Farabi Tıp Dergisi 4(3):63-72,2025
Farabi Medical Journal 4(3):63-72,2025
https://doi.org/10.59518/farabimedj.1745493

FARABİ TIP DERGİSİ
FARABI MEDICAL JOURNAL

Özgün Makale
Original Article

https://dergipark.org.tr/tr/pub/farabimedj

Computational Investigation of the Interaction of Large-scale Plant and Animal-Derived Natural Secondary Metabolites with FOXM1

Geniş Ölçekteki Bitki ve Hayvan Kökenli Doğal İkincil Metabolitlerin FOXM1 ile Etkileşiminin Hesaplamalı Olarak Araştırılması

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ABSTRACT

FOXM1, a transcription factor from the Forkhead box family, serves as a crucial proto-oncogene that plays a role in cancer advancement, spread to distant sites, and resistance to chemotherapy across various human malignancies. The development of selective and therapeutically efficient FOXM1 inhibitors remains a significant challenge in the field. This study employed a multi-step computational approach to identify novel small-molecule compounds that target the DNA-binding domain (DBD) of FOXM1. A structure-guided virtual screening process was conducted using an extensive chemical compound database, evaluated against the crystallographic structure of FOXM1's DNA-binding domain (PDB ID: 3G73). The top-ranking compounds underwent preliminary 10-nanosecond evaluations, subsequently followed by comprehensive 100-nanosecond molecular dynamics (MD) simulations. The binding affinities of the most thermodynamically stable protein-ligand complexes were determined through MM/GBSA calculations. The preliminary computational screening revealed 21 compounds that exhibited docking scores superior to -9.1 kcal/mol. Following 10 ns MD simulations, five compounds were selected, and 100 ns MD simulations confirmed the stable binding of these two compounds (comp_105546, comp_112458). MM/GBSA calculations identified comp_112458 as the most potent binder (-36.25±3.5 kcal/mol). This study successfully identified novel chemical scaffolds with high predicted affinity and stable binding modes against FOXM1, providing a strong foundation for the development of targeted anticancer agents. These promising computational results require validation through *in vitro* and *in vivo* studies.

Keywords: Cancer therapy, FOXM1, MM/GBSA, Molecular dynamics, Virtual screening

ÖZET

FOXM1, Forkhead box ailesinden bir transkripsiyon faktörü olup, çeşitli insan malignitelerde kanserin ilerlemesi, uzak bölgelere yayılması ve kemoterapiye karşı direnç gelişmesinde rol oynayan kritik bir proto-onkogendir. Seçici ve terapötik olarak etkili FOXM1 inhibitörlerinin geliştirilmesi bu alanda önemli bir zorluk olmaya devam etmektedir. Bu çalışma, FOXM1'in DNA-bağlama alanını (DBD) hedefleyen yeni küçük molekül bileşiklerini belirlemek için çok aşamalı bir hesaplamalı yaklaşım kullanmıştır. FOXM1'in DNA-bağlama alanının kristalografik yapısına (PDB ID: 3G73) karşı geniş bir kimyasal bileşik veritabanı kullanılarak yapı-güdümlü sanal tarama işlemi gerçekleştirilmiştir. En yüksek sıralamaya sahip bileşikler, öncelikle 10-nanosaniye değerlendirmelerinden geçirilmiş, ardından kapsamlı 100-nanosaniye moleküler dinamik (MD) simülasyonları ile takip edilmiştir. Termodinamik olarak en kararlı protein-ligand komplekslerinin bağlanma afiniteleri MM/GBSA hesaplamaları ile belirlemiştir. Ön hesaplamalı tarama, -9.1 kcal/mol'den daha iyi docking skorları gösteren 21 bileşik ortaya çıkarmıştır. 10 ns MD simülasyonları sonrasında beş bileşik seçilmiş ve 100 ns MD simülasyonları bu iki bileşiğin (comp_105546, comp_112458) kararlı bağlanmasını doğrulamıştır. MM/GBSA hesaplamaları comp_112458'i en güçlü bağlayıcı (-36.25±3.5 kcal/mol) olarak belirlemiştir. Bu çalışma, FOXM1'e karşı yüksek öngörülen afinite ve kararlı bağlanma modları olan yeni kimyasal iskeletleri başarıyla tanımlamış ve hedefli antikanser ajanlarının geliştirilmesi için güçlü bir temel sağlamıştır. Bu umut verici hesaplamalı sonuçların *in vitro* ve *in vivo* çalışmalarla doğrulanması gerekmektedir.

Anahtar kelimeler: FOXM1, Kanser tedavisi, MM/GBSA, Moleküler dinamik, Sanal tarama

Geliş Tarihi/Received Date: *18.07.2025* | *Kabul Tarihi/Accepted Date:* *19.08.2025*

INTRODUCTION

The Forkhead box (FOX) family of transcription factors represents a large family of evolutionarily conserved proteins which control various cellular functions such as cell division cycles, cellular specialization, programmed cell death, and metabolic processes. Among the FOX family members, Forkhead box M1 (FOXM1) is currently acknowledged as an essential cancer-promoting gene. FOXM1 represents a vital growth-related protein that is abnormally elevated in most human cancers and controls various cancer cell behaviors, such as proliferation, spreading, relapse, and stem cell characteristics.

Under normal physiological conditions, FOXM1 expression is tightly regulated and restricted to proliferating cells, with maximum levels occur during the S and G2/M stages of cell division. Nevertheless, FOXM1 shows abnormal elevation across numerous types of human cancers and, functioning as a gene regulator, it controls various target genes whose disruption contributes to virtually all cancer characteristics.[1] A strong link exists between this dysregulated expression and aggressive tumor characteristics, such as increased proliferation, invasion, metastasis, angiogenesis, and resistance to both chemotherapy and radiotherapy.

Recent studies have further elucidated FOXM1's role in cancer biology and therapeutic resistance. Chemoresistance to various anti-cancer drugs is linked to FOXM1 expression; additionally, multiple studies have verified that inhibiting FOXM1 improves the drug sensitivity of different cancer cell types.[1] Additionally, FOXM1 is frequently found at elevated levels in human malignancies and is closely linked to treatment resistance and reduced patient survival rates, particularly affecting chemotherapy effectiveness for patients with the most aggressive forms.[2]

The development of FOXM1 inhibitors has been an active area of research, with several approaches being pursued. Recent evidence indicates that combining small molecular compounds that block FOXM1 with existing cancer treatments could offer a new treatment approach for chemotherapy-resistant cancers.[1] Recent advances have led to the identification of several promising compounds, including STL001, an initial-generation modified compound that enhances human cancer susceptibility to various cancer treatments, along with NB compounds[2], which demonstrate strong and effective FOXM1 blocking activity in aggressive serous ovarian cancer cells.[1]

Even with these advancements, developing effective FOXM1 inhibitors still faces considerable hurdles. FOXM1-targeting small molecular compounds encounter numerous obstacles, and existing research shows these inhibitors at various developmental phases, requiring examination of present difficulties and future clinical implementation strategies for FOXM1 inhibition.[1] Importantly, while several small-molecule FOXM1 inhibitors have been tested in laboratory settings, none have reached clinical trials, underscoring the pressing need for innovative chemical structures that possess better drug-like qualities and greater therapeutic promise.[3] Computational drug discovery approaches have emerged as powerful tools for identifying novel inhibitors and overcoming traditional drug development challenges. During the initial phases of pharmaceutical development, structure-guided virtual screening functions as an essential methodology.[1] Recent technological advances have significantly enhanced these capabilities, with recently available open-source AI-enhanced virtual screening systems for pharmaceutical research are now accessible.[1,4]

The incorporation of AI and machine learning has dramatically transformed computer-based drug discovery processes. Virtual screening represents a fundamental and continuously developing component of drug discovery methodology, where artificial intelligence plays a broad role in pharmaceutical research to minimize time and resource usage, and when combined with machine learning techniques, VS has evolved into an innovative technology that operates through sophisticated decision-making processes for data organization and compound identification from extensive virtual libraries within minutes or hours.[5] These advances are particularly important considering the major transformation toward adopting computational methods in both academic and pharmaceutical sectors, characterized primarily by the overwhelming amount of information regarding molecular properties and their interactions with therapeutic targets and their three-dimensional configurations, extensive computational resources, and the emergence of accessible virtual collections containing billions of drug-like small compounds.[6]

Recent computational studies have demonstrated the feasibility of identifying novel FOXM1 inhibitors

through *in silico* approaches. Studies have successfully focused on discovering possible FOXM1 blocking compounds through computer-based analysis of drug databases, utilizing pharmacophore modeling with the FOXM1 inhibitor FDI-6, and subsequently conducting virtual screening of DrugBank and Selleckchem repositories.[7] These studies validate the potential of computational approaches for FOXM1 inhibitor discovery and demonstrate the successful application of integrated computational methodologies.

This research utilizes a thorough computational workflow that integrates structure-based virtual screening, all-atom molecular dynamic molecular modeling and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA)-based binding energy computations. Our objective is to discover potent lead compounds with stable binding modes and favorable drug-like properties that could serve as starting points for the development of next-generation FOXM1-targeted cancer therapeutics.

METHODS

Ethical approval

This study does not require ethical approval as it is a computational study.

Protein structure preparation

Coordinates for the 3D configuration of the human FOXM1 DNA-binding region were procured from the RCSB Protein Data Bank (PDB ID: 3G73).[8] This crystal structure, resolved at 2.21 Å, features the FOXM1 DBD in complex with a DNA duplex. For ligand docking purposes, the protein structure was prepared using standard protocols. All non-protein entities including DNA chains, water molecules, and co-solvents were removed. Hydrogenation was performed and protonation states of ionizable amino acids were assigned assuming physiological pH 7.4.[9]

Ligand library and virtual screening

A diverse library of 104,525 small molecules was prepared for virtual screening, with all compound structures sourced from the Human Metabolome Database (HMDB).[10] The ligands were extensively refined using Open Babel[11] before the docking process. This refinement workflow involved generating 3D coordinates from the initial 2D structures, adding hydrogen atoms assuming a physiological pH of 7.4, and then performing energy minimization with the MMFF94 force field to obtain a stable, low-energy initial conformation for each ligand.

Following this preparation, the Amber ff94 force field[12] was employed to assign atomic partial charges to both the protein receptor and the refined ligands, a critical step for an accurate representation of electrostatic interactions. Structure-based virtual screening on the prepared library was then conducted using the Qvina software.[13] The search space was defined by a grid box of 20×20×20 Å, centered on the known inhibitor binding site within the FOXM1 DBD. The docking algorithm in Qvina evaluated multiple binding poses for each of the 104,525 ligands, and these poses were subsequently ranked based on the software's empirical scoring function to identify potential inhibitors.

Molecular dynamics simulations

A multi-stage simulation protocol was used to screen and analyze promising compounds. Initially, the top 21 compounds selected from docking (with binding scores of -8.0 kcal/mol or better) were subjected to a short 10 nanosecond molecular dynamics simulation. The binding free energy of each of these 21 complexes was then calculated from its trajectory using MM/GBSA method. Based on these energy values, compounds exhibiting the most favorable binding were selected for extended analysis. Top five compounds (comp_105546, comp_11184, comp_11309, comp_18920, comp_112458), along with the reference inhibitor FDI-6, were submitted to 100-ns production MD simulations for a thorough assessment of their dynamic behavior and stability.

The GROMACS 2020 software was utilized for conducting all simulations.[14] The protein complexes were modeled using the Amber99SB-ILDN force field.[15] Each system was positioned in an orthorhombic box, hydrated with TIP3P water molecules[16] and neutralized with counterions, then sodium and chloride ions were added to reach 150 mM concentration. The hydrated system was energy-minimized using steepest descent, followed by NVT equilibration (100 ps) and NPT equilibration (1000 ps) before the final molecular dynamics simulation.

Post-MD trajectory analysis

Complex stability was assessed through analysis of 100 ns molecular dynamics trajectories. RMSD values for protein backbone and ligand heavy atoms were determined relative to starting positions. Radius of gyration was calculated to evaluate protein compactness and structural integrity. RMSF values for individual residues were computed to identify flexible areas.

MM/GBSA binding energy computation

Binding free energies for stable protein-ligand complexes were computed using the MM/GBSA approach implemented in gmx_mmpbsa tool.[17] MM/GBSA models provide rapid and cost-effective ligand binding energy prediction methods, with calculations being about 5 times faster than MM-PBSA. Computations were conducted on snapshots from the final 10 ns of molecular dynamics trajectories.

RESULTS and DISCUSSION

Virtual screening identifies high-affinity candidate inhibitors

Structure-guided virtual screening was performed to discover potential FOXM1 blocking compounds from an extensive chemical database. The docking results yielded compounds with favorable predicted binding energies. The top 21 compounds exhibited docking scores ranging from -9.1 to -8.0 kcal/mol, indicating strong theoretical affinity for the target binding site (Figure 1A). The overall distribution of docking scores was skewed, with a large peak of compounds scoring around -2 kcal/mol, and a long tail extending towards higher affinity scores (Figure 1B). The selected hits represent the high-affinity tail of this distribution, suggesting statistical significance.

A

No	Ligand Name	Score
1	comp_19627	-9.1
2	comp_13039	-9
3	comp_20481	-8.8
4	comp_112458	-8.6
5	comp_2821	-8.4
6	comp_11671	-8.4
7	comp_11744	-8.4
8	comp_15258	-8.3
9	comp_18303	-8.2
10	comp_9773	-8.1
11	comp_11964	-8.1
12	comp_21159	-8.1
13	comp_22007	-8.1
14	comp_105546	-8.1
15	comp_1779	-8
16	comp_2857	-8
17	comp_11184	-8
18	comp_11309	-8
19	comp_18920	-8
20	comp_112423	-8
21	comp_112430	-8

B

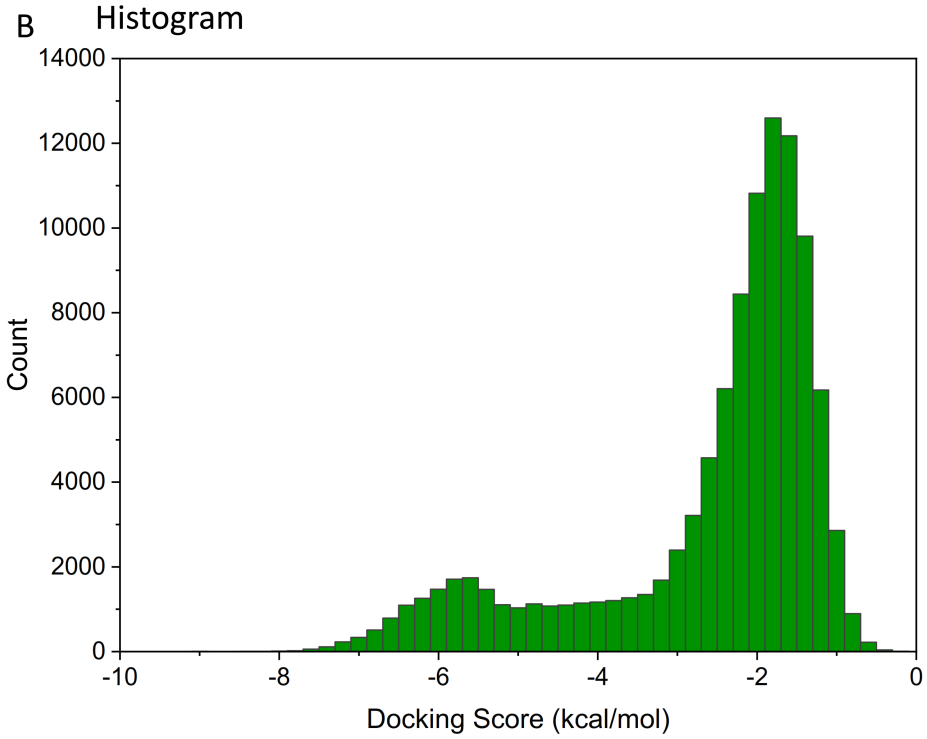


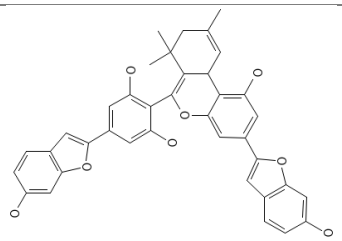
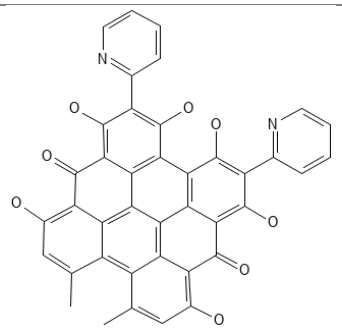
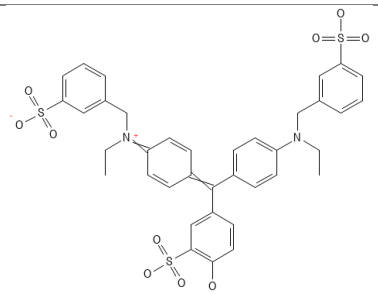
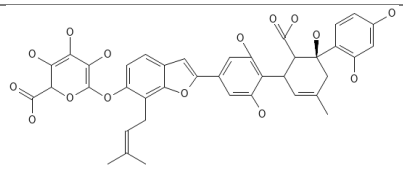
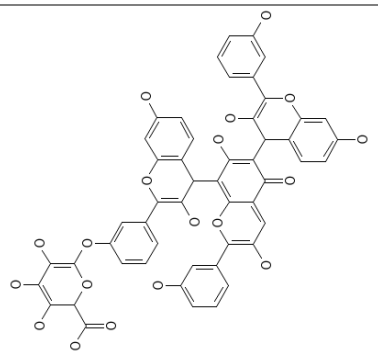
Figure 1. Virtual screening results

(A) Table showing the top 21 compounds identified from virtual screening, ranked by their docking score (kcal/mol), (B) Histogram illustrating the distribution of docking scores across the entire screened compound library, The x-axis represents the docking score, and the y-axis represents the count of compounds within each score bin

The histogram analysis in Figure 1B demonstrates that the vast majority of screened compounds exhibited moderate to poor binding scores, with only a small fraction achieving the high-affinity scores observed in our selected candidates. This distribution pattern validates the screening methodology and confirms that our top-ranked compounds represent genuine high-affinity outliers rather than computational artifacts.

Following initial screening, five compounds (comp_105546, comp_11184, comp_11309, comp_18920 and comp_112458) were selected for molecular dynamics simulations based on docking scores, structural diversity, and visual inspection of binding poses. The chemical structures and identifiers for these compounds are provided in Table 1.

Table 1. HMDB and SMILE codes of compounds with the best scores and stability

Compound	HMDB code	Smile code	2D Conformation
comp_11184	HMDB0030068	cc1=cc2c(c(oc3=cc(=cc(o)=c23)c2=cc3=c(o2)c=c(o)c=c3)c2=c(o)c=c(c=c2o)c2=cc3=c(o2)c=c(o)c=c3)c(c)(c)c1	
comp_11309	HMDB0030196	cc1=cc(o)=c2c(=o)c3=c(o)c(c4ccccn4)=c(o)c4=c3c3=c2c1=c1c(c)=cc(o)=c2c(=o)c5=c(o)c(c6ccccn6)=c(o)c4=c5c3=c12	
comp_18920	HMDB0038220	ccn(cc1=cc(=cc=c1)s(o)(=o)=o)c1=cc=c(c=c1)c(c1=cc(=c(o)c=c1)s(o)(=o)=o)=c1c=cc(c=c1)=[n+](cc)cc1=cc(=cc=c1)s([o-])(=o)=o	
comp_105546	HMDB0126209	[c]1=c([c]=c(c(=c1o)[c]1[c]=c([c])[c][c@]([c]1c(=o)[o])(c1=c([c]=c([c]=[c]1)o)o)o)o)c1=[c]c2=[c]=c(c(=c2o1)[c][c]=c([c])[c])o[c]1[c]([c]([c]([c](o1)c(=o)[o])o)o)o	
comp_112458	HMDB0135052	[c]1c2c(c(c(c(c2o[c]([c]1o)c1=[c]c(=[c][c]=[c]1)o)[c]1c2=c([c]=c([c]=[c]2)o)o[c]([c]1o)c1=[c][c]=[c]c(=[c]1)o[c]1[c]([c]([c]([c](o1)c(=o)[o])o)o)o)o)[c]1c2=[c][c]=c([c]=c2o[c]([c]1o)c1=[c][c]=[c]c(=[c]1)o)o)o	

This approach is consistent with recent successful virtual screening studies for other cancer targets.[18] The virtual screening campaign successfully identified high-scoring compounds, representing the statistical high-affinity tail of the screening library. The validation of our screening methodology is supported by the skewed distribution of docking scores, which also indicates that our chosen compounds are authentic high-potential candidates and not merely computational artifacts.

Molecular dynamics simulations validate complex stability

To evaluate the dynamic stability of ligand-protein interactions predicted by docking, 100 ns all-atom MD simulations were performed for the five selected compounds and the reference inhibitor FDI-6 in complex with the FOXM1 DBD. The stability of each system was assessed by monitoring RMSD of protein backbone and ligand heavy atoms over the simulation period.

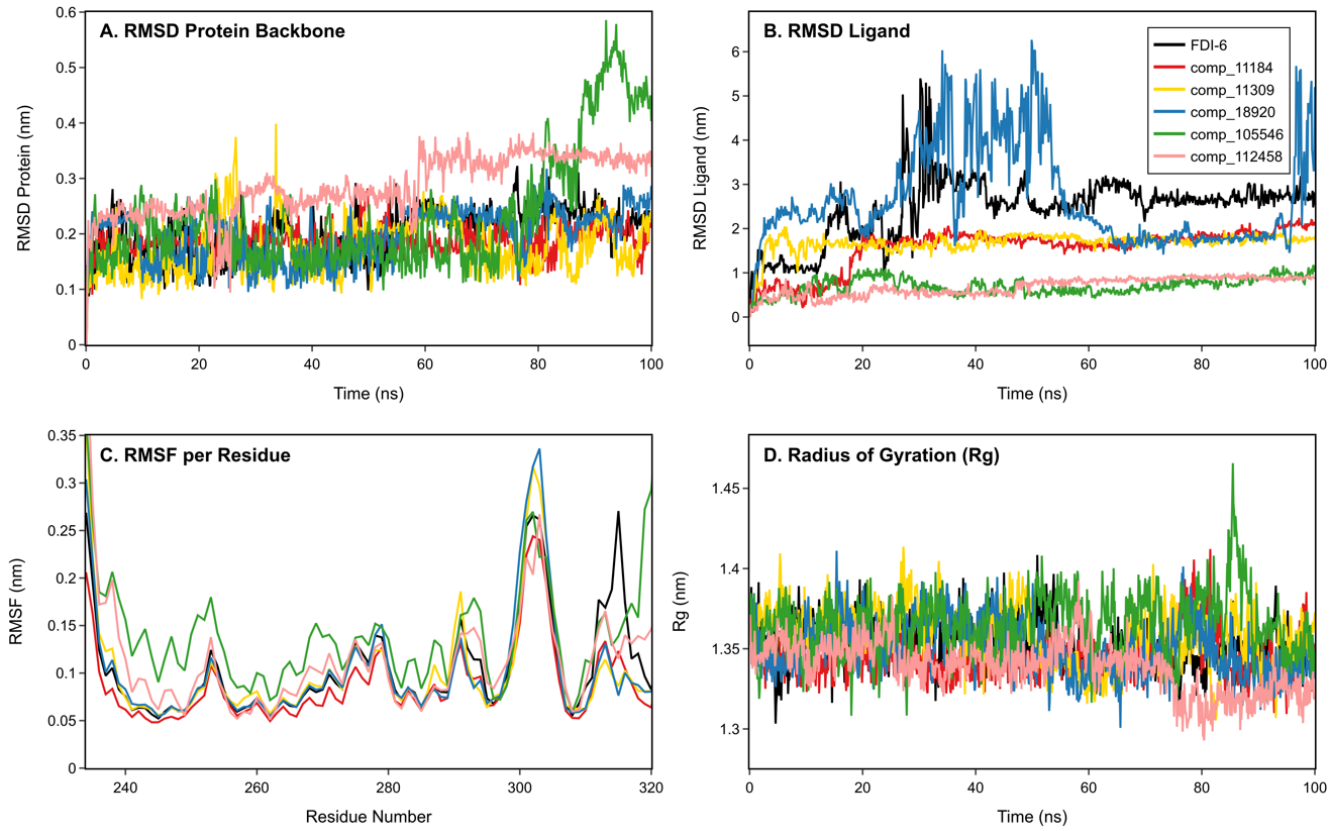


Figure 2. Molecular dynamics simulation analysis of FOXM1-ligand complexes over 100 ns
(A) Protein backbone RMSD trajectories showing equilibration within 20 ns for all complexes, with comp_18920 (blue) displaying the largest deviation (~0.55 nm) and comp_112458 (pink) showing fluctuations exceeding 0.4 nm while maintaining overall protein fold, (B) Ligand heavy atom RMSD demonstrating stable binding for comp_105546 (green) and comp_112458 (pink), contrasting with unstable profiles of FDI-6 (black) and comp_11309 (yellow) indicating dissociation tendency, (C) Per-residue RMSF analysis revealing higher terminal flexibility and critically, low fluctuation values in the binding pocket region (residues 280-310) for stable ligands, indicating effective binding site stabilization, (D) Radius of gyration (Rg) maintaining values around 1.35-1.37 nm throughout simulations, confirming absence of major structural unfolding in all complexes

For the protein backbone RMSD (Figure 2A), all six complexes equilibrated within the first 20 ns. Comp_18920 (blue line) displayed the largest deviation, reaching ≈ 0.55 nm after 80 ns. Comp_112458 (pink line), showed more significant fluctuations that exceeded 0.4 nm towards the end of the simulation, although the overall folded structure of the protein was maintained. Ligand RMSD analysis (Figure 2B) revealed different stability patterns. Comp_105546 (green line) and comp_112458 (pink line) showed stable binding within the active site. In contrast, the reference inhibitor FDI-6 (black line) and comp_11309 (yellow line) displayed an unstable profile, showing a tendency to dissociate from the binding pocket.

The RMSF analysis per residue (Figure 2C) showed that terminal regions exhibited higher flexibility as expected. Importantly, residues constituting the binding pocket (approximately residues 280-310) showed relatively low fluctuation values when bound to stable ligands, suggesting effective binding site stabilization.

Radius of Gyration analysis (Figure 2D) confirmed overall protein structural integrity, with all complexes maintaining Rg values around 1.35-1.37 nm throughout the simulations, indicating no major structural unfolding.

Molecular dynamics simulations provided crucial validation of the static docking predictions. The RMSD analyses revealed that comp_105546 and comp_112458 maintained stable binding throughout the 100 ns simulations. This variation in stability highlights the necessity of dynamic validation within computational drug discovery, since static docking scores by themselves can often be deceptive.[19] The 100 ns simulation length employed in this study is considered sufficient for evaluating protein-ligand complex stability and has been validated in numerous recent studies.[20]

MM/GBSA calculations provide refined binding affinity estimates

While MD simulations confirmed binding stability, MM/GBSA calculations were performed to obtain quantitative binding affinity estimates. The calculations were performed on two different time scales: 10 ns and 100 ns trajectories to assess convergence.

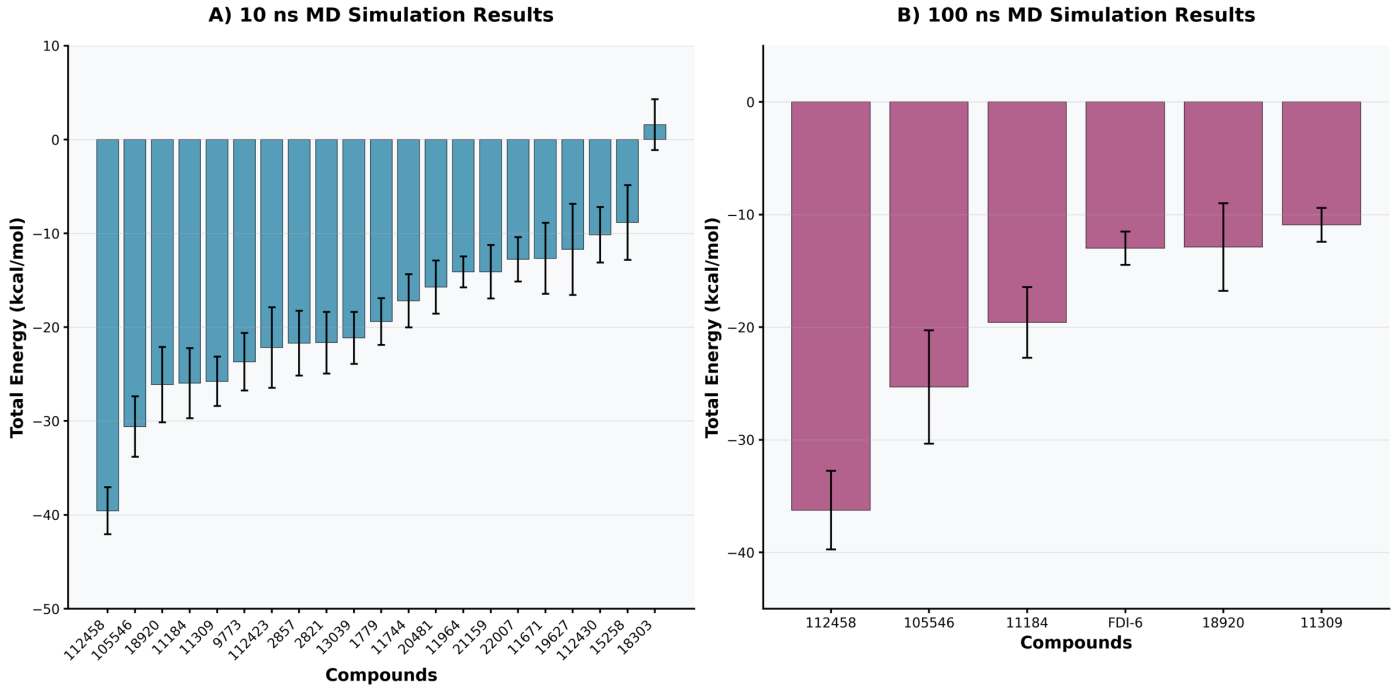


Figure 3. MM/GBSA binding free energy calculations
(A) Binding free energies calculated from 10 ns MD trajectories for multiple compounds, showing the range of binding affinities across the compound library, (B) Refined 100 ns MM/GBSA calculations for the five selected compounds and FDI-6 with error bars representing standard deviations,

The 10 ns MM/GBSA results (Figure 3A) showed varying binding free energies across multiple compounds, with several showing favorable binding energies below -20 kcal/mol. The 100 ns calculations (Figure 3B) were performed for the five selected compounds and the reference inhibitor FDI-6. Comp_112458 emerged as the most potent binder with a binding free energy of -36.25±3.5 kcal/mol, followed by comp_105546 (-25.31 ± 5.04 kcal/mol) and comp_11184 (-19.59±3.14 kcal/mol). The large negative values indicate strong binding affinity, with comp_112458 showing exceptional binding strength. The MM/GBSA calculations provided refined binding affinity estimates, identifying comp_112458 as the most promising candidate with a binding free energy of -36.25 kcal/mol. This exceptionally favorable binding energy, combined with stable MD behavior, suggests that comp_112458 represents a high-quality lead compound for further development. Recent benchmarking studies have demonstrated that MM/GBSA calculations provide reliable binding affinity estimates that correlate well with experimental data.[21, 22]

Implications for FOXM1 inhibitor development

The RMSF analysis revealed that ligand binding effectively stabilizes the functionally important binding pocket region (residues 280-310), a characteristic of effective inhibitors. This localized stabilization suggests that our lead compounds may indeed interfere with FOXM1's normal biological function by preventing DNA binding, which is consistent with the mechanism of action of known FOXM1 inhibitors.[7]

Our findings are particularly significant in the context of recent FOXM1 research. The compounds identified in this study (Table 1) represent novel chemical scaffolds that have not been previously explored for FOXM1 inhibition. Given the challenges associated with existing FOXM1 inhibitors, including limited potency and selectivity issues,[1] these novel scaffolds could provide important alternatives for drug development.

The computational approach employed in this study addresses several limitations of previous FOXM1 inhibitor discovery efforts. Unlike high-throughput screening approaches that are limited by available compound libraries, virtual screening allows exploration of vast chemical space, potentially identifying novel chemotypes not represented in physical libraries.[23] Additionally, the integration of molecular dynamics simulations and free energy calculations provides a more comprehensive evaluation of compound quality compared to docking-based screening alone.

The chemical diversity of our identified compounds is another important consideration. The two lead compounds (comp_105546 and comp_112458) represent distinct chemical scaffolds, providing multiple starting points for medicinal chemistry optimization. This diversity reduces the risk associated with scaffold-specific limitations and increases the likelihood of successful lead optimization.[24]

CONCLUSION

This *in silico* investigation successfully discovered small molecule inhibitors targeting the FOXM1 DNA binding region using an integrated strategy that combines structure-based virtual screening, molecular dynamics simulations, and MM/GBSA binding free energy calculations. The comprehensive screening of a large chemical library yielded 21 high-affinity candidates, from which five compounds (comp_105546, comp_11184, comp_11309, comp_18920 and comp_112458) were selected for detailed validation through 100 ns MD simulations.

The molecular dynamics analysis revealed distinct stability profiles among the two lead compounds, with comp_105546, and comp_112458 all maintaining stable binding interactions throughout the 100 ns trajectory. The MM/GBSA calculations provided refined binding affinity estimates, identifying comp_112458 as the most promising candidate with an exceptionally favorable binding free energy of -36.25 ± 3.5 kcal/mol,

Study limitations and future directions

While these computational predictions are highly promising, several limitations should be acknowledged. First, the predictions are based on a single crystal structure of FOXM1, which may not capture all relevant conformational states of the protein. Future studies could benefit from ensemble docking approaches using multiple conformations.[25]

Second, while MM/GBSA provides improved binding affinity estimates compared to docking scores, experimental validation remains essential to confirm the predicted activities. The next critical step involves synthesizing or procuring these compounds for *in vitro* biochemical assays to measure actual binding affinities and functional inhibition of FOXM1 transcriptional activity.

Third, our study focused on the DNA-binding domain of FOXM1, but recent research has identified other potentially druggable sites, including allosteric binding sites and protein-protein interaction interfaces.[26] Future computational studies could explore these alternative targeting strategies.

Finally, the drug-like properties and potential off-target effects of our lead compounds require careful evaluation. While the compounds were selected from a drug-like library, detailed ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling will be necessary to assess their therapeutic potential.[27]

significantly outperforming existing FOXM1 inhibitors in computational predictions.

The identified compounds represent novel chemical scaffolds that have not been previously explored for FOXM1 inhibition, offering important alternatives to existing inhibitors that face significant challenges in clinical translation. The computational approach employed in this study addresses key limitations of traditional screening methods by enabling exploration of vast chemical space while providing comprehensive evaluation of compound quality through dynamic simulations and free energy calculations.

The binding mode analysis demonstrated that the lead compounds effectively stabilize the functionally critical binding pocket region (residues 280-310), suggesting potential interference with FOXM1's DNA-binding activity. This mechanism of action is consistent with the therapeutic strategy of disrupting FOXM1's transcriptional functions, which are essential for cancer cell proliferation and survival.

The findings are particularly significant given the urgent need for effective FOXM1-targeted therapeutics. Despite FOXM1's recognition as a high-priority cancer target and its designation as "Molecule of the Year" in 2010, no FOXM1 inhibitor has successfully progressed to clinical trials. The novel compounds identified in this study, particularly comp_112458, represent promising starting points for drug development efforts aimed at addressing this critical gap in cancer therapeutics. However, experimental validation remains essential to confirm the computational predictions. Future studies should focus on synthesizing or procuring these compounds for *in vitro* biochemical assays to measure actual binding affinities and functional inhibition of FOXM1 transcriptional activity. Additionally, comprehensive ADMET profiling will be necessary to assess their therapeutic potential and identify any potential off-target effects.

In conclusion, this computational study provides a robust foundation for FOXM1 inhibitor development by identifying high-quality lead compounds with favorable binding properties and novel chemical scaffolds. The integration of multiple computational approaches enhances confidence in the predictions and establishes a validated pipeline for future FOXM1 inhibitor discovery efforts. With appropriate experimental validation and medicinal chemistry optimization, these compounds could contribute to the development of much-needed FOXM1-targeted cancer therapeutics.

Acknowledgement

All numerical calculations in this research were performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

Authorship contribution statement

Consept and desing: ZD, FDK.

Acquisition of data: EA.

Analysis and interpretation of data: ZD.

Drafting of the manuscript: ZD.

Critical revision of the manuscript for important intellectual content: ZD, FDK.

Statistical analysis: ZD, EA.

Declaration of competing interest: None of the authors have potential conflicts of interest to be disclosed.

Ethical approval: This study does not require ethical approval as it is a computational study.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Funding: This research was supported by Giresun University Scientific Research Projects Commission (Project No: SAĞ-BAP-A-250221-45).

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To Cite: Duzgun Z, Demirtas-Korkmaz F, Alp E. Computational Investigation of the Interaction of Large-scale Plant and Animal-Derived Natural Secondary Metabolites with FOXM1. Farabi Med J. 2025;4(3):63-72.