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Original Article

Mechanistic Insight and Inhibitory Potential of Fluvastatin-Derivatives against Dyslipidemia

Ali Mahzari[1,*], Eman H. Khalifa[1], Ali A Zaeri[1], Hanan E Alyahyawi[1], Wanian M Alwanian[2], Saad M. Wali[3], Ohood Almuzaini[3], Yazeed Alshuweishi[4]

[1]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, SAUDI ARABIA.
[2]Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah, SAUDI ARABIA.
[3]Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah, SAUDI ARABIA.
[4]Chair of Medical and Molecular Genetics Research, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, SAUDI ARABIA.

ABSTRACT

Background: Cardiovascular diseases comprehend a array of situations that disturb the heart and blood vessels, making them a principal cause of morbidity and mortality worldwide. Dyslipidemia, an imbalance in lipids, such as cholesterol, Low-Density Lipoprotein Cholesterol (LDL-C), triglycerides and High-Density Lipoprotein (HDL), is a significant risk factor for cardiovascular disease and contributes to numerous deaths annually. The development of cholesterol-degrading drugs, known as statins, began in the mid-1970s and their effects on intracellular processes have been well documented. **Materials and Methods:** Fluvastatin, a statin widely used in cardiovascular disease, inhibits hydroxymethyl glutaryl-coenzyme A, a vital enzyme in cholesterol biosynthesis. This study aimed to discover new therapeutic drugs to prevent cardiovascular diseases by deriving new compounds from fluvastatin. **Results:** The investigation involved several processes including derivative generation, molecular docking, ADME analysis, PASS prediction, rodent toxicity assessment and identification of possible adverse effects. After evaluating a vast dataset of 194 derivatives, compounds that met stringent criteria across all parameters were selected. **Conclusion:** Two promising compounds from this filtered set were further assessed through Molecular Dynamics (MD) simulation and Principal Component Analysis (PCA), along with the control.

Keywords: Cholesterol, CVDs, Drug designing, Dyslipidemia, MD simulation, Molecular docking, PCA.

Correspondence:
Dr. Ali Mahzari
Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, SAUDI ARABIA.
Email: amoosa@bu.edu.sa

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INTRODUCTION

Cardiovascular Diseases (CVDs), a broad term encompassing conditions affecting the heart and blood vessels, pose a significant global health threat, leading to millions of deaths worldwide. CVDs are categorized as noncommunicable diseases, contributing to a decline in overall life expectancy (Sadeghi *et al.*, 2017). The global prevalence of CVD is on the rise and key risk factors include obesity, hypertension, dyslipidemia and Diabetes Mellitus (DM) (Mathers and Loncar, 2006). Unhealthy lifestyles, physical inactivity, smoking, alcohol consumption, aging, and a family history of cardiovascular diseases are major contributors to the risk of CVD (Després *et al.*, 2008; Martirosyan *et al.*, 2007; Ordovas, 2006). Dyslipidemia, characterized by abnormal blood lipid levels, is a key modifiable risk factor for CVD. It involves elevated Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), and Triglycerides (TG) levels, along with decreased High-Density Lipoprotein Cholesterol (HDL-C) concentration (Wu *et al.*, 2018; Klop *et al.*, 2013; Guzzaloni *et al.*, 2000). Dyslipidemia significantly influences the development and progression of atherosclerosis, a condition associated with stroke, heart failure, myocardial infarction and kidney failure. Factors such as elevated LDL cholesterol, low HDL cholesterol, and high triglyceride concentrations are connected with an increased cardiovascular risk (O'Meara *et al.*, 2004; Joshi *et al.*, 2014; Brown *et al.*, 2000; Hadaegh *et al.*, 2006).

As individuals age, the prevalence of dyslipidemia tends to rise (Hedayatnia *et al.*, 2020). The primary diagnostic method for dyslipidemia is a fasting lipid panel that includes measurements of total cholesterol, LDL, HDL, and triglycerides. The age at which dyslipidemia screening should commence remains a matter of debate. Furthermore, genetic factors can contribute to dyslipidemia, with familial hypercholesterolemia as a notable example. Despite advancements in cardiovascular medicine,

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CVDs remains a growing public health concern, particularly in developing and economically challenged nations. Individuals at moderate to high risk of developing cardiovascular diseases are often prescribed statins, with atorvastatin being a commonly used medication among various statins (Grundy and Stone, 2019; Anderson *et al.*, 2016). The rising prevalence of cardiovascular illnesses such as heart attack, angina and artery disease has contributed significantly to the increased use of this class of medications (Mihaylova *et al.*, 2012; Taylor *et al.*, 2013).Some studies have reported that statins reduce both cholesterol levels and CVD risk with no side effects. Unlike various individuals from the statin group, atorvastatin has proven to be a functional compound and does not require any attention to activate.

HMG-CoA reductase plays a major role in cholesterol regulation. HMG-CoA reductase was the first enzyme to be involved in the mevalonate pathway, which is considered to be responsible for the rate-limiting step of cholesterol production. Statins competitively inhibited HMG-CoA reductase. Fluvastatin, an HMG-CoA reductase inhibitor treated as an inhibiting drug compound was discovered at about the same time as atorvastatin with greater efficacy (Sikorski, 2007). In individuals who grew up, fluvastatin was prescribed from person to person with hypercholesterolemia and mixed dyslipidemia to diminish cholesterol levels in the bloodstream. In particular, this medication lowers low-density lipoprotein cholesterol and triglycerides while extending high-density lipoproteins. From every aspect of view, the inhibitory activity of HMG-CoA enzyme has been proven to reduce cholesterol synthesis, resulting in an increase in the liver's absorption of low-density lipids via LDL receptor modification. More explicitly, statin prescriptions were proven to seriously repress HMG-CoA reductase (Moghadasian, 1999), which catalyzes the conversion of HMG-CoA to mevalonic acid (Liao and Laufs, 2005). The impact of dyslipidemia can shift from one individual to another and geographical area, depending on age, illness, climate, dietary routine, and lifestyle factors. This study demonstrated that the synergistic action of dyslipidemia and other CVD risk factors in cardiac patients accelerates the progression of atherosclerosis, cardiovascular morbidity, and death. Recognizing the probable cause of dyslipidemia in patients with CVD is pivotal in dealing with the illness and decreasing further complexities. Therefore, the study aimed to identify fluvastatin derivatives to prevent CVDs with minimal side effects, utilizing the advancement of bioinformatics tools and databases to focus on derivatives as new pharmaceutical alternatives for better and safer control of cardiovascular disorders. The analysis was based on distinct parameters, including pharmacokinetics, drug-likeliness features, toxicity profiling assessment, molecular interactions and molecular dynamics. The protein chosen for the study was HMG-CoA reductase with atorvastatin (PDB ID 1HWK). Using these leveraging bioinformatics techniques, we were able to thoroughly assess research contributing to gaining an effective compound (Mihaylova *et al.*, 2012; Taylor *et al.*, 2013; Sikorski, 2007; Moghadasian, 1999; Liao and Laufs, 2005).

MATERIALS AND METHODS

Retrieval and preparation of target protein

The target protein, 1HWK, with a resolution of 2.22 Angstroms, was gained from the Protein Data Bank in PDB format, which was sourced from rcsb.org (Domínguez *et al.*, 2021).Afterwards, the macromolecule was prepared, which involved the removal of water particles and the formation of hydrogen bonds, using the BIOVIA Discovery Studio Visualizer 2021 (Singh *et al.*, 2021).

Bioisosteric substitution and preparation of ligands database

Retrieving the parent compound in standard SMILE format was the starting point. To generate the primary molecule, the authoritative SMILE ID was inputted into the web-based tool MolOpt (Modee *et al.*, 2023). Subsequently, the bio isosteric substitution technique was applied, in which the functional groups of molecules were exchanged to enhance their potency and efficacy (Dick and Cocklin, 2020). The data mining (fast) replacement technique provided rules for drawing derivatives. The server automatically generated derivatives with SMILE IDs, which were then converted into the PDB format using the ChemDB chemoinformatic gateway for further analysis (Langdon *et al.*, 2010).

Virtual Screening

Molecular docking procedures were conducted using DockThor, a widely recognized and versatile docking web server (Santos *et al.*, 2020), to evaluate the binding affinity of the acquired ligand analogs in comparison to the control. The proteins and analogs were carefully uploaded to ensure a smooth execution. The binding pocket was defined and other parameters, such as docking runs and evaluations per docking, remained unchanged. For individual runs, the server provides the best pose based

Table 1: Binding affinities of ligands using DockThor tool.

Original Ligand Name	Coded Ligand Name	Binding Affinity (kcal/mol)
control.pdb	ligand_e787dfc97e	-7.04
mol_000.pdb	ligand_6f4099b31b	-6.92
mol_001.pdb	ligand_ef3441670f	-6.89
mol_002.pdb	ligand_068ab1	-7.00
mol_003.pdb	ligand_46a0627	-8.00
mol_004.pdb	ligand_a1096914f4	-7.52
mol_005.pdb	ligand_a61ae1016	-7.37
mol_008.pdb	ligand_62c5696a2a	-7.83
mol_012.pdb	ligand_bbd5403f04	-7.75
mol_014.pdb	ligand_f1ef0b	-7.50

on binding energies and clustering probability. The results were retrieved and saved in an Excel file for in-depth analysis. Biovia Discovery Studio was used to identify the most favorable compound interactions. The DockThor score, similar to delta G, was used to measure the binding affinity and rank different ligand molecules (Santos *et al*., 2020; De Magalhães *et al*., 2014).

Evaluation of drug-likeness and pharmacokinetics predictions

In evaluating the pharmacokinetic properties of drugs, ADMET examines various parameters such as surface area, number of hydrogen bond donors and BBB permeation. Profiling is a crucial step in drug development and highlights its importance (Singh *et al*., 2021; Azzam, 2023). Profiling helps to identify compounds with drug-like properties and favorable pharmacokinetics, indicating their potential as drug candidates. To ensure the accuracy of our results, we employed Swiss ADME (Brenk *et al*., 2008; Muegge *et al*., 2001; Daina *et al*., 2017; Lipinski, 2004) and pkCSM (Ze-jun *et al*., 2023; Pires *et al*., 2015). prediction tools.

In silico Prophecy of Activity Spectra for compounds (PASS)

The PASS prediction tool was utilized to investigate a wide array of biological activities, such as antifungal, antidote and antiviral effects (Dmitriev *et al*., 2019). (https://www.way2drug.com/passonline/index.php). First, the compound structures were transformed into the SMILES format using the online tool Chemdb. These data were then uploaded to the system to analyze the mass spectrum activities. The values were determined based on the principles of Pa and Pi, which represent the probability of active and inactive molecules, respectively. For a compound to be deemed acceptable, its Pa and Pi values must fall within the range of 0.000-1.00, with Pa being greater than Pi.

Anticipating compound toxicity and side effects

To predict severe acute toxicity and potential secondary effects in rats, researchers have used the online tools GUSAR and ADVER-Pred. GUSAR (http://www.way2drug.com/gusar/) (Lagunin *et al*., 2011) was employed to predict rat toxicity based on different routes of administration, including intraperitoneal, intravenous, oral and subcutaneous. Meanwhile, ADVER-PRED (http://www.way2drug.com/adverpred) (Ivanov *et al*., 2018) was utilized to determine potential side effects.

Performing Molecular Dynamics

Molecular Dynamics has emerged as a valuable approach for understanding the atomic-level chemistry of biological macromolecules (Khan *et al*., 2018).Through rigorous analysis of various trajectories, each parameter was thoroughly examined, leading to refinement of the compounds. The top-hit compounds underwent Molecular Dynamics (MD) simulation studies conducted at 300 K using GROMACS software version 5.1.2 (Stenberg and Stenqvist, 2020).The gmx grep module of the GROMACS package was utilized to extract and compile complexes and topologies. The affected compounds were subjected to a force field CGenFF (CHARMm General Force Field) (Fischer *et al*.,

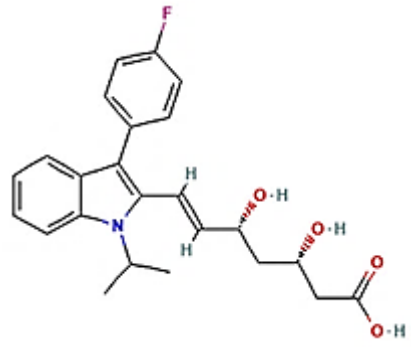

Figure 1: 2D representation of parent compound Fluvastatin.

Table 2: ADME properties of analogs.

Compounds	Formula	MW*	RB*	HBA*	HBD*	BBB*	BS*	RO5	TPSA
control.pdb	$C_{24}H_{26}FNO_4$	411.47	8	5	3	No	0.56	Yes	82.69
mol_000.pdb	$C_{20}H_{19}FN_2O_4$	370.37	7	5	3	No	0.56	Yes	91.56
mol_001.pdb	$C_{21}H_{22}FNO_5$	387.4	6	6	4	No	0.56	Yes	102.92
mol_002.pdb	$C_{21}H_{22}FNO_3$	355.4	6	4	2	Yes	0.85	Yes	62.46
mol_003.pdb	$C_{21}H_{22}FNO_5$	387.4	6	6	3	No	0.56	Yes	98.07
mol_004.pdb	$C_{21}H_{22}FNO_5$	387.4	6	6	4	No	0.56	Yes	102.92
mol_005.pdb	$C_{21}H_{22}FNO_5$	387.4	10	6	3	No	0.56	Yes	103.7
mol_008.pdb	$C_{21}H_{22}FNO_3$	355.4	6	4	2	Yes	0.85	Yes	62.46
mol_012.pdb	$C_{21}H_{22}FNO_4$	371.4	6	5	3	No	0.56	Yes	82.69
mol_014.pdb	$C_{21}H_{22}C_lFO_4$	392.85	8	5	1	No	0.85	Yes	63.6

*MW: molecular weight, RB: ratable bond, HBA: hydrogen bond acceptor, HBD: hydrogen bond donor, BBB: blood-brain barrier, BS: bioavailability, RO5: Lipinski rule

Table 3: Drug-likeness and toxicity profiling of selected compounds.

Parameters	ANALOGS									
	Control	Mol_000	Mol_001	Mol_002	Mol_003	Mol_004	Mol_005	Mol_008	Mol_012	Mol_014
Absorption										
Skin perm. (log mol/l)	-2.735	-2.734	-2.735	-2.735	-2.735	-2.735	-2.735	-2.773	-2.735	-2.734
Water solubility (Log Kp)	-3.454	-4.567	-2.946	-4.097	-3.829	-3,162	-2.827	-5.089	-3.481	-5.656
Distribution										
CNS perm.	-2.411	-2.822	-3.203	-2.272	-3.325	-3.259	-3.081	-2.225	-2.3	-2.099
BBB perm.	-1.194	-0.871	-1.435	0.209	-1.015	-1.353	-0.959	-0.342	-1.165	-0.453
Metabolism										
CYP2D6 subs.	Yes	No	No	No	No	No	No	No	No	No
CYP3A4 subs.	Yes	No	No	Yes	Yes	No	No	No	No	Yes
CYP1A2 inh.	No	No	No	Yes	No	No	No	Yes	No	No
CYP2C19 inh.	No	No	No	No	No	No	No	No	No	No
CYP2C9 inh.	Yes	No	No	Yes	No	No	No	No	No	No
CYP2D6 inh.	No	No	No	No	No	No	No	No	No	No
CYP3A4 inh.	No	No	No	No	No	No	No	No	No	No
Excretion										
Total clearance	0.507	0.644	0.414	0.531	0.073	0.334	0.26	0.586	0.4	-0.031
RenalOCT2 subs.	No	No	No	No	No	No	No	No	No	No
Toxicity										
AMES	No	No	No	No	No	No	No	No	No	No
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No	No	No	No	No	No
Hepatotoxicity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

*Perm: Permeability, subs: Substrate, inh: inhibitor.

2015). Subsequently, the TIP3 model was employed to solvate each complex within a cubic box. After applying the boundary conditions, salts were introduced to equilibrate the charges. The framework underwent 25,000,000 steps using the steepest descent algorithm for energy minimization. The temperature was increased while maintaining the stable NVT and NPT conditions. Following the equilibration phase, a mesh was generated using the Ewald method (Grant *et al.*, 2006; Petersen, 1995).The simulation lasted for 10 ns, providing in-depth insights into submolecular dynamics (Yu and Dalby, 2020; Zhang *et al.*,2020).

Principal component analysis in protein dynamics

Principal Component Analysis (PCA) offers valuable insights into the overall behavior of a protein during various simulations (Maisuradze *et al.*,2009). Specifically, it provides information on a protein's large-scale average motion and exposes the underlying structures and atomic fluctuations (David and Jacobs, 2014). In the context of the galaxy framework, PCA was used to explore conformational changes induced by complex interactions between proteins and drugs (Khan *et al.*, 2023). The 10 ns trajectory of the targeted protein and selected compounds was carefully analyzed to emphasize the parameters and conformations (Patel *et al.*, 2024).

RESULTS

Ligands library

Fluvastatin, the parent compound, was used by the MolOpt webserver to generate diverse analogs with improved pharmacokinetic profiles. To achieve this, bioisosteric replacement sites were identified, resulting in 194 derivatives. Information for these compounds was obtained from a previous study. CSV file for subsequent molecular docking analysis. Figure

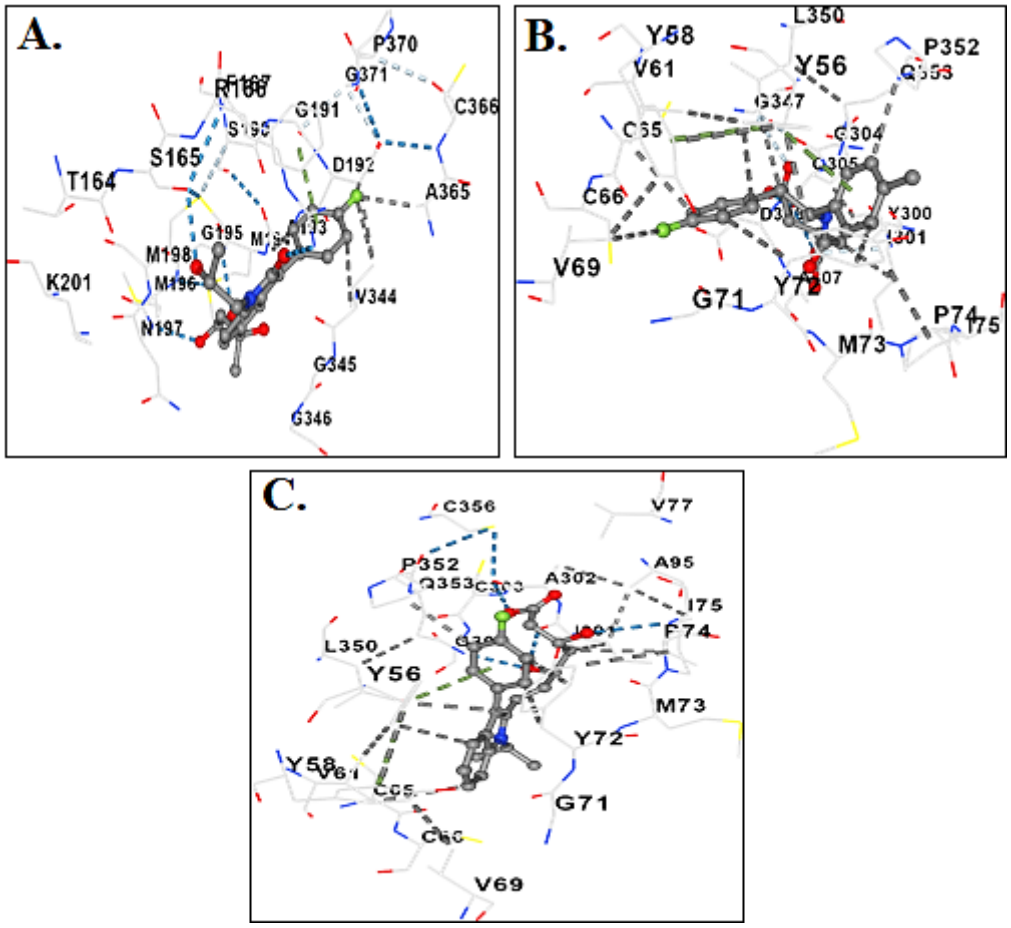

Figure 2: 3-dimensional molecular structures surrounded with Interacting amino acid residues. A: Compound 03 and B: Compound 05 and C: Control.

Table 4: PASS prediction.

Compounds	Cardio protectant		Cardiotonic		Cholesterol absorption inhibitor		Cholesterol antagonist		Anti-hyper cholesterolemic		Anti-inflammatory	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
control.pdb	0,286	0,094	0,223	0,143	0,038	0,038	NA	NA	0,248	0,080	0,283	0,084
mol_000.pdb	NA	NA	NA	NA	NA	NA	NA	NA	0,155	0,148	0,252	0,210
mol_001.pdb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
mol_002.pdb	0,204	0,183	NA	NA	NA	NA	0,215	0,197	0,215	0,102	0,409	0,091
mol_003.pdb	0,273	0,106	0,250	0,115	0,043	0,019	0,308	0,103	NA	NA	NA	NA
mol_004.pdb	0,246	0,131	NA	NA	NA	NA	0,265	0,138	NA	NA	NA	NA
mol_005.pdb	NA	NA	NA	NA	NA	NA	0,267	0,136	0,395	0,038	NA	NA
mol_008.pdb	NA	NA	NA	NA	NA	NA	0,308	0,103	0,289	0,060	0,359	0,117
mol_012.pdb	0,265	0,113	NA	NA	NA	NA	0,277	0,127	NA	NA	NA	NA
mol_014.pdb	NA	NA	NA	NA	NA	NA	NA	NA	0,392	0,039	0,473	0,065

1 illustrates the chemical structure of fluvastatin as shown in a 2D representation.

Virtual screening of derivatives

The DockThor web server, a flexible docking tool, was used to determine the binding energies of the 194 derivatives. This method involves assigning unique ligand identities and predicting the binding energy, taking into account additional intermolecular interaction energy, which encompasses the sum of van der Waals and electrostatic energy, van der Waals energy and electrostatic energy. A range of docking scores was recorded for different derivatives, with values ranging from -8.30 to -6.06 kcal/mol. The top nine molecules, exhibiting superior binding energies and interactions, were selected along with the control compound for further evaluation (Supplementary Table 1).

Drug-likeness profiling and ADMET evaluation

The investigation of absorption, desorption, metabolism, excretion and toxicity properties was conducted with great care using the ADMET profile, which is of great importance in drug discovery (Vardhan and Sahoo, 2020).The properties of the chosen compounds were thoroughly assessed to determine their drug-likeness and it was observed that all of them met the ADME criteria and adhered to Lipinski's rule (Supplementary Table 2). Furthermore, Swiss ADME conducted an extensive examination of the ADME profile (Hanee *et al.*, 2021) Beyond drug-likeness parameters, other molecular properties, including skin permeability, various metabolic parameters, AMES and hepatotoxicity, were uncovered using the pkCSM tool, which is used for toxicity profiling (Chen *et al.*, 2005). All parameters of the selected compounds were predicted and analyzed with precision (Supplementary Table 3).

PASS prediction spectrum analysis

The online tool way2 drug was utilized to examine the selected compounds through PASS prediction. This examination was showed in accordance with the procedures set forth (Filimonov *et al.*, 2014). In their inactive state, the chosen medicinal compounds exhibited lipid-related features, including cardioprotectant, cardiotonic, cholesterol absorption inhibitor, antagonist, anti-hypercholesterolemic and anti-inflammatory properties, which were assessed for their risk of cardiovascular disease (Kimball *et al.*, 2019). The PASS prediction involves determining the likelihood of active and inactive compounds, as outlined (Islam *et al.*, 2022). The primary goal of the PASS prediction was to thoroughly analyze the parameters and ensure that each compound demonstrated a Pa value greater than Pi, as shown in (Supplementary Table 4).

Assessment of acute rat toxicity and side effects

All compounds were subjected to a thorough evaluation of acute toxicity in rats, as shown in (Supplementary Table 5). Acute rat toxicity in rodent models was predicted using the GUSAR database. It is crucial to assess potential adverse side effects in the pursuit of lead candidacy. The Adver-pred web service was employed to analyze the compounds and the outcomes are reported along with the values of Pa and Pi, as presented in (Supplementary Table 6).

This study focused on the comprehensive analysis of mol-003 and mol-005, two compounds that demonstrated non-toxic properties, as well as negative AMES results and no inhibition of hERG channels or hepatotoxicity. These findings indicated that these compounds have minimal side effects. The interaction details and interacting residues of these compounds are provided in (Supplementary Table 7) and Figure 2, respectively.

Molecular Dynamic Simulation

Molecular Dynamics (MD) simulations were executed for a period of 10 ns using the GROMACS setup with the aim of evaluating stability and gaining insights into the identified hits. To comprehensively analyze the stability of the complexes comprising ligands and proteins, several trajectories were incorporated (Tarique *et al.*, 2017). Plots, including Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation

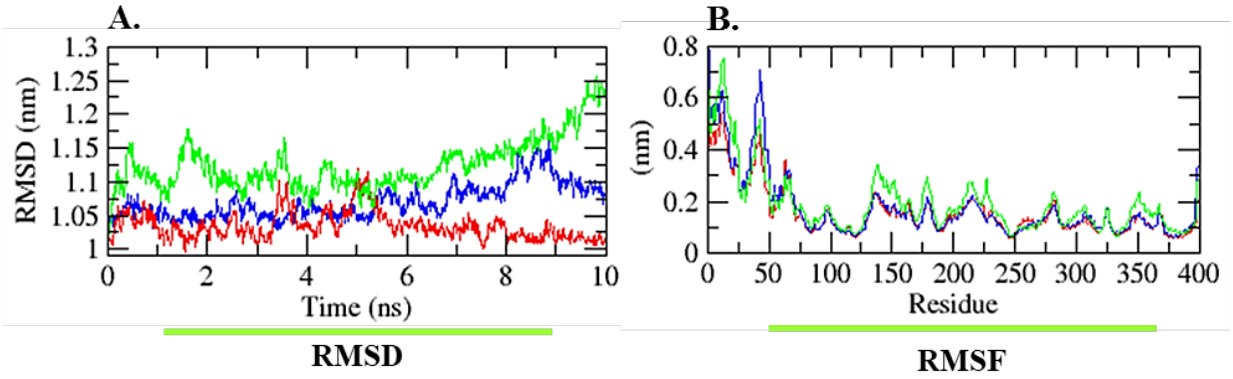

Figure 3: Time-dependent (A) RMSD and (B) RMSF plots as function of time obtained for compound 03 as red color, blue color represented as compound 05, while the green color represented the control.

Table 5: Acute rat toxicity prediction.

Rodent Parameters of Compounds										
	Control	Mol_000	Mol_001	Mol_002	Mol_003	Mol_004	Mol_005	Mol_008	Mol_012	Mol_014
Units/Class	(Rat IP LD_{50} Log 10)									
(mmol/kg)	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD	-0,460 in AD
(mg/kg)	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD	147,600 in AD
CLASS	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV
	(Rat IV LD_{50} Log 10)									
(mmol/kg)	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD	-0,508 in AD
(mg/kg)	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD	132,000 in AD
CLASS	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV
	Rat oral LD_{50} Log 10									
(mmol/kg)	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD	-0,133 in AD
(mg/kg)	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD	313,700 in AD
CLASS	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV
	Rat SC LD_{50} Log 10									
(mmol/kg)	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD	-0,542 in AD
(mg/kg)	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD	122,200 in AD
CLASS	III	III	III	III	III	III	III	III	III	III

*Class out of AD, IP-Intraperitoneal route of administration, IV-Intravenous route of administration, Oral-Oral route of administration, SC-Subcutaneous route of administration.

(RMSF), Solvent Accessible Surface Area (SASA), and Radius of gyration (Rg), were generated to assess the stability of ligand-protein interactions (Khan *et al*., 2022).

Root-Mean-Square Deviation (RMSD) and Fluctuation (RMSF) analysis

To evaluate the reliability of the protein, RMSD and RMSF analyses were conducted over a 10 ns MD trajectory. Lower values indicate greater stability. Specifically, RMSD was analyzed for the protein backbone and ligand, revealing the higher reliability of compound 03 with a value of 1.03 nm, while the other hit showed a value of 1.07 and the control exhibited a value of 1.12 nm (Kausar *et al*., 2022). RMSF analysis depicted how protein residues fluctuated during ligand binding throughout progression (Ghahremanian *et al*., 2022). Notably, from the RMS-fluctuation analysis, minimum deviations were observed for compound 03, demonstrating its superior stability. The RMSF values for both compounds were recorded as 0.16 nm and 0.17, respectively, compared to the control's 0.19. Plots of RMSD and RMSF are revealed in Figure 3.

Quantification of compactness and SASA Determination

The Radius of Gyration (Rg) was determined to evaluate fluctuations in protein compactness during the simulation. This method provides a profile that illustrates the uniformity of the protein throughout the process. The root-mean-square average of the broken distances showed the overall information content of the protein to a greater extent. This study also revealed the size of the macromolecule; with smaller values indicating greater compactness (Figure 4A) (Ahmad Khan and Almarshad, 2023). The Rg values were noted to be 2.48 nm and 2.53 nm for both compounds, compared to the control at 2.57 nm.

A Solvent-Accessible Surface Area (SASA) representation was used to explore the stability and conformational changes after ligand binding. This approach provided in-depth insights from the 10 ns trajectory of all hits, including the control drug. A higher SASA value indicates that the protein structure has expanded, allowing for increased solvent access to a larger surface area (Rathod, 2021).The mean SASA alone does not fully

Table 6: Evaluation of side effects.

Compounds	Pa	Pi	Side Effects
Control.pdb	0.979	0.007	Hepatotoxicity
	0.94	0.005	Nephrotoxicity
mol_000.pdb	0.776	0.069	Hepatotoxicity
	0.576	0.032	Myocardial infarction
	0.547	0.036	Cardiac failure
	0.453	0.065	Nephrotoxicity
mol_001.pdb	0.4	0.07	Myocardial infarction
	0.333	0.126	Nephrotoxicity
	0.289	0.184	Cardiac failure
mol_002.pdb	0.779	0.067	Hepatotoxicity
	0.511	0.05	Cardiac failure
	0.489	0.045	Myocardial infarction
	0.444	0.068	Nephrotoxicity
mol_003.pdb	0.394	0.088	Nephrotoxicity
mol_004.pdb	0.445	0.067	Nephrotoxicity
	0.29	0.224	Myocardial infarction
	0.273	0.199	Cardiac failure
mol_005.pdb	0.755	0.078	Hepatotoxicity
	0.284	0.175	Nephrotoxicity
mol_008.pdb	0.752	0.079	Hepatotoxicity
	0.44	0.069	Nephrotoxicity
	0.412	0.099	Cardiac failure
	0.373	0.087	Myocardial infarction
mol_012.pdb	0.377	0.205	Arrhythmia
	0.345	0.118	Nephrotoxicity
	0.33	0.322	Hepatotoxicity
	0.298	0.203	Myocardial infarction
mol_014.pdb	0.526	0.182	Hepatotoxicity
	0.465	0.061	Nephrotoxicity
	0.354	0.128	Cardiac failure
	0.323	0.157	Myocardial infarction
	0.979	0.007	Hepatotoxicity

convey the impact of ligand binding on stability, necessitating careful analysis of the trajectory during the simulation. The mean values for compounds 03 and 05 were 26.51 nm² and 26.55 nm², respectively (Figure 4B).

Principal component analysis for stability assessment

The application of Principal Component Analysis (PCA) in this study expected to evaluate and equivalence the trajectories of docked complexes by simplifying the complexity while preserving the maximum variance. The extent of diversity delineated by PC1 for compounds 1 and 2 was remarkably similar, representing 22.1% and 22.6% of that of compound 2, respectively. This finding suggested that the pathways associated with each protein were comparable. By contrast, the variance in the control group was 37.8%. It is widely acknowledged that lower variance proportions in PCA plots indicate greater stability. A visual representation of the plots is shown in Figure 5A-C. The effectiveness of PCA lies in its capacity to produce Principal Components, which are small variables based on variance estimation theory. These components capture the maximum variance in the original data.

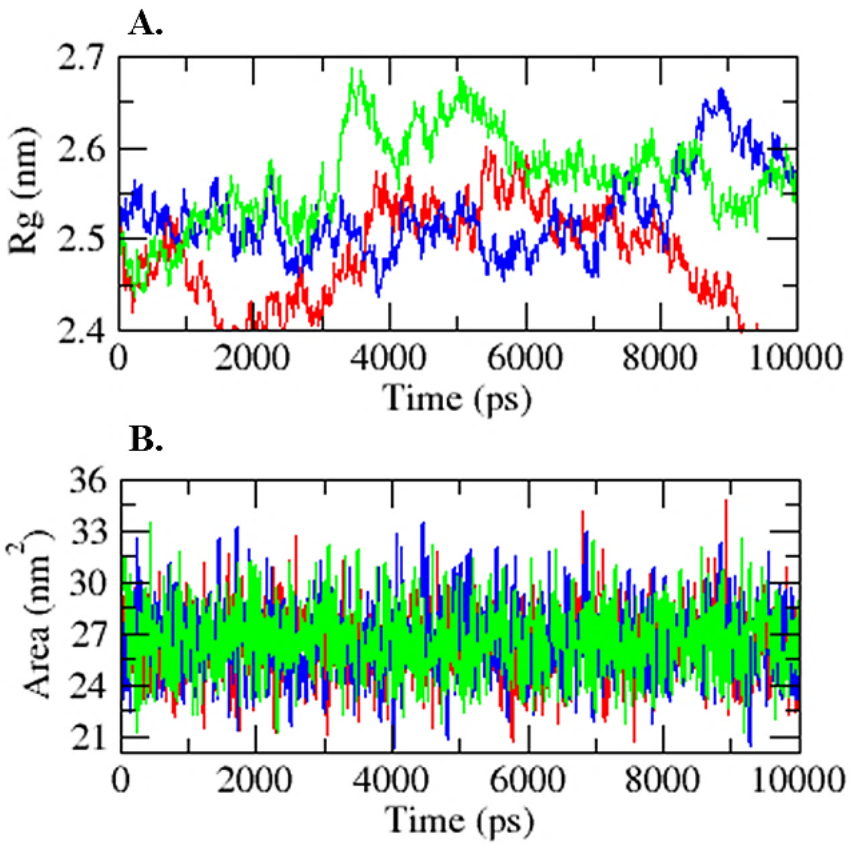

Figure 4: (A) Time-dependent Radius of Gyration graph. the red line corresponds to Compound 03, the blue line represents Compound 05 and the green line denotes the control. (B) SASA analysis. red color represents compound 03, Blue color represented as compound 05 while the green color represents the control.

Table 7: Interacting residues of top hits.

Compounds	Interacting Residues
Control	TYR56 TYR58 VAL61 CYS65 CYS66 VAL69 GLY71 TYR72 MET73 PRO74 ILE75
	VAL77 ALA95 ILE301 ALA302 CYS303 GLY304 LEU350 PRO352 GLN353
03	LEU51 ARG54 ASP55 TYR56 TYR58 VAL61 CYS66 VAL69 ILE70 GLY71 TYR72 MET73 PRO74 ILE75 VAL77 ALA95
	TYR300 ILE301 ALA302 CYS303 GLY304 GLN305 ASP306 ALA307 GLY347 LEU350 PRO352 GLN353 CYS356
05	TYR56 TYR58 VAL61 CYS66 VAL69 GLY71 TYR72 MET73 PRO74 TYR300 ILE301
	GLY304 GLN305 ALA307 GLY347 LEU350 PRO352 GLN353

DISCUSSION

The focus of our study was Cardiovascular Disease (CVD), specifically dyslipidemia. Traditionally, statins have been considered as the primary treatment option, demonstrating efficacy in reducing the risk of major vascular events by lowering LDL-C levels. However, our investigation aimed to enhance efficacy and overcome drug resistance by developing derivatives of the well-known first-line treatment, fluvastatin, using the web-based server MolOpt. The resulting dataset underwent molecular docking for screening and the screened compounds, detailed in (Supplementary Table), were subjected to further analyses.

The drug-likeness and ADME profiles, as outlined in (Supplementary Tables 2 and 3), provided comprehensive information. Notably, all compounds adhered to the Rule of Five (RO5), while compounds 02 and 08 exhibited Blood-Brain Barrier (BBB) penetration. For pharmacological impact, the PASS prediction indicated broad-ranging properties, with compounds 03 and 05 standing out for having more features than the rest. Subsequent evaluations using the GUSAR and ADVER-PRED databases have provided insights into acute rodent toxicity and adverse effects. This additional filtration reinforced the prominence of compounds 03 and 05, which demonstrated minimal side effects compared to the other compounds, including the control.

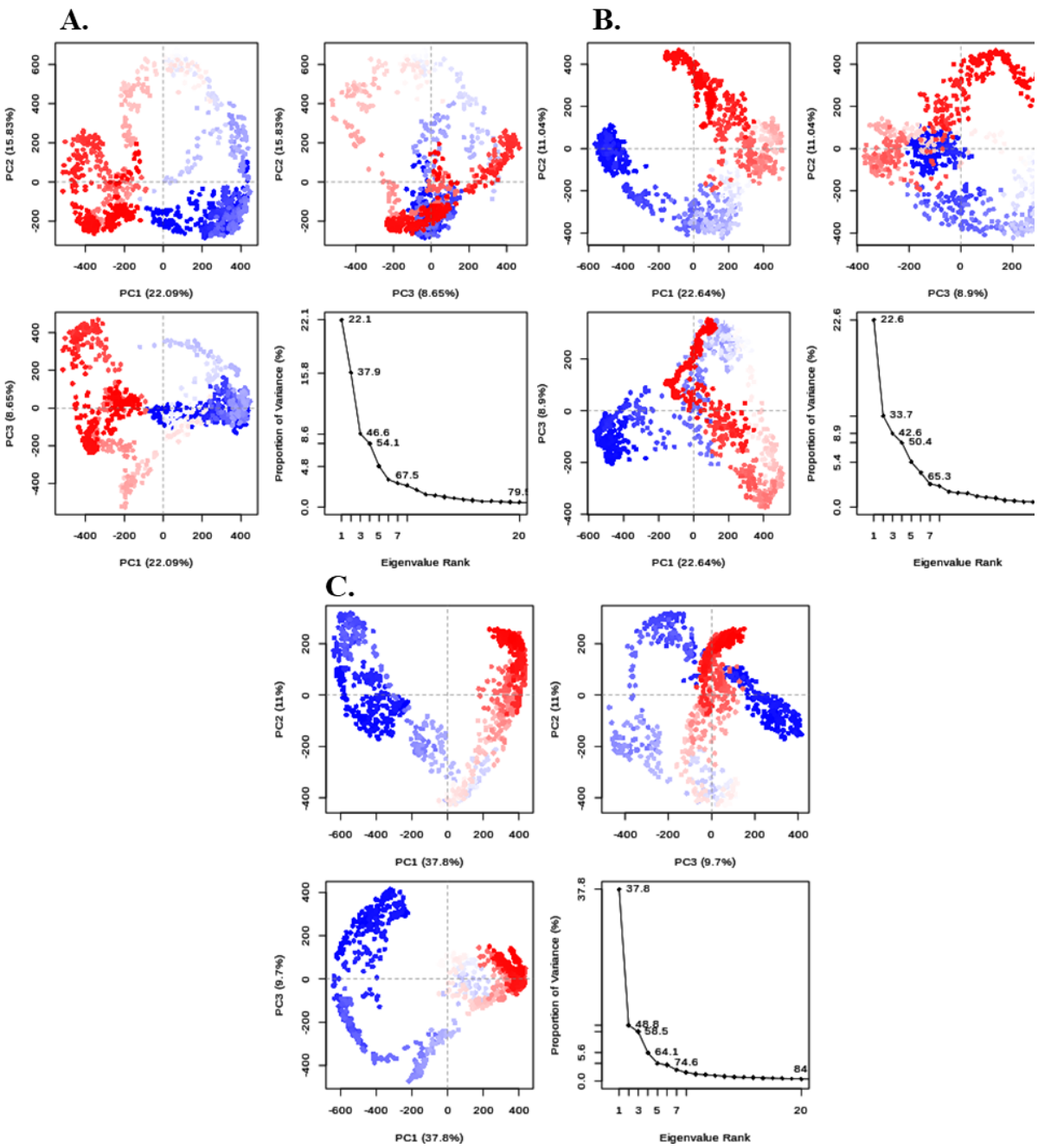

Figure 5: (A) Graphic illustrations of the plot mol-003. (B) Plot for mol-005. (C) Plot of Control.

The refined selection of compounds, identified as promising candidates, underwent molecular dynamics simulation and principal component investigation for further scrutiny. Simulation studies encompassing root-mean-square deviations, root-mean-square fluctuations, solvent-accessible surface area, and Radius of Gyration revealed that both compounds, mol_03 and mol_05, outperformed the control compound, fluvastatin. Mol_03 exhibited superior stability, with an RMSD of 1.03 nm, which was lower than that of the parent compound and other derivatives. Fluctuation analysis indicated ranges of 0.16 nm and 0.17 nm for mol_03 and mol_05, respectively, compared to the parent compound's value of 0.19 nm, as illustrated in Figure 3. The radius of gyration and Solvent-Accessible Surface Area (SASA) further supported this assessment, as shown in Figure 4A-B.

Additionally, principal component studies provided supporting evidence by showing lower variance for mol_03 and mol_05 compared with the control, as revealed in Figure 5A, B and C. Collectively, these findings suggest that mol_03 and mol_05 exhibit favorable characteristics in terms of stability and dynamics, making them hopeful candidates for additional investigation in cardiovascular disease treatment.

CONCLUSION

The development of novel drugs to address severe cardiac diseases has become a pressing concern that has garnered significant attention. In light of these challenges, our investigation highlights the promising potential of fluvastatin derivatives or analogs that exhibit anti-hypercholesterolemia, anti-cardiac effects and cholesterol inhibition, particularly in the context of diagnosing dyslipidemia within the spectrum of Cardiovascular Diseases (CVDs). Of all the molecules assessed, mol_03 emerged as the most promising candidate. This compound exhibited remarkable binding affinity, along with a lack of toxicity and only one negligible side effect, as evidenced by various parameters. To further explore this potential, MD simulation studies and Principal Component Analysis (PCA) were conducted. The results of the MD studies indicated lower fluctuations, whereas PCA served as a supporting pillar, reinforcing the robustness of the outcomes. In conclusion, mol_03, a derivative of fluvastatin, is a promising candidate for further development and optimization in the field of drug discovery. However, it is essential to emphasize the need for wet-lab verification to validate and solidify these findings before considering practical applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LDL-C: Low-density lipoprotein cholesterol; **HDL:** High-density lipoprotein; **MD:** Molecular dynamics; **PCA:** Principal component analysis; **CVDs:** Cardiovascular diseases; **DM:** Diabetes mellitus; **TC:** Total cholesterol; **RMSD:** Root Mean Square Deviation; **RMSF:** Root Mean Square Fluctuation; **SASA:** Solvent Accessible Surface Area; **Rg:** Radius of Gyration; **BBB:** Blood-brain barrier.

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