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Repurposing Drugs to Modulate Sortilin: Structure-Guided Strategies Against Atherogenesis, Coronary Artery Disease, and Neurological Disorders

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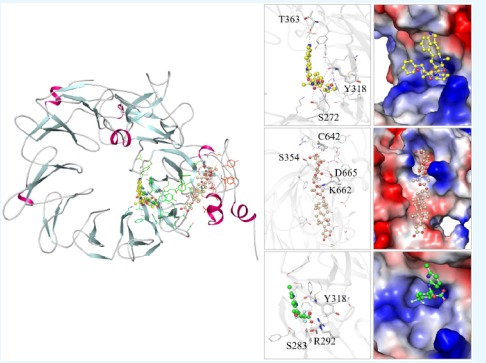
ABSTRACT: Sortilin (SORT1) is a multifunctional protein intricately involved in atherogenesis, coronary artery disease (CAD), and various neurological disorders. It has materialized as a potential pharmacological target for therapeutic development due to its diverse biological roles in pathological processes. Despite its central role under these conditions, effective therapeutic strategies targeting SORT1 remain challenging. In this study, we introduce a drug repurposing strategy guided by structural insights to identify potent SORT1 inhibitors with broad therapeutic potential. Our approach combines molecular docking, virtual screening, and molecular dynamics (MD) simulations, enabling the systematic evaluation of 3648 FDA-approved drugs for their potential to modulate SORT1. The investigation reveals a subset of repurposed drugs exhibiting highly favorable binding profiles and stable interactions within the binding site of SORT1. Notably, two hits, ergotamine and digitoxin, were carefully chosen based on their drug profiles and subjected to analyze their interactions with SORT1 and stability assessment via all-atom MD simulations spanning 300 ns (ns). The structural analyses uncover the complex binding interactions between these identified compounds and SORT1, offering essential mechanistic insights. Additionally, we explore the clinical implications of repurposing these compounds as potential therapeutic agents, emphasizing their significance in addressing atherogenesis, CAD, and neurological disorders. Overall, this study highlights the efficacy of structure-guided drug repurposing and provides a solid foundation for future research endeavors aimed at the development of effective therapies targeting SORT1 under diverse pathological conditions.



1. INTRODUCTION

Atherogenesis, coronary artery disease (CAD), and neurological disorders represent distinct yet interlinked domains of human health that collectively impose a significant global health burden.[1] While diverse in their etiology and clinical manifestations, these conditions share a common thread in the form of the multifunctional protein sortilin (SORT1).[2] SORT1 is a type I transmembrane receptor that belongs to the Vps10p-domain receptor family.[3] It has emerged as a critical molecular player that intricately coordinates several biological pathways and processes relevant to these pathologies.[4] SORT1 was initially identified as a neuronal sorting receptor which has garnered substantial attention due to its rich biology and profound implications in various disease states.[5] It exerts its diverse roles through its involvement in intracellular trafficking, protein sorting, and interaction with an array of ligands.[6] Importantly, SORT1 has been linked to lipid metabolism, inflammation, and cellular stress responses, all of which are pivotal in atherogenesis and CAD pathogenesis.[7]

In the context of cardiovascular disease, SORT1 has been recognized as a critical modulator of low-density lipoprotein cholesterol (LDL-C) metabolism.[8] SORT1 facilitates the intracellular sorting and degradation of proatherogenic LDL receptor-related protein 1 (LRP1), thereby regulating LDL-C levels in circulation.[9] Dysregulation of SORT1-mediated LDL-C clearance contributes to the accumulation of atherosclerotic plaques in the arterial walls, a hallmark of atherogenesis and CAD.[2] Beyond its cardiovascular implications, SORT1 has also been implicated in cancer[10] and a spectrum of neurological disorders, including Alzheimer's disease (AD)[11] and Parkinson's disease (PD).[12] SORT1 has been linked to the trafficking and processing of key neurodegenerative disease-associated proteins, such as the amyloid precursor protein (APP) and alpha-synuclein, thereby influencing the pathophysiological cascades characteristic of these disorders.[13]

Given the pivotal roles of SORT1 in these diverse pathologies, it represents a fascinating therapeutic target.[3,8,14] However, the development of specific and effective SORT1-targeting therapeutics remains a formidable challenge.[15]

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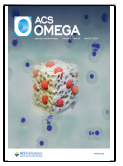

Traditional drug discovery approaches often require substantial time and resources, making them less viable for rapid intervention in conditions with urgent clinical needs.[16] In response to this challenge, our study presents a drug repurposing approach guided by structural insights to identify potent SORT1 inhibitors with therapeutic potential across the realms of atherogenesis, CAD, and neurological disorders.[17] By harnessing the power of molecular docking, virtual screening, and molecular dynamics (MD) simulations, we systematically screened a library of FDA-approved drugs to uncover a subset of compounds demonstrating promising binding affinities and stability within the SORT1 binding site. Subsequently, we provide a comprehensive account of our methodology including the computational tools employed for drug screening and molecular analysis. We present the findings of our study, highlighting the lead candidates identified through this approach, designated as ergotamine and digitoxin, and detail their interaction profiles with SORT1.

To gain a deeper understanding of the binding mechanisms and stability of ergotamine and digitoxin within SORT1, we conducted an extensive analysis using all-atom MD simulations spanning 300 ns (ns). These simulations allowed us to observe the dynamic behavior of the drug−protein complexes, evaluate their stability, and pinpoint key interactions that contribute to their inhibitory potential. By integrating various computational approaches, we selected two molecules as repurposed drugs, namely, ergotamine and digitoxin, with appreciable binding potential against SORT1. Overall, this study highlights the utilization of computational methods in expediting drug repurposing approaches in the discovery of novel therapeutic options for SORT1-related complexities. The elucidated compounds in this study mark a significant advancement in addressing the multifaceted role of SORT1 under various pathological conditions, providing hope for innovative and accelerated therapeutic strategies.

2. MATERIALS AND METHODS

2.1. Data Collection and Preparation. In order to perform virtual screening and MD simulation experiments, a diverse set of bioinformatics software was utilized, including MGL AutoDock Tools,[18] AutoDock Vina,[19] Discovery Studio Visualize,[20] and GROMACS 2020 beta.[21] Additionally, a combination of online and standalone tools played a crucial role in data retrieval, evaluation, and analysis. These tools encompassed the RCSB Protein Data Bank,[22] DrugBank,[23] SwissPDB-Viewer,[24] and XMGrace.[25] The GPT-3.5 large language model of OpenAI was used for text refinement and error correction with its default parameters. The crystal structure of human SORT1 served as the basis for our structure-based investigations and was downloaded from the RCSB Protein Data Bank (PDB ID: 5MRI, resolution: 2.00 Å). This structure underwent a careful examination and preparation process to ensure its suitability for subsequent analyses. To improve its readiness for further investigations, an optimization procedure was carried out using SwissPDB-Viewer and MGL tools. During the virtual screening phase, we thoroughly assembled a comprehensive library comprising 3,648 small molecules. This library exclusively consisted of FDA-approved drug molecules. To maintain consistent and accurate representations of chemical structures, ligand structures were preprocessed and optimized using MGL AutoDock tools and PyMOL.[26]

2.2. Structure-Based Molecular Docking. Molecular docking studies were carried out employing the AutoDock Vina software with additional support from multiple Perl scripts. The preparation of the SORT1 crystal structure was performed carefully, including the addition of hydrogen atoms, the assignment of charges, and the determination of appropriate atom types. The binding site of the SORT1 protein was delineated without any restriction, encompassing the entire protein search space. Ligands were introduced freely into the SORT1 binding site by using a blind grid-based strategy. The dimensions of the grid were set as follows: 97 Å for the *X*-axis, 91 Å for the *Y*-axis, and 73 Å for the *Z*-axis, with a central reference point at coordinates of −17.626 for *X*, −66.55 for *Y*, and 23.357 for *Z*. The grid spacing was consistently maintained at 1.00 Å, and the exhaustiveness parameter was fine-tuned to a value of 8. These docking parameters were systematically optimized to ensure a comprehensive exploration of the ligand conformations and orientations. For each ligand, individual docking simulations were conducted, and the resulting poses were ranked based on their binding affinity scores and interaction energies. The molecular docking calculations produced binding affinity scores, which were subsequently analyzed to identify potential candidates with a high affinity for SORT1. Ligands were further categorized based on their docking scores, and an in-depth analysis of their interactions with crucial residues in the active site was carried out to assess their consistency with known SORT1−ligand interactions.

2.3. Validation of Docking Protocol. The reliability of the Vina standard docking protocol was thoroughly established through a rigorous validation process employing a well-documented scientific technique, known as redocking. This validation procedure focused on a comprehensive assessment involving the redocking of a cocrystallized reference inhibitor (AF38469) with SORT1. Subsequently, a structural comparison was made between the poses obtained from the docking process and the poses of the cocrystallized inhibitor. The outcomes of this validation procedure explicitly prove the precision and dependability of our docking procedure. Remarkably, our methodology consistently predicted a binding pose for the AF38469 that perfectly matched the position of the cocrystallized AF38469 within the SORT1 binding site (RCSB ID: 4N7E). The superimposed binding poses are visually presented in Figure S1, showing the docked and crystallographic determined AF38469 with SORT1. The superimposition of these binding poses between the docked and cocrystallized AF38469 provides convincing confirmation supporting the accuracy and efficacy of our docking protocol.

2.4. Analysis of Molecular Interactions. Compounds that displayed favorable binding affinities and possessed suitable drug profiles were selected as potential candidates for further investigation. To elucidate possible binding modes for each compound, we utilized the Vina Splitter program after an initial evaluation of the docking data and identified those with stronger binding affinities. These conformations were subsequently subjected to a thorough examination to evaluate their potential interactions with SORT1. This comprehensive analysis was conducted using PyMOL and Discovery Studio Visualizer. Only compounds that exhibited specific interactions with SORT1 binding-site and active-site residues were chosen based on the interaction analysis.

2.5. Biological Property Prediction and Selection. To evaluate the biological properties of our chosen compounds,

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we utilized the PASS server (http://www.way2drug.com/passonline/predict.php). These predictions played a pivotal role in prioritizing compounds with favorable pharmacokinetic and safety profiles for further evaluation. This tool utilizes the chemical structure of compounds to analyze their potential biological characteristics.[27] It generates predictive scores for various biological attributes based on the "probability to be active (Pa)" and "probability to be inactive (Pi)" ratio. A higher Pa value suggests a greater likelihood of compounds possessing specific biological properties. Our primary focus in this study was to identify biological indicators associated with SORT1 inhibitory properties. Consequently, compounds that showed promise in these predictions were subjected to further investigation through MD simulations, capitalizing on their potential as SORT1 inhibitors.

2.6. MD Simulations. We conducted comprehensive all-atom MD simulations for SORT1 both before and after binding with the identified compounds, namely, ergotamine and digitoxin. These simulations were carried out over a duration of 300 ns at a temperature of 300 K, utilizing the GROMOS 54A7 force field[28] within GROMACS 2020 beta.[21] Prior to initiating the MD simulations, we prepared the SORT1−ligand complexes. These complexes were immersed in the SPC216 solvent model and enclosed within a periodic cubic box with an edge distance of 1 nm (nm). To maintain overall charge neutrality, an appropriate number of counterions were added. To resolve any potential steric clashes within the systems, we conducted energy minimization involving 1500 steps of the steepest descent algorithm over a 1000 ps (ps) period. Before proceeding to the production MD phase, we took preliminary steps to ensure system relaxation and establish a stable starting point. This included energy minimization and equilibration in both the NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles. After a 1000 ps equilibration period under constant volume conditions and the application of periodic boundary constraints at a constant 1 bar pressure, the temperature of all systems gradually increased from 0 to 300 K. Subsequently, all four systems underwent a final MD simulation run spanning 300 ns. Trajectories were recorded at regular intervals of 2 fs to facilitate subsequent analysis. These resulting trajectories were thoroughly examined using GROMACS' built-in tools and were visually represented using software applications such as VMD and XMGrace.

2.7. MD Trajectory Analysis. The analysis of our MD trajectories encompassed an evaluation of several critical aspects, including structural stability, conformational dynamics, and intermolecular interactions, within the SORT1-ligand complexes. To assess the stability and fluctuations in specific regions of these complexes, we conducted calculations for root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (R_g), solvent accessibility surface area (SASA), hydrogen bonding, and dynamics of secondary structures. Furthermore, we delved into hydrogen bonding patterns and other noncovalent interactions to gain valuable insights into the binding mechanisms and essential interactions at play. In addition, we utilized principal component analysis (PCA) and free energy landscape (FEL) techniques to extract dominant conformational modes and identify representative structures within the complexes. This multifaceted analysis provided a comprehensive understanding of the behavior of the SORT1−ligand complexes during the MD simulations.

2.8. Density Functional Theory (DFT) Study. Density functional theory (DFT) is a cornerstone of contemporary computational chemistry, increasingly employed in drug design endeavors to complement experimental approaches in the pursuit of novel therapeutic agents.[38] This quantum mechanical method offers precise descriptions of intermolecular interactions and facilitates the assessment of a candidate drug's reactivity. In this study, we employed DFT to investigate the electronic structures of the screened compounds. The molecular structures of the compounds were optimized, and the representations of their highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were developed within the solvent phase using Gaussian 09 software (https://gaussian.com/). These calculations were pivotal in elucidating the energetics and properties of the screened compounds that provide valuable insights into their potential pharmaceutical applications.

3. RESULTS AND DISCUSSION

3.1. Molecular Docking-Based Screening. In pursuit of our primary goal, which is to identify repurposed drugs with the potential to modulate SORT1, we executed molecular docking-based virtual screening techniques.[29] Our strategy involved the evaluation of a curated library consisting of 3648 FDA-approved drug molecules from the DrugBank repository. The purpose of this screening was to systematically assess each compound's capacity to engage with the binding site of SORT1, with the aim of pinpointing molecules that demonstrated favorable binding characteristics. Our analysis unveiled a noteworthy outcome, as the top 10 selected compounds exhibited remarkable affinity for SORT1. Their binding scores ranged from −10.9 to −9.8 kcal/mol, as delineated in Table 1. Notably, every single one of these compounds demonstrated superior binding affinity compared to the reference inhibitor, AF38469 which shows a binding score of −7.7 kcal/mol. This compelling result strongly suggests that these selected compounds possess substantial potential in terms of their ability to bind to SORT1 and, consequently, inhibit its activity effectively. These findings underscore the importance of further investigation into these compounds as they hold the promise of serving as potent binding partners of SORT1, potentially opening new avenues for therapeutic development.

Table 1. List of the Top 10 hit Molecules and Their Docking Scores with SORT1

sl. no.	drug	binding affinity (kcal/mol)	ligand efficiency (kcal/mol/non-H atom)	torsional energy
1.	rifaximin	−10.9	0.1912	2.1791
2.	ergotamine	−10.4	0.2419	1.5565
3.	keracyanin	−10.2	0.2429	4.9808
4.	digitoxin	−10.1	0.187	3.7356
5.	midostaurin	−9.9	0.2302	1.8678
6.	temoporfin	−9.9	0.1904	2.4904
7.	eptifibatide	−9.9	0.1737	3.4243
8.	cefpiramide	−9.9	0.2357	3.7356
9.	picloxydine	−9.8	0.3063	0.6226
10.	eltrombopag	−9.8	0.297	2.1791
11.	AF38469	−7.7	0.3348	0.9339

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3.2. Selection of Promising Candidates and Pass Analysis. Following the molecular docking screening, our attention shifted to the identification of the most promising candidates, ultimately leading us to focus on two compounds, designated as ergotamine and digitoxin. This selection process was carefully carried out considering various factors, such as drug profiles, structural characteristics, and docking scores. To further assess the potential of these compounds, we turned to the PASS webtool, a web-based resource renowned for its ability to predict a wide array of biological properties, encompassing approximately 4,000 distinct attributes. Leveraging the PASS server, we embarked on an evaluation of the potential biological properties of ergotamine and digitoxin. The outcomes of this analysis substantiated the promise of these compounds, as they displayed significant attributes that aligned closely with our research objective. Specifically, both compounds exhibited noteworthy antineurodegenerative diseases, anti-inflammatory, and vascular dementia treatment potential. Notably, the Pa (probability to be active) values for ergotamine exceeded 0.846, and for digitoxin, they surpassed 0.446, as presented in Table 2. This marked the superiority of Pa over Pi (probability to be inactive), further confirming the potential of ergotamine and digitoxin in the context of SORT1 inhibition. Overall, ergotamine and digitoxin were predicted to have favorable properties related to SORT1 inhibition.

Table 2. Screened Compounds with Their PASS BiologicalProperties

drug	Pa	Pi	biological activity
ergotamine	0.992	0.002	5-hydroxytryptamine antagonist
	0.969	0.003	antimigraine
	0.961	0.002	vascular (periferal) disease treatment
	0.926	0.005	nootropic
	0.846	0.004	neurodegenerative diseases treatment
digitoxin	0.950	0,003	cardiotonic
	0.938	0.002	proliferative diseases treatment
	0.694	0.002	dementia treatment
	0.619	0.002	vascular dementia treatment
	0.446	0.075	anti-inflammatory
AF38469	0.772	0.004	5-hydroxytryptamine release inhibitor
	0.389	0.047	antidiabetic
	0.395	0.098	anti-inflammatory
	0.290	0.022	pulmonary hypertension treatment
	0.343	0.092	superoxide dismutase inhibitor

3.3. Pharmacokinetic Assessment. The pharmacokinetic assessment of the screened molecules and the reference inhibitor was carried out to explore their ADMET characteristics. The pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) and SwissADME (http://www.swissadme.ch/) were utilized for the pharmacokinetic assessment of ergotamine, digitoxin, and AF38469. The analysis showed that all three molecules have favorable pharmacokinetic properties (Table 3). They lack any toxic patterns that make them potentially more effective and secure drug candidates. Overall, the results demonstrate that ergotamine, digitoxin, and AF38469 have favorable pharmacokinetic characteristics without any PAINS patterns, indicating that they may be effective leads for drug development.

3.4. Binding Modes and Interactions: Molecular Docking Investigation. Our investigation delved deeper into the binding modes that underpin the interaction of ergotamine and digitoxin with SORT1. The examination of these interactions provided valuable insights into the potential inhibitory mechanisms of these molecules in Figure 1. Both ergotamine and digitoxin exhibited consistent and substantial interactions with important amino acid residues situated within the SORT1 binding site, thereby reinforcing their potential as inhibitors of this target protein. This is visually depicted in Figure 1. During the binding process, both compounds formed crucial hydrogen bonds that played a pivotal role in stabilizing the complex, emphasizing the efficacy of ergotamine and digitoxin in precisely targeting SORT1. Ergotamine binds with multiple residues, forming three hydrogen bonds with Ser272, Tyr318, and Thr363. This interaction pattern directly mirrored those observed in the cocrystallized reference SORT1 inhibitor, AF38469, showcasing a commendable complementarity fit. In contrast, digitoxin interacts with Ser354, Cys642, Lys662, and Asp665. These interactions were not limited to polar interactions alone as several hydrophobic interactions also contributed to complex formation. In summary, the findings underscore the potential of elucidated molecules in interfering with SORT1 function and suggest their viability as candidates for therapeutic development.

In the pursuit of a deeper understanding of the interaction mechanism between SORT1 and the elucidated molecules, we conducted a comprehensive analysis. This analysis is pivotal in shedding light on the nature and precise locations of the intramolecular connections between the compounds and the protein. Particularly, we focused on ergotamine and digitoxin, scrutinizing their interactions in 2D plots, which served as invaluable visual tools for elucidating these intricate connections. The visual representations of the resulting 2D plots for both compounds can be found in Figure 2A,rB. Remarkably, the analysis revealed that ergotamine exhibited a similar binding pattern to the cocrystallized reference SORT1 inhibitor, AF38469, as demonstrated in Figure 2C. Specifically, ergotamine was found to establish close interactions with the binding site, primarily engaging with Tyr318. In contrast, digitoxin exhibited six hydrogen bonds with four residues of the SORT1 binding site. These interactions were not limited to hydrogen bonding alone; they also encompassed a network of hydrophobic interactions. Collectively, these interactions contributed significantly to the overall stability of the complex formed between the compounds and SORT1. This emphasizes the substantial promise of ergotamine and digitoxin as potent SORT1 inhibitors, underscoring their potential as valuable

Table 3. ADMET Properties of the Screened Molecules Along with the Reference Inhibitor[a]

sI. no.	molecule	absorption (HIA)	distribution (VDss, log *L*/kg)	metabolism (CYP3A4 inhibitor)	excretion (renal OCT2 substrate)	toxicity (AMES)
1.	ergotamine	high, 64.27%	1.338	yes	no	no
2.	digitoxin	high, 74.29%	0.259	no	no	no
3.	AF38469	high, 93.14%	−0.974	no	no	no

[a]HIA, human intestinal absorption; VDss, steady-state volume of distribution.

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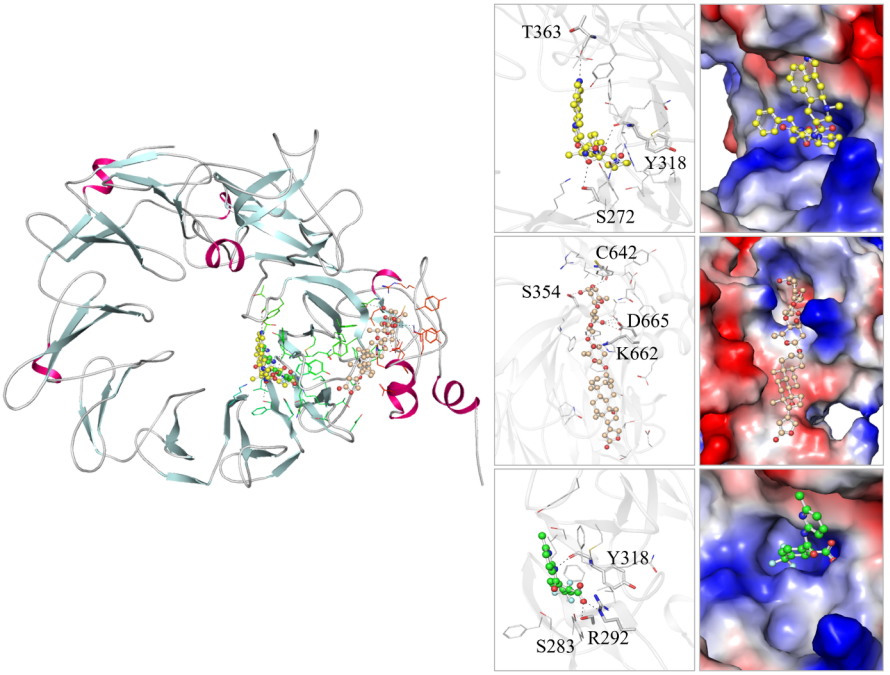

Figure 1. SORT1 in complex with the selected molecules. Left panel: SORT1 with ergotamine (yellow), digitoxin (red salmon), and AF38469 (green). Middle panels: a close-up view of SORT1 interaction with ergotamine and digitoxin. Right panels: the surface potential view of the SORT1 binding pocket with the elucidated molecules.

candidates for further development as therapeutics with significant clinical relevance.

3.5. MD Simulation Analysis. In our quest to unravel the intricacies of the binding mechanisms, stability, and time-dependent dynamics of ergotamine and digitoxin within the SORT1 binding site, we embarked on an extensive series of all-atom MD simulations spanning a duration of 300 ns. The objective behind these MD simulations was to gain profound insights into the dynamic behavior of the drug−protein complexes, evaluate their overall stability, and pinpoint the pivotal interactions that underpin their inhibitory potential.

3.5.1. Structural Deviation and Compactness. The binding of a ligand to the binding site of a protein can induce significant conformational changes in the protein's structure, with potential consequences for its biological activity.[30] To probe these conformational shifts in SORT1 before and after interacting with ligands, we harnessed the RMSD as a valuable analytical tool.[31] This study focused on examining the RMSD variations in the SORT1 protein before and following its interaction with ergotamine and digitoxin. The RMSD values over time for all four systems are depicted in Figure 3A. Remarkably, the plot showcases minimal fluctuations, suggesting the overall stability of the systems under investigation. It is particularly noteworthy that the RMSD plot illustrates that the ergotamine- and digitoxin-bound states exhibit comparably minor fluctuations compared to the free state of SORT1. As we assessed RMSD values across all of the systems, a consistent equilibrium was maintained throughout the 300 ns of simulation, with no pronounced conformational alterations. Some slight RMSD fluctuations, approximately in the range of 0.1 nm, were observed within the 100−130 ns time frame following the ligand binding, as depicted in Figure 3A. This provides strong evidence for the overall stability of the systems under investigation.

To gain a more comprehensive understanding of the flexibility of individual amino acids within SORT1, we analyzed the RMSFs. RMSF serves as a valuable metric for assessing the residual vibrations within the protein structure, shedding light on its dynamic behavior. The RMSF plot, as presented in Figure 3B, exhibits a consistent pattern across all four systems. Notably, these residual fluctuations within the protein structure signify stability, with a noteworthy observation being the reduction in RMSF values upon the binding of ergotamine and digitoxin when compared with the free SORT1. This reduction in RMSF values underscores the enhanced stability of the complexes formed by ergotamine and digitoxin. Furthermore, our analysis revealed that the specific residues interacting with ergotamine and digitoxin exhibited exceptional stability throughout the study. This observation reinforces the notion that ergotamine and digitoxin are adept at establishing robust interactions with SORT1, which in turn contribute to the overall stability of the resulting complexes.

To assess the compactness of the protein structure, we turned to R_g calculations from the MD trajectory. In this examination, we closely scrutinized the time-dependent behavior of R_g to ascertain the level of compactness demonstrated by SORT1 in the presence of ergotamine and digitoxin. The analysis encompassed R_g values for SORT1−ergotamine, SORT1−digitoxin, and free SORT1. The trajectories of all three systems consistently demonstrated stable R_g values, distributed within the range of 2.75−2.85 nm (Figure 4A). These comparative findings strongly indicate that SORT1 maintains its conformational stability and maintains its structure when interacting with both ergotamine and digitoxin.

In parallel, we assessed the SASA from the simulated trajectory as the protein's surface area accessible to the surrounding solvent.[32] To estimate the folding dynamics of SORT1 in the presence of ergotamine and digitoxin, we conducted an analysis of SORT1's SASA over time. The SASA

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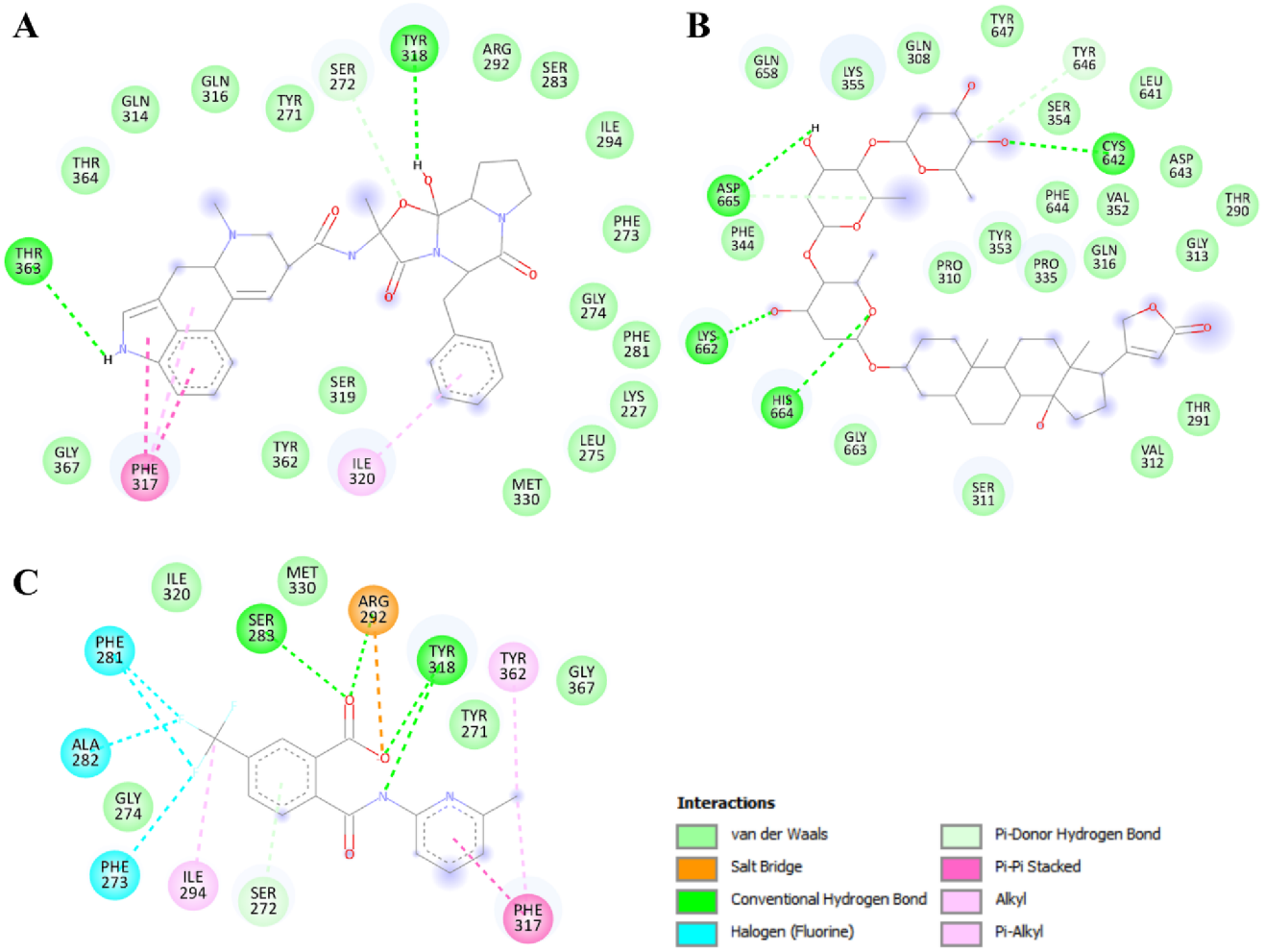

Figure 2. Interactive plots of SORT1 with (A) ergotamine, (B) digitoxin, and (C) AF38469.

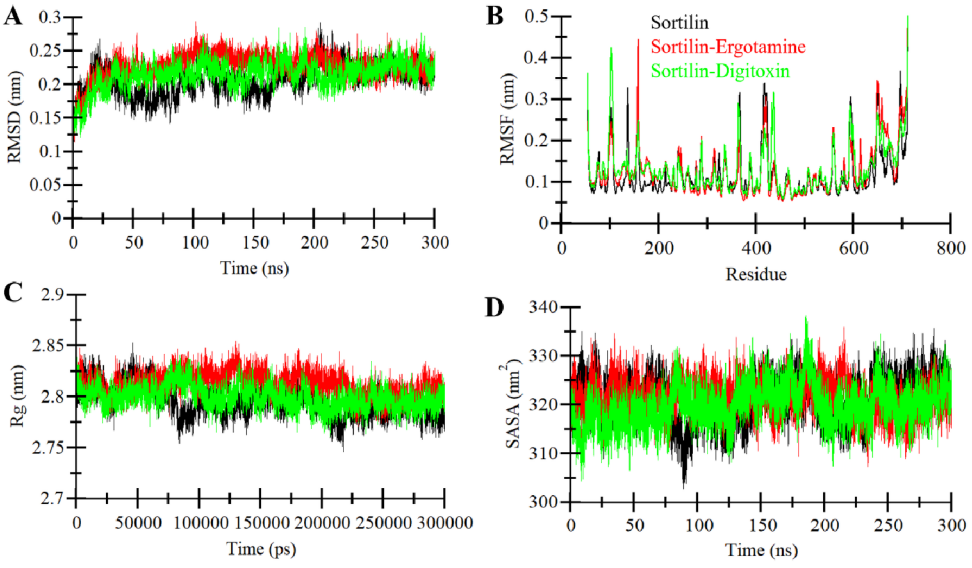

Figure 3. SORT1 dynamics and compactness with ergotamine and digitoxin binding. (A) RMSD plot of SORT1 with ergotamine and digitoxin. (B) Average residual fluctuations (RMSF) of SORT1 and its complexes with ergotamine and digitoxin. (C) The time evolution of SORT1 R_g before and after ergotamine and digitoxin binding. (D) SASA distribution plot of SORT1 before and after ergotamine and digitoxin binding.

plot unveiled a steady pattern, with negligible variations detected in SASA over the duration of the simulation (Figure 4B). The consistent SASA values indicate the strong stability of the protein−ligand complexes. Similar to the R_g analysis, the SASA also remained constant, showing no fluctuations in the structural folding or compactness throughout the simulation.

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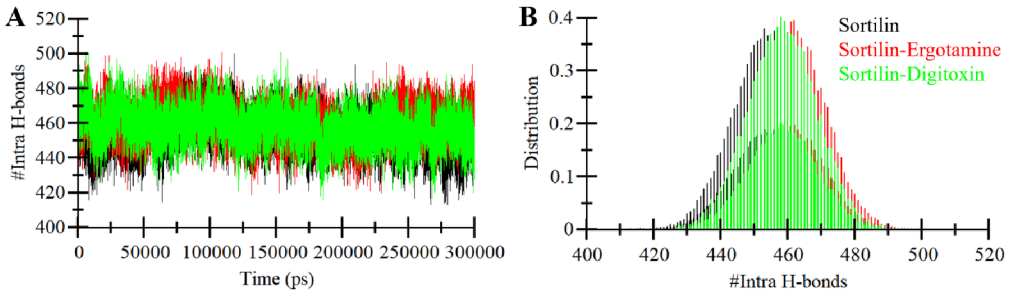

Figure 4. Dynamics of intramolecular hydrogen bonding. (A) Intramolecular hydrogen bonding in SORT1 before and after ergotamine and digitoxin binding. (B) The PDF plot of intramolecular hydrogen bonding in SORT1.

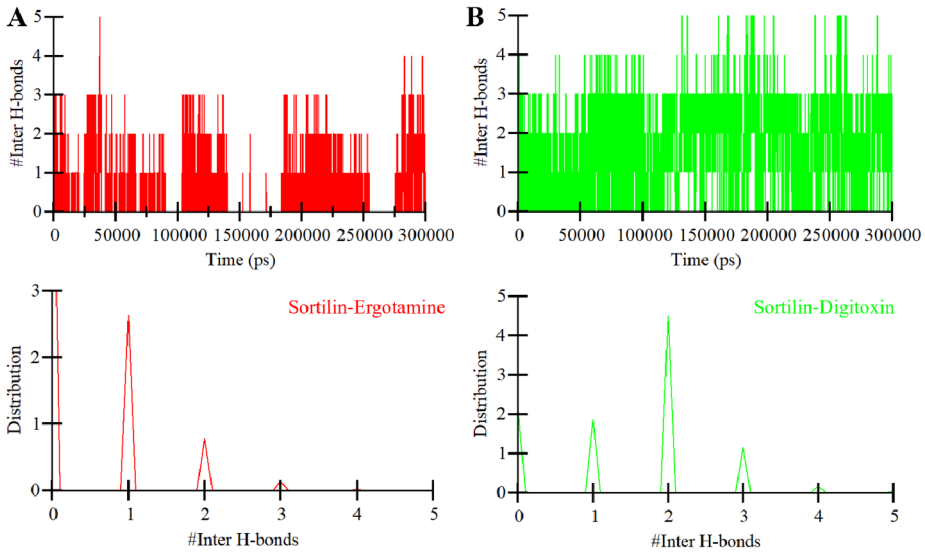

Figure 5. Intermolecular hydrogen bonding in SORT1 and (A) ergotamine and (B) digitoxin as a time function.

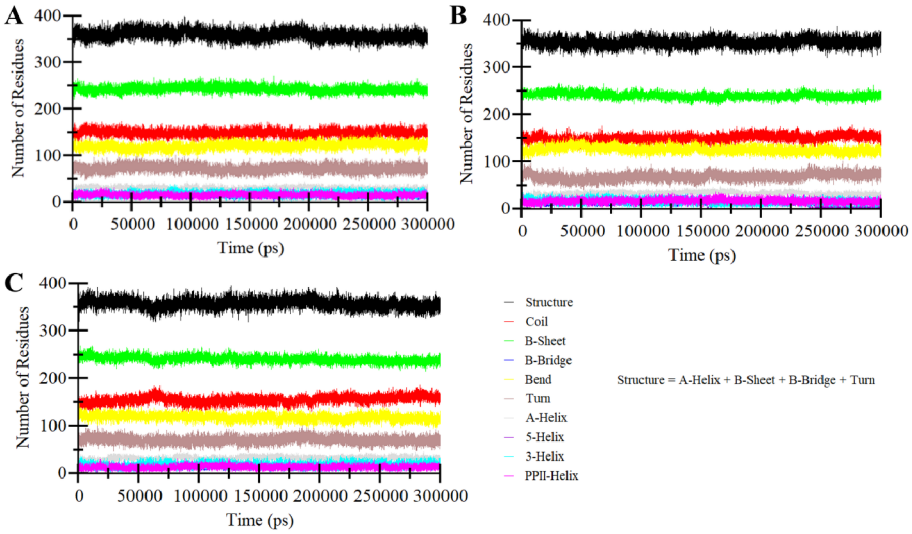

Figure 6. Secondary structure dynamics in (A) SORT1, (B) SORT1−ergotamine, and (C) SORT1−digitoxin as time function.

Taken together, the study underscores the structural compactness of SORT1 in the presence of ergotamine and digitoxin, further highlighting their potential as effective ligands for the protein.

3.5.2. Hydrogen Bond Analysis. The maintenance of protein structural stability significantly relies on intramolecular hydrogen bonding.[33] Analyzing hydrogen bonds can offer valuable insights into the compactness within the protein structure.[33] In this study, we delved into the dynamics of hydrogen bonding in the SORT1. The results of this analysis are visually depicted in Figure 4, which illustrates the dynamics of intramolecular hydrogen bond formation with ergotamine and digitoxin in SORT1. Notably, the plot reveals that even upon binding with ergotamine and digitoxin, the formation of

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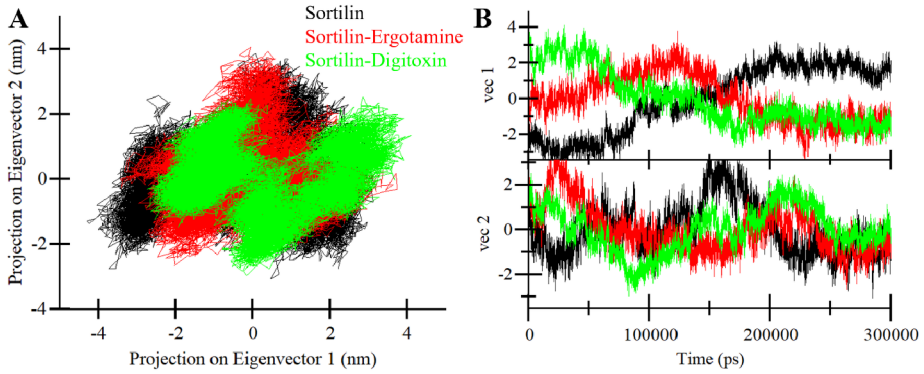

Figure 7. PCA of SORT1 and its docked complexes. (A) 2D projection and (B) time dynamics of EV1 and EV2.

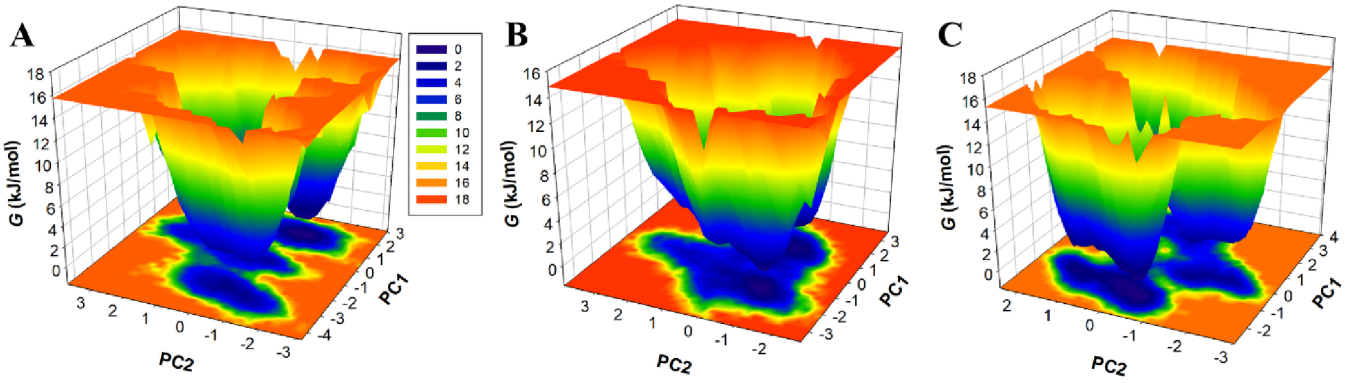

Figure 8. Free energy landscapes of (A) SORT1, (B) SORT1−ergotamine, and (C) SORT1−digitoxin.

hydrogen bonds within SORT1 remains constant (Figure 4A). Moreover, the probability density function (PDF) illustrates a uniform trend across all three systems (Figure 4B). This observation reinforces the notion that the binding of ergotamine and digitoxin to SORT1 does not compromise intramolecular hydrogen bonding.

To further delve into the stability of polar interactions between SORT1 and ergotamine, as well as digitoxin, we directed our focus toward examining intermolecular hydrogen bonds. The directionality and specificity of these bonds are of paramount importance in gaining insights into protein kinetics.[34] In the SORT1−ergotamine complex, the results revealed the formation of 1−3 hydrogen bonds, irregularly increasing to 4−5 (Figure 5A). In contrast, the SORT1−digitoxin showed 2−3 hydrogen bonds, irregularly reaching up to 5 bonds (Figure 5B). The PDF analysis showed a distribution of intermolecular hydrogen bonds, with a prominent occurrence of one hydrogen bond (Figure 6, lower panels). The outcomes pinpointed that the binding of ergotamine and digitoxin to SORT1 is underpinned by intermolecular hydrogen bonds, which play a crucial role in the stability of protein−ligand complexes. This robust interaction network further substantiates the stability of the protein−ligand complexes and supports their potential as reliable binders within the SORT1 system.

3.5.3. Secondary Structure Dynamics. To gain a comprehensive understanding of the temporal changes in structural content and assess the impact of ergotamine and digitoxin binding on the secondary structure of SORT1, we conducted an extensive analysis (Figure 6). Our analysis revealed that the secondary structure composition of SORT1 remained constant during the simulation when it was not bound to any ligands (Figure 6A). Even upon binding with ergotamine and digitoxin (Figure 6B,C), the secondary structure makeup of SORT1 demonstrated a high degree of stability. Notably, the alterations in the secondary structure of SORT1 following binding with ergotamine and digitoxin were relatively minor. This finding underscores the robustness and persistence of the secondary structure of SORT1 in the presence of ergotamine and digitoxin. This preservation of secondary structure underscores the stability and fidelity of the native protein conformation in the presence of ergotamine and digitoxin, further supporting the notion of a well-preserved SORT1−ergotamine and SORT1−digitoxin complex. These results highlight the potential of ergotamine and digitoxin as ligands that do not disrupt the native secondary structure of SORT1, making them promising candidates for further study and development as SORT1 inhibitors.

3.5.4. Principal Component Analysis. PCA is a valuable tool for acquiring insights into the conformational folding of a protein's structure.[35] In this investigation, we performed PCA of SORT1 and its complexes with ergotamine and digitoxin to analyze their conformational landscape. The results of PCA, as depicted in Figure 7, vividly illustrate the conformational landscape of these three complexes. A noteworthy observation is that the SORT1−ergotamine and SORT1−digitoxin complexes primarily occupy the same essential subspace as SORT1 in its unbound and cocrystallized states (Figure 7A). These results from the eigenvalue (EV) plots provide compelling evidence of the complexes' stability throughout the simulation, suggesting that the binding of the ligands, ergotamine and digitoxin, does not exert a substantial influence on the SORT1's conformational exploration (Figure 7B). The convergence of the systems further supports the stability and

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potential of the SORT1−ergotamine and SORT1−digitoxin complexes as promising candidates for therapeutic development.

3.6. Free Energy Landscape. FELs provide a graphical representation of the intricate folding process a protein undergoes as it converges to its native state, reaching the global energy minimum.[36] FELs are valuable tools for assessing the stability of proteins and protein−ligand complexes during MD simulations.[37] Within FELs, the color gradient symbolizes the energy level of the protein with deeper blue regions indicating lower energy levels that closely approximate the native state of the protein. In this analysis, we employed a combination of two principal components (PCs) to extract energy minima and conformational profiles of SORT1, SORT1−ergotamine, and SORT1−digitoxin complexes. The FELs of these complexes, as presented in Figure 8, illustrate alterations in the size and location of confined phases containing 2−3 global minima upon binding ergotamine and digitoxin to SORT1. Across these FELs, the deeper blue hues signify lower energy levels that are closer to the global minima for all three systems. Specifically, SORT1 primarily occupies three global minima, extending to encompass 3 basins (Figure 8A). Similarly, SORT1−ergotamine and SORT1−digitoxin were also confined to 2−3 global minima, each characterized by 2−3 basins (Figure 8B,C). The FEL analysis effectively demonstrates that SORT1 maintains stable conformations throughout the simulations, even in the presence of bound ligands. This valuable insight aids in a deeper understanding of the binding mechanism and persistence of the elucidated molecules, contributing to the advancement of SORT1-targeted therapeutics.

3.7. Density Functional Theory (DFT) Analysis. The molecular orbitals HOMO and LUMO serve as pivotal descriptors for understanding a molecule's stability and reactivity upon interaction with other species. We calculated the physicochemical descriptors and thermodynamic parameters of ergotamine and digitoxin, alongside the reference molecule AF38469, to assess their chemical reactivity and stability. To estimate the chemical reactivity and stability, we calculated the band gap ($\Delta E = E_{LUMO} - E_{HOMO}$) for the screened compounds. The energy gap between HOMO and LUMO significantly influences the bioactivity and intermolecular charge transfer. A higher energy gap corresponds to a lower reactivity of the complex. Table 4 presents the HOMO−LUMO energy values and energy gap (ΔE) for ergotamine, digitoxin, and AF38469. The band energy gap values ranged from 0.0276 to 0.12485 kcal/mol, indicating narrow energy gaps and high reactivity properties. This observation implies that the compounds demonstrate significant reactivity toward SORT1. The energies of both HOMO and LUMO provide insights into the electrophilic and nucleophilic nature of the screened compounds (Figure 9).

Table 4. HOMO, LUMO, and Band Energy Gap for the Screened Compounds

sI. no	compound	E_{HOMO} (kcal/mol)	E_{LUMO} (kcal/mol)	band gap ($\Delta E = E_{LUMO} - E_{HOMO}$) (kcal/mol)
1.	ergotamine	−0.16613	−0.11410	0.05203
2.	digitoxin	−0.08529	−0.05769	0.02760
3.	AF38469	−0.21376	−0.08891	0.12485

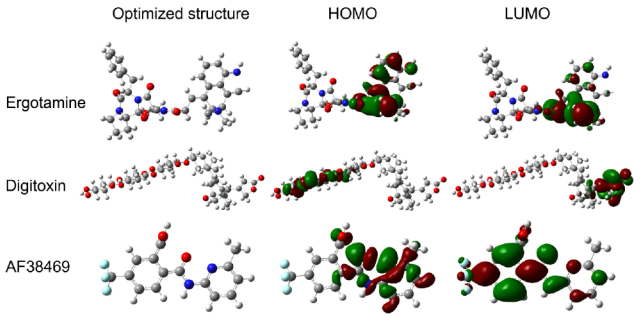

Figure 9. Frontier molecular orbitals (HOMO and LUMO) and optimized geometry of ergotamine, digitoxin, and AF38469.

4. CONCLUSIONS

In pursuit of identifying potent SORT1 inhibitors with broad therapeutic potential, we employed a multifaceted approach that combined computational modeling, virtual screening, MD simulations, and in-depth structural analyses. Our study yielded several key findings and insights, underscoring the significance of our research in the context of atherogenesis, CAD, and neurological disorders. First, our molecular docking and virtual screening endeavors identified a subset of repurposed drugs exhibiting highly favorable binding profiles with SORT1. Notably, the top 10 compounds demonstrated binding affinities superior to those of the reference inhibitor, AF38469. This outcome emphasizes the potential of these compounds as effective SORT1 inhibitors, thereby opening new avenues for therapeutic intervention in diseases associated with SORT1 dysregulation.

Following the screening process, two lead candidates, ergotamine and digitoxin, were selected based on their drug profiles, structural characteristics, and predicted binding affinities. Leveraging the PASS tool, we uncovered significant antineurodegenerative, anti-inflammatory, and vascular dementia treatment potential associated with both ergotamine and digitoxin, aligning closely with our initial research objectives. This identification strengthens the candidacy of these compounds for SORT1 inhibition and broadens their therapeutic relevance across a spectrum of conditions. Our structural analyses delved deeper into the binding modes and interactions of ergotamine and digitoxin with SORT1, elucidating the key mechanisms underpinning their potential as effective inhibitors. These compounds formed crucial hydrogen bonds and engaged in robust polar and hydrophobic interactions with crucial residues within the SORT1 binding pocket, mirroring interaction patterns observed in the reference SORT1 inhibitor, AF38469. These findings underscore the efficacy of ergotamine and digitoxin in precisely targeting SORT1 and inhibiting its activity.

Furthermore, MD simulations provided valuable insights into the stability and dynamics of the SORT1−ergotamine and SORT1−digitoxin complexes. The analysis revealed minimal conformational changes in SORT1, underscoring the stability of the protein−ligand complexes. The secondary structure of SORT1 remained largely unaltered during the simulation. Intermolecular hydrogen bond analysis indicated that these compounds establish robust interactions with SORT1, further supporting the stability of the complexes. PCA and FEL analyses confirmed the stability of the SORT1−ergotamine and SORT1−digitoxin complexes, highlighting their convergence with the unbound state of SORT1. The study

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provides compelling evidence that ergotamine and digitoxin are promising SORT1 inhibitors with potential clinical relevance in atherogenesis, CAD, and neurological disorders. The structural insights and stability that these compounds demonstrate underscore their candidacy for further exploration and development as therapeutic agents. The promising steps made in this study represent a significant leap toward addressing unmet medical needs and offer hope for patients affected by SORT1-associated diseases.

■ ASSOCIATED CONTENT

Data Availability Statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00470.

Representation of binding pose for the AF38469 that perfectly matched the location of the cocrystallized AF38469 within the SORT1 structure (PDF)

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Notes

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