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Exploring the Potential of Natural Products as FoxO1 Inhibitors : an *In Silico* Approach

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Abstract

FoxO1, a member of the Forkhead transcription factor family subgroup O (FoxO), is expressed in a range of cell types and is crucial for various pathophysiological processes, such as apoptosis and inflammation. While FoxO1's roles in multiple diseases have been recognized, the target has remained largely unexplored due to the absence of cost-effective and efficient inhibitors. Therefore, there is a need for natural FoxO1 inhibitors with minimal adverse effects. In this study, docking, MMGBSA, and ADMET analyses were performed to identify natural compounds that exhibit strong binding affinity to FoxO1. The top candidates were then subjected to molecular dynamics (MD) simulations. A natural product library was screened for interaction with FoxO1 (PDB ID-3CO6) using the Glide module of the Schrödinger suite. *In silico* ADMET profiling was conducted using SwissADME and pkCSM web servers. Binding free energies of the selected compounds were assessed with the Prime-MMGBSA module, while the dynamics of the top hits were analyzed using the Desmond module of the Schrödinger suite. Several natural products demonstrated high docking scores with FoxO1, indicating their potential as FoxO1 inhibitors. Specifically, the docking scores of neochlorogenic acid and fraxin were both below -6.0. These compounds also exhibit favorable drug-like properties, and a 25 ns MD study revealed a stable interaction between fraxin and FoxO1. Our findings highlight the potential of various natural products, particularly fraxin, as effective FoxO1 inhibitors with strong binding affinity, dynamic stability, and suitable ADMET profiles.

Key Words: Docking, *In silico*, ADMET screening, FoxO1, MMGBSA, Molecular dynamics

INTRODUCTION

The Forkhead transcription factor (Fox) family in humans includes four members belonging to the "O" subclass, specifically FoxO1, FoxO4, FoxO3a, and FoxO6. FoxO2 serves as a homolog of FoxO3, while FoxO5 is found solely in Danio rerio (Eijkelenboom and Burgering, 2013; Lee and Dong, 2017). Distributed across a variety of organisms, FoxOs are crucial for regulating lifespan, cell proliferation, metabolism, stress resistance, and apoptosis (Xing *et al*., 2018; Xu and Wang, 2021).

FoxO1, a member of the FoxO family, possesses significant transcriptional regulatory functions (Kandula *et al*., 2016). It is a key regulator of metabolic processes in the liver, adipose tissue, and hypothalamus (Nakae *et al*., 2008). FoxO1 modulates hepatic gluconeogenesis and glycogenolysis by responding to insulin signaling and stimulating the transcription of two vital enzymes, namely G6PC (glucose 6-phosphatase) and PEPCK (phosphoenolpyruvate carboxykinase) (Peng *et al*., 2020). In diabetic db/db mice, the inhibition of hepatic FoxO1 activity led to decreased expression levels of G6PC and PEPCK, thereby reducing hepatic gluconeogenesis and fasting hyperglycemia. Moreover, FoxO1 targets apolipoprotein C-3 (ApoC3), a direct participant in plasma lipid

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metabolism (Lee *et al*., 2020). Overexpression of FoxO1 has been shown to increase plasma triglyceride levels (Peng *et al*., 2020). It also plays an essential role in muscle development and differentiation by modulating glucose and lipid metabolism in skeletal muscle. In particular, FoxO1 enhances the activity of PDK4, a key regulator of pyruvate flow during the Krebs cycle. Additionally, it controls lipoprotein lipase (LPL) activity, allowing muscle cells to utilize fatty acids (Gross *et al*., 2008). FoxO1 is also involved in apoptosis regulation by upregulating the transcription of FasL and transactivating Bim, a pro-apoptotic member of the Bcl-2 family involved in the intrinsic mitochondrial apoptotic pathway. Furthermore, the phosphorylation of FoxO1 in the cytoplasm activates cyclin D1 and Cdk4 (cyclin-dependent kinase), facilitating the transition of the cell cycle from the G0 to the G1 phase and initiating DNA synthesis (Gan *et al*., 2009; Yang *et al*., 2009; Zhang *et al*., 2011). Consequently, its suppression could serve as a potential therapeutic target for addressing carcinogenesis, metabolic disorders, and skeletal muscle differentiation (Lu and Huang, 2011).

Nevertheless, despite FoxO1's established role in a wide range of cellular functions, the exploration of potential targets remains limited. To date, only a few direct FoxO1 inhibitors have been identified. For instance, AS1842856 is a small molecule that has been shown to bind directly to FoxO1, as confirmed through mass spectrometric affinity screening (Zou *et al*., 2014). Additionally, during a cell-based high-throughput screening of more than 170,000 small molecules, AS1708727 emerged as a potent FoxO1 inhibitor (Tanaka *et al*., 2010). Both academic and industrial researchers have expended significant time and resources over many years in the search for novel FoxO1 inhibitors. Natural products, owing to their unique chemical diversity and drug-like properties, have garnered attention as potential sources of inhibitors. These compounds have long been used in traditional medicines, particularly as active ingredients in herbal treatments, predating the development of contemporary chemical pharmacology. In the present study, we aimed to identify natural inhibitors of FoxO1 (PDB ID-3CO6) utilizing an *in silico* approach.

MATERIALS AND METHODS

Protein-protein interaction analysis

Protein-protein interactions play a crucial role in the regulation and execution of a protein's biological activities. The STRING 11.0 (Swiss Institute of Bioinformatics, Lausanne, Switzerland) database is employed to predict the top ten proteins most likely to interact with the target gene, FoXO1. This web-based resource is among the limited platforms capable of aggregating and integrating publicly available data on protein-protein interactions, thereby establishing a comprehensive network of both direct (physical) and indirect (functional) protein associations. Predictions are formulated based on the expected interactions between proteins, as computed by the STRING database (https://string-db.org). The STRING algorithm relies on multiple information sources, including gene fusion, co-expression, functional annotations, and experimental data, to offer insights into the potential interaction partners for a specific protein. A scoring system quantifies the level of interaction for each protein, assigning values ranging from "0" to "1," where "0" indicates the least likelihood of interaction and "1" denotes the maximum likelihood of interaction (Sakle *et al*., 2020).

Molecular docking studies

Ligand preparation: A collection of 550 natural products was sourced from the APExBIO database (https://www.apexbt.com). The natural products library was optimized using LigPrep (Ver 2021, Schrödinger, LLC, NY, USA) from the Schrödinger suite. This optimization employed Epik 2.0 and considered a pH range of 7.0 ± 2.0 to generate various ionization states, tautomers, and ring conformations for each input ligand. A single, energy-minimized, low-energy conformer for each molecule was generated using the OPLS 2005 force field (John *et al*., 2016; Sahu *et al*., 2020).

Protein preparation: The three-dimensional (3D) crystal structure of human FoxO1 (PDB 3CO6) was obtained from the Protein Data Bank (https://www.rcsb.org/structure/3CO6). Subsequent processing of the retrieved protein structure was carried out using the Protein Preparation Wizard (Epik, Schrödinger, LLC). The initial steps involved the assignment of appropriate bond orders, incorporation of hydrogen atoms, and establishment of disulfide and zero-order metal bonds. Water molecules beyond 5 Å from hetero groups were removed. Various ionization states for the protein structure were generated using Epik (Ver 2021, Schrödinger, LLC), and the most stable state was selected for further analysis. Missing chains and loops were completed using Prime (Ver 2021, Schrödinger, LLC) from the Schrödinger suite. Sample water orientation was enhanced, and protonation states at pH 7.0 were generated using PROPKA (Epik, Schrödinger, LLC). Lastly, a controlled reduction was executed to align heavy atoms to a root mean square deviation (RMSD) of 0.30, employing the OPLS3e force field (Sastry *et al*., 2013; Guan *et al*., 2021).

Active site prediction and grid generation

The protein target was subjected to SiteMap analysis (Ver. 2021, Schrödinger, LLC) to identify potential binding sites. Default parameters were utilized for identifying potential druggable sites based on D and site scores. SiteMap conducts exhaustive searches to examine the features of binding sites, identifying regions conducive to ligand-receptor interactions. A minimum requirement of 15 site points was set for the sites. The OPLS-3e force field, a standard grid with a resolution of 1.0, and restricted hydrophobicity specifications were employed. The grid box was determined based on the centroid of the residues identified by SiteMap. Default settings for grid generation were used, including a Van der Waals scaling factor of 1.00, an OPLS-3e force field, and a partial charge cutoff value of 0.25 (Halgren, 2009; Gudipati *et al*., 2018).

Molecular docking

This study utilized molecular docking techniques to examine the binding affinity of natural compounds to FoxO1. Preprocessed compounds were docked onto the FoxO1 receptor. The extra precision (XP) feature of the Glide module facilitated flexible docking within the predefined receptor grid. Ligand-receptor interactions were not subject to any restrictions. The resulting output was configured as a pose viewer file and subsequently visualized using the pose viewer tool (Kalirajan *et al*., 2019).

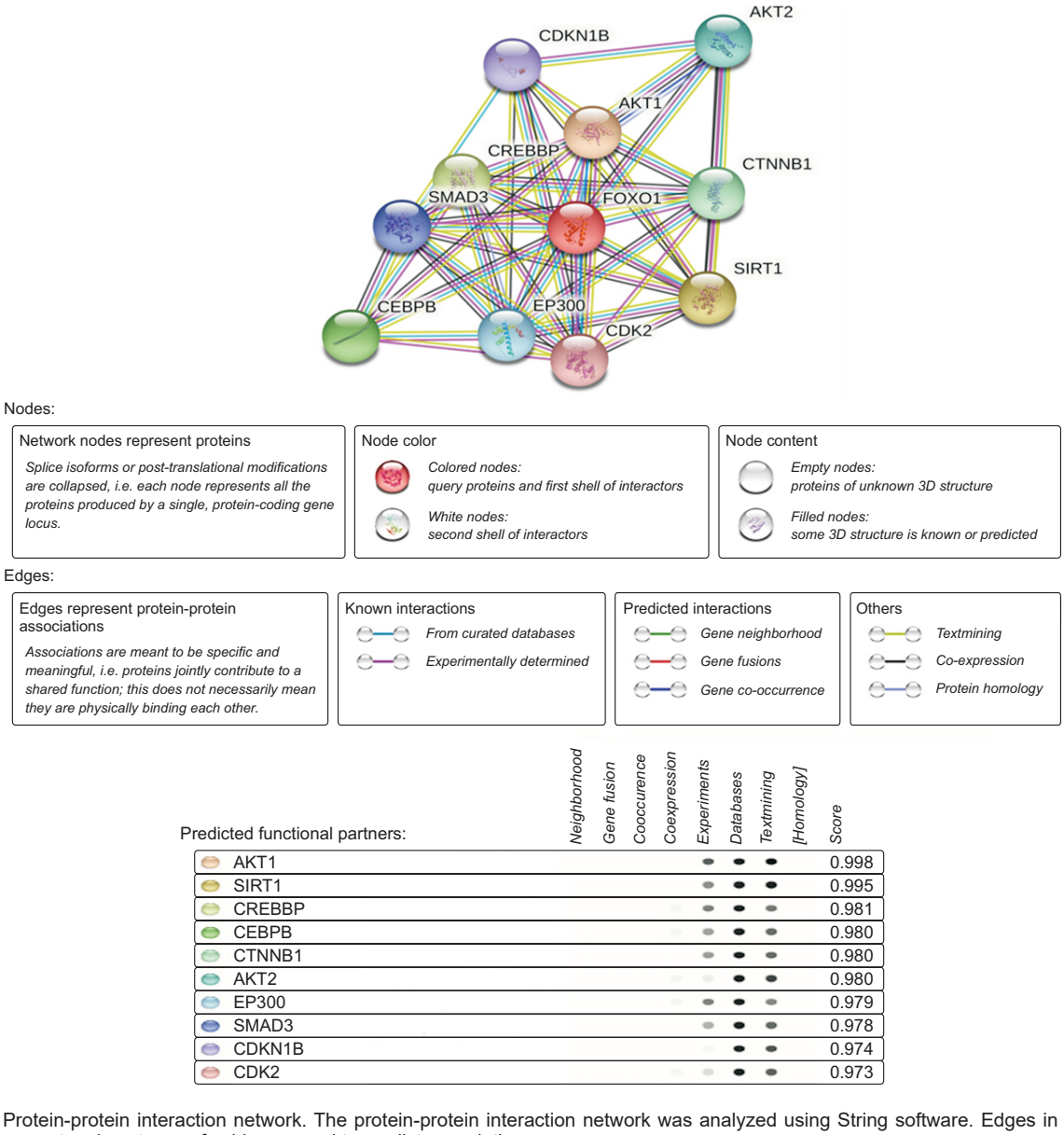

Fig. 1. Protein-protein interaction network. The protein-protein interaction network was analyzed using String software. Edges in the diagram represent various types of evidence used to predict associations.

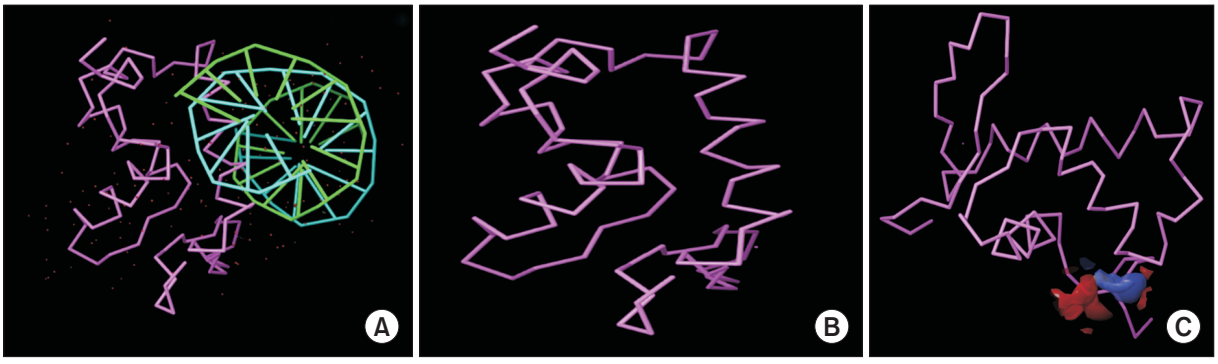

Fig. 2. Structure of FoxO1 (PDB ID-3CO6). (A) The raw crystal structure of human FoxO1, sourced from the Protein Data Bank. (B) The protein after undergoing processing via the Protein Preparation Wizard. (C) The active site identified using SiteMap.

Binding free analysis

The obtained ligand poses were further validated and re-scored through MMGBSA, executed via the Prime module in the Schrödinger suite (Ver. 2021, Prime, Schrödinger, LLC). The XP dock pose viewer file served as the input for these calculations. The relative energy of the complexes was evaluated using the OPLS3 force field in conjunction with the VSGB solvation method. The binding free energy was calculated using the following equation (Choudhary *et al*., 2020).

$$\Delta G \text{ bind} = E \text{ complex (minimized)} - E \text{ ligand (minimized)} - E \text{ receptor (minimized)} \quad (1)$$

ADMET analysis

The ADMET properties of the selected ligands were assessed using two computational platforms: the SwissADME (http://www.swissadme.ch/) web-server, provided by the Swiss Institute of Bioinformatics, and pkCSM. SwissADME offers predictions for various parameters including physicochemical descriptors, ADME attributes, pharmacokinetic properties, drug-likeness, and medicinal chemistry compatibility of small compounds. It utilizes a support vector machine (SVM) algorithm for these predictions. For the analysis, molecules were input into the SMILES list field based on their distinct structures. The calculations were initiated by clicking the run button, and the subsequent output featured a comprehensive set of values for each molecule, displayed in sequence. The data were then exported as a unified CSV file. Additionally, the ADMET properties of the chosen ligands were investigated using the pkCSM web-server, which is available at http://biosig.unimelb.edu.au/pkcsm/. pkCSM is an open-access machine learning platform specialized in analyzing and optimizing pharmacokinetics and toxicity characteristics through graph-based signatures. For this assessment, the SMILES string was uploaded to the web-server, and the prediction mode was set to ADMET. The computed results were promptly displayed in a tabular format, covering all relevant ADMET parameters (Pires *et al*., 2015; Daina *et al*., 2017; Irfan *et al*., 2023; Khan *et al*., 2023).

Molecular dynamics (MD) analysis

MD simulations of the two most ADMET-favorable natural compounds were performed using the Desmond module within the Schrödinger suite. The workflow involved sequential steps: system building, energy minimization, and molecular dynamics simulations. The single point charge (SPC) solvent model was employed during system building, with an orthorhombic boundary box. Following this, energy minimization of the model system was carried out. Simulations of the corresponding complexes proceeded using the NPT ensemble for 25 ns. The Nose-Hoover thermostat algorithm and the Martyna-Tobias-Klein barostat algorithm were employed to maintain a constant pressure of 1 atm and a temperature of 300 K. Short-range interactions were evaluated using a cutoff value of 9.0 for coulombic interactions (John *et al*., 2015; Kumar *et al*., 2019).

RESULTS

Functional protein association network analysis

The current investigation focused on examining the protein network related to FoxO1 using the STRING database. Lines

Table 1. Docking properties and MMGBSA of the selected compounds as determined using Glide and Prime

Name	Glide RB	Docking Score	Glide evdw	Glide ecoul	Glide Energy	Glide emodel	XP H Bond	XP Lipo EvdW	ΔG Bind	ΔG Bind Coulomb	ΔG Bind Covalent	ΔG Bind Hbond	ΔG Bind Lipo	ΔG Bind Solv GB	ΔG Bind vdW
Cellobiose	12	–6.709	–10.273	–16.425	–26.698	–31.509	–4.427	–0.913	–36.69	–11.75	4.51	–3.07	–23.9	15.22	–17.7
Scutellarin	10	–6.45	–16.919	–19.688	–36.607	–46.56	–4.438	–1.138	–44.09	–78.47	6.36	–2.22	–20.58	76.96	–25.63
Neochlorogenic acid	10	–6.32	–20.392	–19.984	–40.376	–47.112	–3.367	–1.482	–54.57	–81.48	2.32	–3.45	–21.1	67.99	–18.85
Chondroitin sulfate	12	–6.27	–19.021	–23.79	–42.81	–49.687	–3.951	–1.016	–35.23	–126.64	2.5	–6.29	–12.27	131.81	–24.34
Amygdalin	15	–6.199	–27.001	–15.539	–42.54	–47.955	–3.799	–1.999	–52.27	–25.99	6.62	–2.74	–24.14	23.93	–29.95
Fraxin	9	–6.13	–15.889	–15.324	–31.212	–34.046	–4.82	–0.931	–45.22	–31.18	3.22	–2.49	–17.77	19.55	–16.54
Salvianolic acid A	15	–6.127	–30.411	–14.253	–44.664	–54.774	–4.302	–2.042	–54.57	–97.3	13.37	–4.25	–20.86	86.15	–31.53
Desacetyl asperulosidic acid	11	–6.126	–14.86	–21.901	–36.761	–33.28	–4.205	–0.688	–44.41	–107.58	3.85	–4.08	–11.78	91.27	–16.08
Quercitrin	10	–6.107	–24.43	–16.271	–40.701	–52.418	–4.099	–2.126	–54.3	–30.22	3.97	–1.96	–21.74	23.18	–27.53

Glide evdw, Van der Waals energy; Glide ecoul, Coulomb energy; Glide energy, Modified Coulomb-van der Waals interaction energy; Glide emodel, Model energy, Emodel; H Bond, H-bond pair term; Lipo EvdW, Lipophilic term derived from hydrophobic grid potential at the hydrophobic ligand atoms; Coulomb, Coulomb energy; Hbond, Hydrogen-bonding correction; Lipo, Lipophilic energy; Solv GB, Generalized Born electrostatic solvation energy; vdW, Van der Waals energy; MMGBSA, Molecular mechanics-generalized Born surface area.

connecting the protein nodes in this graphical representation correspond to distinct evidence types, suggesting their role in establishing functional associations. The colors of the connecting lines represent various types of evidence. Moreover, the distance between nodes represents the confidence level of observed associations, which ranged from 0.998 to 0.973 (Fig. 1).

Virtual screening

The crystal structure of FoxO1 was obtained from the protein databank and displayed in simple mode (Fig. 2A). The downloaded protein was subsequently processed using the Protein Preparation Wizard (Fig. 2B). Its active site was identified with the SiteMap module within the Schrödinger suite (Fig. 2C). Previous work by Damayanti *et al*. (2017) demonstrated that rutin, xylopine, and anonaine bind to the same FoxO1 site.

Docking analysis revealed that over 80 natural products in the test library achieved a docking score below –5.0. Further investigation was conducted on the nine compounds with the most favorable docking scores, namely, cellobiose, scutellarin, neochlorogenic acid, chondroitin sulfate, amygdalin, fraxin, salvianolic acid A, desacetyl asperulosidic acid, and quercitrin. These scores ranged from –6.70 to –6.11. The analysis also indicated that hydrogen bonding, covalent energy, and Van der Waals forces largely contributed to the optimal binding of these selected compounds (Table 1). Fig. 3 depicts the best docking poses and interaction diagrams for the selected ligands.

MMGBSA analysis

To provide additional validation, MMGBSA calculations were executed on the nine selected natural compounds. MMGBSA serves to rank a congeneric series of ligands according to their free energies. Table 1 presents the relative binding free energies (ΔG bind) for these selected natural compounds, as calculated through the Prime module. These binding free energies ranged from –35.23 to –54.57 kcal/mol, signifying a high affinity toward FoxO1.

ADMET screening of nine top docked natural drugs

The ADMET properties of the nine selected compounds were assessed using SwissADME and pkCSM (Table 2).

Molecular docking simulations of best docking poses

MD simulations for neochlorogenic acid and fraxin were undertaken to corroborate our findings. RMSDs were employed to gauge the stability of the protein-ligand complexes over 25 ns MD simulations. For smaller proteins, RMSD fluctuations within the range of 1-3 Å are considered acceptable. In our study, RMSDs varied between 1.6 and 3.2 Å for the interactions involving neochlorogenic acid and FoxO1. RMSD plots

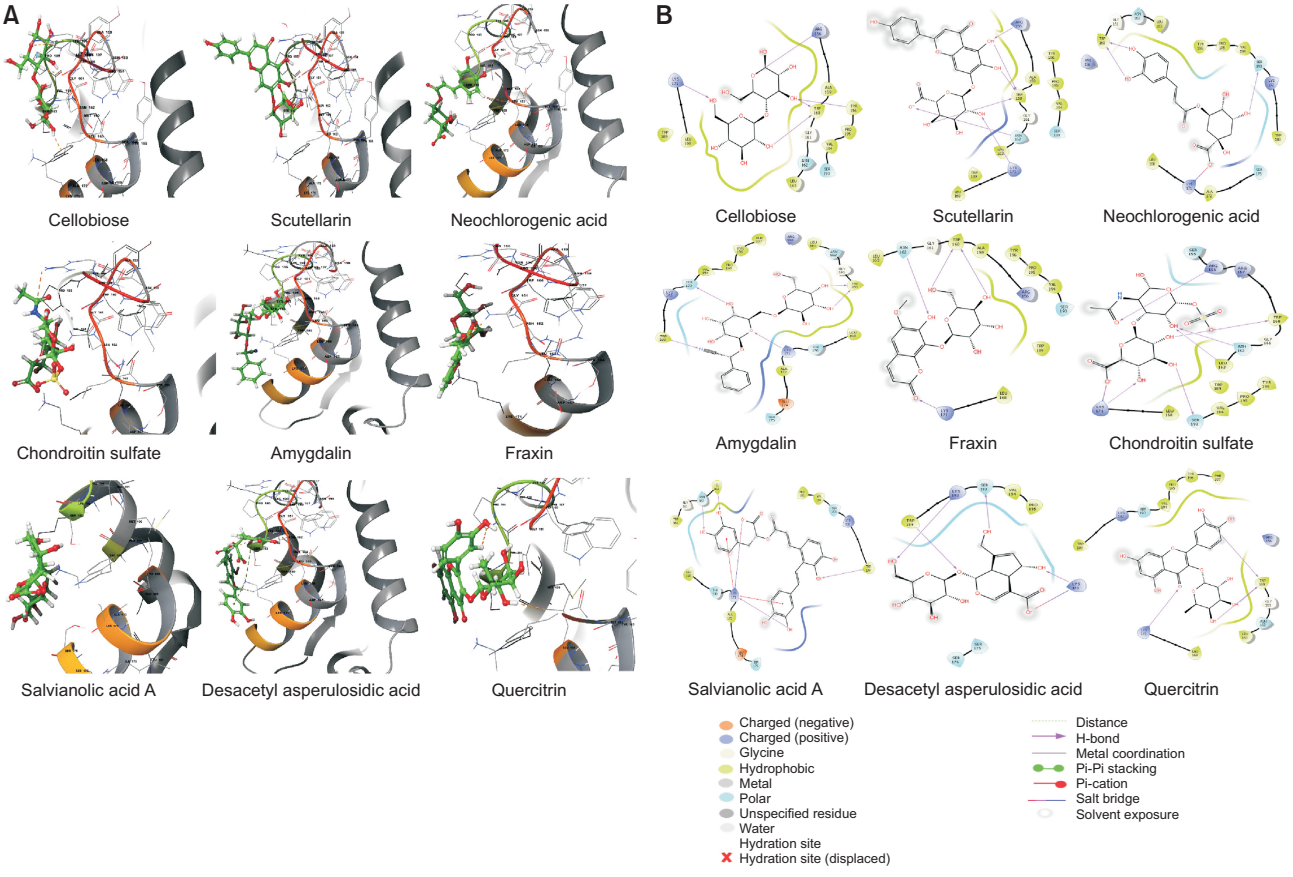

Fig. 3. Docking poses and ligand interactions. (A) 3D docking poses of the nine top-scoring compounds against FoxO1. (B) 2D ligand interaction diagram illustrating the various types of contacts between the nine top-scoring compounds and FoxO1.

Table 2. ADMET properties of the nine selected compounds as determined using SwissADME and pkCSM

Name	Mol. Weight	No. of Heavy Atoms	CYP2D6/ CYP2D6/ CYP2C9 Inhibitor	BBB Per.	Log S (ESOL)	Sol. Class	Lipinski Violations	SA	Intestinal Abs. (%) (Human)	Pgp I/II Inhibitor	AMES Tox.	hERG I/11 Inhibitor	Liver Tox.	Skin Sensation
Cellobiose	342.3	23	No	No	0.55	HS	2	5.41	4.807	No	No	No	No	No
Scutellarin	462.36	33	No	No	–3.27	S	2	5.12	13.836	No	No	No	No	No
Neochlorogenic acid	354.31	25	No	No	–1.62	VS	1	4.16	36.377	No	No	No	No	No
Chondroitin sulfate	463.37	30	No	No	0.75	HS	2	5.45	0	No	No	No	No	No
Amygdalin	457.43	32	No	No	–0.65	VS	2	5.41	26.722	No	No	No	No	No
Fraxin	370.31	26	No	No	–1.76	VS	0	5.03	47.525	No	No	No	No	No
Salvianolic acid A	494.45	36	No	No	–5.15	MS	1	4.18	26.73	No	No	No	No	No
Desacetyl asperulosidic acid	390.34	27	No	No	0.45	HS	2	5.86	0.139	No	No	No	No	No
Quercitrin	448.38	32	No	No	–3.33	S	2	5.28	52.709	No	No	No	No	No

Mol. Weight, Molecular Weight; Per., Permeability; SA, Synthetic Accessibility; Abs., Absorption; Pgp, P-glycoprotein; Tox., Toxicity.

indicated that the interactions with neochlorogenic acid stabilized after 15 ns, while interactions with fraxin remained stable for the entire duration of the simulation (Fig. 4). Additionally, ligand interaction diagrams highlighted that ARG 156, TRP 160, LYS 171, LYS 200, ASP 199, ALA 159, and TYR 196 were pivotal in facilitating the interactions between FoxO1 and both neochlorogenic acid and fraxin (Fig. 5).

DISCUSSION

FoxO1 is a multifunctional protein implicated in various cellular processes including apoptosis, cell cycle regulation, glucose metabolism, muscle growth, and differentiation (Lu and Huang, 2011). Previous studies have shown that Akt phosphorylates FoxO proteins, leading to their nuclear exclusion and subsequent loss of activity (Gross *et al*., 2008). Additionally, under conditions of oxidative stress, SIRT1 interacts with FoxO1 in mice and deacetylates specific lysine residues K242, K245, and K262 (Greer and Brunet, 2005). Protein-protein interaction analyses using STRING have identified the top 10 functional partners that directly interact with FoxO1. These partners include AKT1, SIRT-1, CREBBP, CEBPB, CTNNB1, AKT2, EP300, SMAD3, CDKN1B, and CDK2, with confidence scores ranging from 0.998 to 0.973.

While substantial data exist on the role of FoxO1 in various biological processes, there is limited information on direct inhibitors of FoxO1. In our study, we sought to investigate the inhibitory potentials of 550 natural products against FoxO1. Docking scores for the nine selected compounds—namely, cellobiose, scutellarin, neochlorogenic acid, chondroitin sulfate, amygdalin, fraxin, salvianolic acid A, desacetyl asperulosidic acid, and quercitrin—ranged from –6.70 to 6.11. These scores suggest a good binding affinity of the selected compounds with FoxO1, which was further corroborated by MMGBSA analysis.

DMPK studies, also referred to as ADMET investigations, play a crucial role in drug discovery and development by elucidating drug-likeness properties. Data suggest that nearly 50% of drug candidates fail at the clinical trial stage due to insufficient effectiveness, while approximately 40% fail because of unacceptable toxicity. For instance, mibefradil, soruvidine, and phenylpropanolamine hydrochloride were removed from the market due to adverse drug-drug interactions or toxic effects. Recognizing the significance of these metrics, both regulatory agencies and pharmaceutical companies have shifted their focus to include ADME and Tox evaluations as integral components of drug quality assessment and success prediction. Consequently, such studies are increasingly being initiated earlier in the drug development timeline. Given that conducting ADMET experiments on a large scale during early development phases is often impractical because of logistical and financial constraints, *in silico* ADMET predictions have gained prominence. The advent of high-quality *in silico* ADMET models facilitates concurrent analysis and optimization of compound efficacy and druggability. In the present study, we utilized free web servers, specifically SwissADME and pkCSM, for ADMET profiling.

The molecular weight of a drug substantially influences its oral bioavailability, with a molecular weight of ≤ 500 being optimal (Ya'u Ibrahim *et al*., 2020). In this study, the molecular weights of the nine evaluated compounds ranged from 320 to

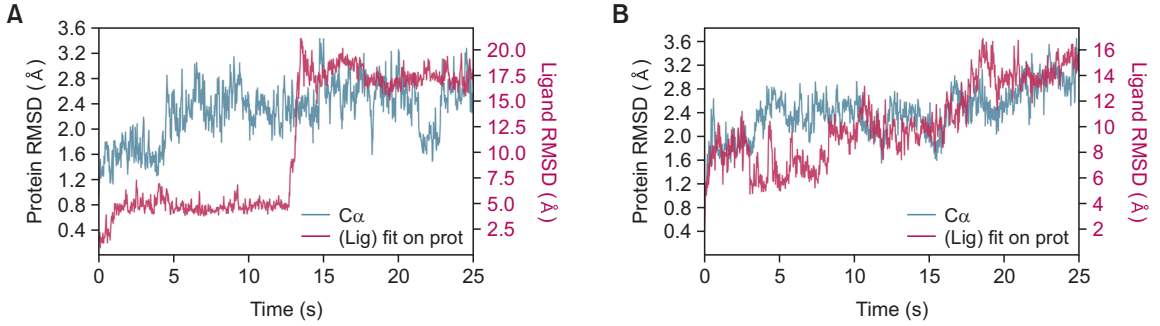

Fig. 4. RMSD plots of docked compounds. The RMSD plots show (A) neochlorogenic acid and (B) fraxin over 25 ns simulation runs against FoxO1.

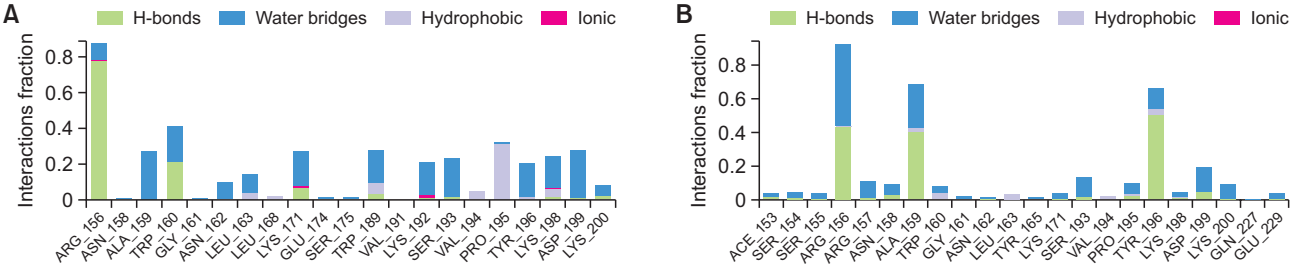

Fig. 5. Protein-ligand interactions. Protein-ligand interaction plots for (A) neochlorogenic acid and (B) fraxin over 25 ns simulation runs against FoxO1.

495, and the number of heavy atoms varied between 23 and 36. Evaluating blood-brain barrier (BBB) permeability is critical in drug development, as candidate compounds must traverse this barrier to exert pharmacological effects on the brain. Interestingly, none of the studied compounds displayed BBB permeability. Additionally, understanding interactions with cytochrome P450 (CYP) is vital, given this isoenzyme's significant role in metabolic drug clearance. Between 50 and 90% of contemporary drugs are substrates for CYP isoforms such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The inhibition of these isoforms can lead to pharmacokinetic-related drug-drug interactions and potentially toxic or undesirable side effects due to impaired drug clearance (Daina *et al*., 2017). Notably, none of the chosen compounds demonstrated inhibitory activity against CYP2D6 or CYP2C9, suggesting a lower risk of side effects. Moreover, synthetic accessibility (SA) is an important characteristic of drug development, as some computer-designed compounds are not feasible for synthesis. SA scores range from one (very easy) to ten (extremely difficult). ADME profiling with SwissADME indicated that all nine compounds had SA scores ranging from 3.50 to 5.86, signifying their potential for synthesis. Human intestinal absorption (HIA) is another key ADMET property, given that drug absorption by the body is a complex process that presents challenges for analysis. HIA is a vital step in the delivery of drugs to their intended targets (Srivastava *et al*., 2022). The intestine serves as a primary site for the absorption of orally administered drugs. Our results revealed that compounds such as neochlorogenic acid, fraxin, quercitrin, and dihydromyricetin exhibited good intestinal absorption rates of greater than 30%.

P-glycoprotein (P-gp) regulates cellular uptake and transport of xenobiotics and toxins (Amin, 2013), and its inhibition has been implicated in multiple clinical drug-drug interactions (Keogh, 2012). For instance, clarithromycin interferes with the transport of digoxin (a P-gp substrate), resulting in elevated plasma levels and diminished renal clearance (Wakasugi *et al*., 1998). In the present study, none of the nine chosen compounds exhibited P-gp inhibitory activity.

The Ames test, which utilizes the bacterial strain *Salmonella typhimurium*, assesses the mutagenic potential of drugs. pkCSM analysis revealed that none of the selected compounds exhibited Ames toxicity, thereby indicating a lack of mutagenic risk. Conversely, human ether-a-go-go-related gene (hERG) channels play a critical role in cardiac function. Impaired hERG function can extend ventricular action potentials, prolong electrocardiographic QT intervals, and increase the risk of lethal ventricular arrhythmias. Importantly, none of the selected compounds demonstrated hERG inhibitory activity, suggesting an absence of cardiotoxic risk. Liver toxicity, another major factor contributing to drug development failure, was not observed for any of the tested compounds (Wang *et al*., 2019). Additionally, none of the compounds were predicted to cause skin sensitization (Bucao and Solidum, 2022). Among the nine compounds examined, all displayed negligible toxicity. However, most of them—except for neochlorogenic acid, salvianolic acid A, and fraxin—had more than two Lipinski violations and were therefore excluded from further study. Notably, salvianolic acid A exhibited intestinal absorption greater than 30%. Consequently, only neochlorogenic acid and fraxin were selected for subsequent molecular dynamics studies.

MD simulations typically model the motions of atoms using Newton's equations, while docking provides a static snapshot of a compound's binding pose within the active site of a

specified protein. To assess the stability of the protein-ligand complex, we employed MD simulations, consistent with previous studies (Choudhary *et al*., 2020). Our MD simulations confirmed that the interaction with fraxin remained stable throughout the simulation period.

FoxO1 has been implicated in various physiological processes, including carcinogenesis, skeletal muscle differentiation, and metabolic disorders. In the present study, we explored interactions between FoxO1 and other proteins such as AKT and SIRT1 to gain preliminary insights into FoxO1's functions. A library of 550 natural products underwent docking and MMGBSA analyses. Nine compounds that exhibited the highest docking scores (below –6.0) and MMGBSA binding energies (below –35 kcal/mol) were selected for further study. Among them, neochlorogenic acid and fraxin displayed promising drug-like properties. Specifically, MD simulations verified the stable interaction between fraxin and FoxO1.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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