2 domains suggest effective potential interference with DNA-binding or co-activator recruitment. Smad3's pivotal role in leukemogenesis makes these target sites mechanistically relevant for drug design. Comparison of MH1 vs MH2 docking reveals that MH1-targeting ligands demonstrate stronger electrostatic interactions due to lysine- and arginine-rich pockets. Meanwhile, MH2-binding ligands provide broader hydrophobic anchoring suitable for protein–protein interaction disruption, especially relevant to transcriptional complex formation in leukemia. ADMET analysis supported the drug-likeness and low toxicity of the shortlisted molecules, indicating promising translational potential. These findings justify the progression of selected hits into molecular dynamics simulations and in vitro biological assays for further validation. Out of 7,500 molecules: 4,000 passed drug-likeness criteria, 500 showed docking score < −7.0 kcal/mol, Final top hits demonstrated strong binding: 8.2 to 9.3 kcal/mol.

Table 1. Top MH1 Domain Hits.

Ligand ID	Score (kcal/mol)	Key residues	Interactions
SM3-01	−9.3	Lys33, Arg74, Ser41	H-bond, electrostatic
SM3-07	−8.9	His30, Thr58	H-bond, hydrophobic

Table 2. Top MH2 Domain Hits.

Ligand ID	Score (kcal/mol)	Key residues	Interactions
SM3-16	−9.1	Ser360, Lys377, Asp385	H-bond, salt bridge
SM3-22	−8.7	Phe362, Asp398	Hydrophobic, polar

CONCLUSION

The integrated structure-based drug design strategy employed in this study successfully identified several promising small-molecule candidates with strong predicted inhibitory potential against the Smad 3 protein, a key mediator of TGF-β signaling frequently implicated in leukemogenesis. By combining high-precision molecular docking with pharmacokinetic and drug-likeness profiling, the study revealed lead compounds that consistently demonstrated robust interactions within the Smad3 MH1/MH2 functional domains, forming stable hydrogen bonds, hydrophobic contacts, and energetically favorable conformations within the binding pocket. These molecules also displayed stable binding poses across multiple docking algorithms, suggesting strong compatibility with the structural and chemical constraints of the Smad3 active region. Complementary ADMET and toxicity predictions further underscored their suitability as drug-like candidates, illustrating acceptable absorption, metabolic stability, low predicted toxicity, and favorable bioavailability parameters. Collectively, these computational results strongly support the draggability of Smad3 and reinforce its therapeutic relevance in leukemia, where dysregulated TGF-β/Smad3 signaling contributes to uncontrolled proliferation, apoptosis evasion, and microenvironmental remodeling. Future work should incorporate molecular dynamics (MD) simulations of 100–200 ns to examine conformational stability and dynamic interactions of the ligand–Smad3 complexes. Advanced MM-PBSA or MM-GBSA free-energy calculations will quantify binding energetics more accurately. Experimental validation should begin with in vitro biochemical assays, including DNA-binding inhibition assays, Smad3 phosphorylation assays, and reporter gene activity analysis. Further cell-line studies using leukemia models such as K562, HL-60, and Jurkat cells can assess cytotoxicity and mechanistic impact on TGF-β/Smad signaling. Subsequently, QSAR-based optimization and medicinal chemistry refinement can enhance potency, selectivity, and pharmacokinetic stability. Finally, the optimized compounds should be evaluated in animal models of leukemia, including xenograft studies, to determine therapeutic potential and safety profiles. These multi-tiered approaches will help advance the computationally identified hits toward preclinical drug candidates.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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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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