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In silico approach for identification of potential tetracyclic triterpenoids from mushroom as HMG-CoA reductase inhibitor

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ABSTRACT

Cardiovascular disease is estimated to be responsible for one-third of all global deaths annually. It occurs mostly due to hyperlipidemia, a condition where excessive cholesterol deposits in blood vessels. A favorable target for treating hyperlipidemia involves the crucial role of inhibition of a specific enzyme known as 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase). The primary goal of this present study is to identify potential HMG-CoA reductase inhibitors containing tetracyclic triterpene nucleus derived from mushrooms. A library of 86 myco-constituents bearing a tetracyclic triterpene scaffold was prepared and screened to identify potential HMG-CoA reductase inhibitors targeting proteins 1HW8 and 1HW9. For this purpose, molecular docking, ADME prediction, and molecular dynamics (MD) simulation studies were performed on this in-house prepared database. The virtual screening results exhibited **M_02(c)** as the best hit with promising SP Glide scores compared to standard statin drugs. In order to assess the stability and interactions, a 100 ns MD simulation was performed. Further, **M_02(c)** was also analysed for MMGBSA binding energy to access and validate the thermodynamic stability of the protein-ligand complex. The results of this study revealed that M_02(c) is a promising hit molecule and may emerge as a potent HMG-CoA reductase inhibitor in preventing and treating hyperlipidemia.

1. Introduction

Cardiovascular disease (CVD) is one of the leading non-communicable health issues in the world, causing almost one-third of all global fatalities annually in developed and developing countries (Cosenza et al., 2019; Schmieder et al., 2007; Shukr et al., 2019). CVD is typically associated with hyperlipidemia, one of the main contributing factors of cardiovascular disease (Cosenza et al., 2019; Schmieder et al., 2007). If this condition is left untreated, there is a chance of accumulation of fatty deposits resulting from increased plasma lipids, including total cholesterol and cholesterol triglycerides on the inner surface of arterial walls (atherosclerosis), and simultaneously an increased risk of blood clots (Fuster et al., 2005). In addition, it is associated with damage to arteries in vital organs, viz., brain, heart, kidneys, and eyes (Junaidin et al., 2022; Surya et al., 2017). Moreover, cholesterol is produced endogenously in the human body via the mevalonate pathway to manage the need for physiological requirements. However, the elevated cholesterol concentration may increase the chances of cardiovascular disease (Kinosian et al., 1994). The mevalonate concentration is meticulously controlled through 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) enzyme activity in human cells (Istvan et al., 2000; Junaidin et al., 2022). This particular enzyme plays a vital role in endogenous cholesterol biosynthesis in our liver. The substrate HMG-CoA conversion (3-hydroxy-3-methylglutaryl-CoA) is catalyzed by HMG-CoA reductase to mevalonate, the precursor of sterols biosynthesis, including cholesterol. Accordingly, this reductase enzyme could be a plausible target for developing drugs for hyperlipidemia (Hasimun et al., 2018). The decrease in mevalonate production is achieved by inhibiting HMG-CoA reductase, and subsequently, cholesterol synthesis is reduced (Tricarico et al., 2015). For this purpose, drugs like statins are prescribed to treat the threat of coronary heart disease by decreasing cholesterol synthesis and promoting protein receptor

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upregulation.

Statin-class drug molecules act as competitive inhibitors of HMG-CoA and hinder the function of HMG-CoA reductase by conquering the enzyme's active site as an auxiliary substrate (Yeganeh et al., 2014). It inhibits the mevalonate biosynthesis from HMG-CoA and reduces cholesterol synthesis (Istvan, 2003). Though the statin group has been widely used to treat hyperlipidemia condition, it has numerous adverse effects, including headache, enlarged serum liver transaminase levels, distal muscle paleness, and sleep disorders (Calderon et al., 2010). In addition, long-term consumption of statins is found to cause eczema, peripheral neuropathy, sensory instabilities, and depression-like symptoms (Junaidin et al., 2022).

Nature is a most valuable source of bioactive molecules that can be studied for the discovery and development of new chemical entities. Bioactive molecules found in edible plants and foods play an essential role for human health, though their importance still remains unrecognized (Ghosh and Deb, 2014; Mazumder et al., 2024). Triterpenoid is one of the most significant bioactive molecules synthesized naturally from squalene. It exhibits varied pharmacological activities, including cardioprotective and anti-hyperlipidemic (Ghosh and Sandeep, 2020; Han and Bakovic, 2015; Rascon-Valenzuela et al., 2017; Zhao et al., 2023). Triterpenoids can be found in various natural sources such as plants, ferns, fungi, and marine sources. There are more than 200 different triterpenoidal nuclei known to be derived from both natural sources and enzymatic reactions while being divided into acyclic, monocyclic, bicyclic, tricyclic, tetracyclic, and pentacyclic frameworks (Hill and Connolly, 2020; Rascon-Valenzuela et al., 2017). The most widely explored triterpenoids involve tetracyclic derivatives with dammarane, cycloartane, euphane, lanostane, and cucurbitane types (Noushahi et al., 2022) (Fig. 1). The present state of research on tetracyclic triterpenes is robust and multidimensional reflecting their diversified activities and potential therapeutic benefits. Tetracyclic triterpenes like ginsenosides, cycloastragenol, and astragalosides are extensively utilized and studied for their countless biological activities (Aly et al., 2024; Ghiulai et al., 2020; Ghosh and Sandeep, 2020; Hamid et al., 2015; Zhao et al., 2023). In the recent past, systematic investigations were conducted to assess the benefit of triterpenes in several pharmacological activities. The results of these studies indicated their efficacy and captured the attention of medicinal chemists (Agra et al., 2015; Ghosh and Sandeep, 2020; Han and Bakovic, 2015). Most recent reports have demonstrated the cardioprotective properties of tetracyclic triterpenoids that can potentially ameliorate cardiovascular as well as other metabolic disorders (Hamid et al., 2015; Machaba et al., 2014; Mosa et al., 2014; Nguyen et al., 2021; Sangweni et al., 2019).

Considering the high functional values and delicacy, cultivation, and production of different mushrooms named *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes*, and *Flammulina velutipes*, are increased worldwide, especially in China and Southeast Asia (Valverde et al., 2015). Mushrooms are even reported as significant sources of bioactive compounds and rapidly increasing attraction as medicinal and nutritional food worldwide, which could be attributed to the presence of several metabolites such as triterpenoids, phenolic compounds, sterols, fatty acids, peptides, and lectins (Cateni et al., 2021; Dasgupta and Acharya, 2019). Several researchers exhibited that intake of mushrooms in diet potentially reduces the chances of CVD (Guillamón et al., 2010). In the recent past, various bioactive compounds viz., cordycepin, ergosterol, chitosan, mannitols, eritadenine, β-glucans, tocopherols, Gamma-aminobutyric acid (GABA), and ergothioneine are reported from mushrooms act as leading molecules with potential effects on heart diseases (Rauf et al., 2023), however, these metabolites are yet to be thoroughly investigated for their cardioprotective benefits. Moreover, aqueous extracts of 26 mushrooms, including *Agaricus bisporus*, *Lentinula edodes, Pleurotus* spp., and *Flammulina velutipes,* were explored for potential inhibitory activity of the critical enzyme, 3-hydroxy-3-methyl--glutaryl CoA reductase in the cholesterol biosynthesis (Gil-Ramírez et al., 2013; Gil-Ramírez et al., 2013; Hipol et al., 2020). It was also observed that the genus *Pleurotus*, especially species *P. ostreatus, P. sapidus, and P. saca*, were reported as favorable sources for the occurrence of hypo-cholesterolemia agent, mevinolin (Gunde-Cimerman et al., 1993). As edible fungi, mushrooms can provide a natural and dietary approach to cardiovascular health. The vast diversity of mushrooms can provide the possibility of discovering newer compounds, specifically tetracyclic triterpenoids, with more target specific benefits (Bell et al., 2022; Chugh et al., 2022; Gupta et al., 2019; Kumar et al., 2021). Two molecules namely, α, β-amyrin, closely related natural compounds of triterpene class, were reported to have

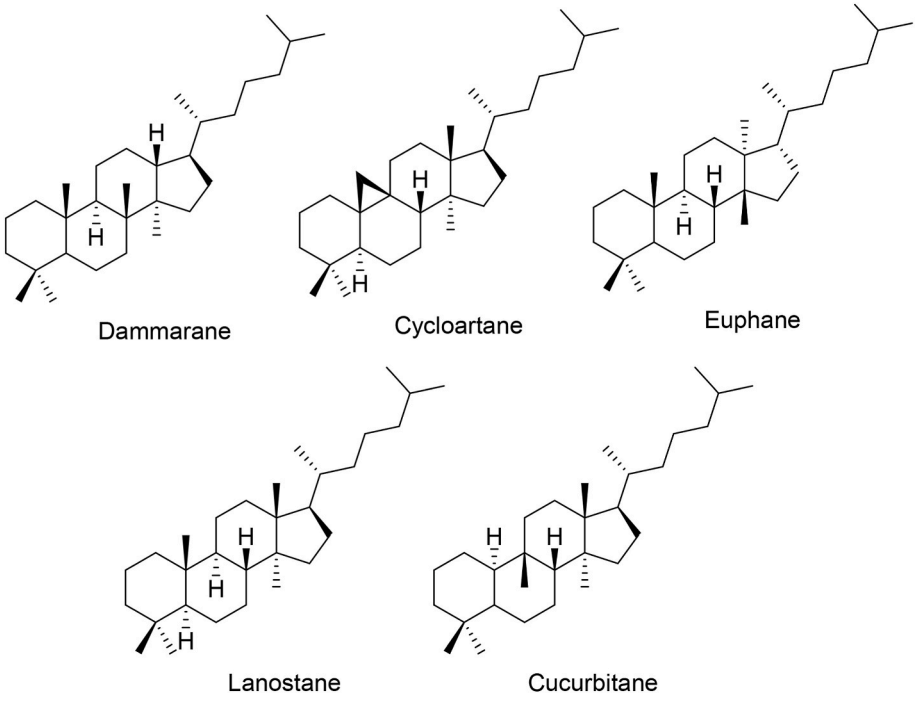


Fig. 1. Examples of tetracyclic triterpenes.

anti-hyperglycemic and lipid-lowering effects. Recently, it was also documented that triterpenes have a high medicinal benefit and have been used in treating several diseases (Jeitler et al., 2020; Santos et al., 2012).

Therefore, potential novel candidates as inhibitors for HMG-CoA reductase are a prerequisite. Recently, phytochemicals have been enlightened for their potential in treating various CVDs, mainly due to the limitations of existing therapy. The present study used molecular docking and molecular dynamics simulation to predict potential bioactive molecules bearing tetracyclic triterpene frameworks from an in-house library database of myco-constituents reported for mushrooms. It is primarily opted based on the literature survey and four existing statin drugs, viz., compactin and lovastatin of natural origin and simvastatin and pravastatin of semisynthetic developments. These compounds are also known as active HMG-CoA reductase inhibitors, whereas naturally derived lovastatin and compactin are produced predominantly by *Aspergillus* and *Penicillium* species. Contrarily, simvastatin and pravastatin are semisynthetic derivatives of lovastatin and compactin, respectively. Herein, specific attention is provided to natural inhibitors of HMG-CoA reductase due to the increasing adverse effects of existing HMG-CoA reductase inhibitors, viz., hepatic toxicity, tendonitis, cataracts, renal disorders, hyperglycemia, and atheroma (Huisman, 2014; Khatiwada and Hong, 2024; McLean et al., 2015; Pinal-Fernandez et al., 2018). These observations prompted us to screen out potential tetracyclic triterpenes derived from mushrooms, which may provide new ideas for discovering molecular compounds with potential remedies against CVD. Moreover, the exciting research findings of various researchers about the anti-cholesterol activity of edible and medicinal mushrooms encourage us to design this work. With this approach, molecules with tetracyclic triterpenes from myco-constituents were evaluated via molecular docking followed by molecular dynamics simulation studies to assess the binding stability of the hit molecule at the HMG-CoA reductase active site.

2. Material and methods

2.1. Dataset, ligand preparation, protein preparation, and receptor grid generation

The computational simulations and visualizations were performed using Dell Inc. System Model-Precision 5820 Tower; Processor: Intel(R) Xeon(R) W-2245 CPU@ 3.90 GHz, OS: Ubuntu 22.04.1 LTS, 64-Bit. The software utilized in this study includes Schrödinger, Maestro 2022–4, and Desmond. In this present study, a set of 86 myco-constituents bearing tetracyclic triterpene core from mushrooms was retrieved from extensive literature searches (Lindequist et al., 2005; Öztürk et al., 2015; Thu et al., 2020). The ligand set is based on recent studies involving mushrooms and other tetracyclic terpenes obtained from different plant sources, which were examined and reported for their cardioprotective potential. Since terpenes, especially tetracyclic triterpenes are among the abundant constituents attained from mushrooms. The primary focus was given to the tetracyclic triterpenes reported from mushrooms, which were designed to recognize the cardioprotective potential of these selected natural tetracyclic triterpenes. Also, four known statin drugs, either naturally attained or semisynthetic developments, namely, compactin, lovastatin, pravastatin, and simvastatin, were considered reference standards. All 86 structures (Fig. S1) were drawn utilizing ChemDraw Professional 15.1 and saved in sdf format for future use. The structures were built and optimized using the Ligprep Module of Schrödinger, Maestro 2022–4. Based on the literature review, target proteins 1HW8 and 1HW9 of HMG-CoA reductase were selected due to their natural and semisynthetic nature of co-crystallized ligands, viz. compactin and simvastatin. These target proteins were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (Istvan and Deisenhofer, 2001). The target proteins were prepared using "Protein Preparation Wizard" (Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2021). During protein preparation, protein energy was optimized using the OPLS_2005 force field and, subsequently, restrained. In both cases, the 15 Å receptor grids were generated by selecting the centre of the co-ligands.

2.2. Molecular docking

Molecular Docking studies of known inhibitors (standards) and prepared molecules were performed using Schrodinger Glide (Grid-based Ligand Docking with Energetics) Program on a distinct receptor grid, and flexible docking was executed using the standard precision (SP) feature of the Glide module (Schrödinger LLC, New York, NY, 2022–04). This method became a valuable technique for ascertaining ligand hits. Subsequently, the docked conformers were further evaluated using the Glide score.

2.3. Validation of docking

A set of co-crystallized HMG-CoA Reductase inhibitors was fragmented from the receptor and imperiled to SP Glide corresponding to the grid of HMG-CoA Reductase to assess the capability of SP Glide docking. Docking capability was calculated using the Root Mean Square Deviation (RMSD)' of the finest docked structural stance by covering its inherent co-ligand in X-ray crystallographic assembly. The RMSD values of co-ligand of 1HW8 and 1HW9 were 0.77 Å and 1.23 Å respectively. SP Glide docking predicted the accurate binding temperament of all co-ligands with an RMSD $<$2.0 Å, which indicated that the Glide reproduced the instinctive conformation fruitfully, as shown in Fig. 2 (Hevener et al., 2009; Sarkar et al., 2024a).

2.4. Molecular dynamics

Molecular Dynamics (MD) simulations were performed to inspect the best ligand-protein complexes' variability and conformational variations of different binding modes. The ordered protein-ligand complexes were presented to the OPLS_2005 force field pre-defined SPC solvent model within the Desmond Module for the MD simulations (Jorgensen et al., 1996). The Orthorhombic box boundary was set up to postulate the restating unit's shape and size, allowing for a 10 Å buffer region between the complex atoms and box sides. The system was counter balanced by adding proper counter Na^+/Cl^- ions (Mark and Nilsson, 2001). After constructing the solvated system comprising a protein-ligand complex, the system was exposed to 300 K temperature and 1.01325 bar pressure for 100 ns of simulations using the NPT ensemble class (Martyna, 1994; Toukmaji and Board, 1996), and molecular dynamics trajectories were recorded at 100 ns intervals. Subsequently, the MD trajectories' RMSD vacillations, hydrogen bonding, and other binding interactions were scrutinized over time (Bhaumik et al., 2024; Selvaraj and Singh, 2014).

2.5. Molecular mechanics with generalized born and surface area solvation (MMGBSA) binding free energy calculation

The binding free energy calculations showed the alterations in the binding affinity of the different structurally-unlike ligands and the binding pocket. MMGBSA is generally used to explore the energetic contribution of protein-ligand binding affinities(Sarkar et al., 2024b).

$$\Delta G_{\text{binding}} = G_{\text{complex}} - [G_{\text{protein}} + G_{\text{ligand}}]$$

2.6. Absorption, distribution, metabolism, elimination (ADME) predictions

A drug could be effective when it must reach targets in sufficient concentration in the body and stay inside the body in bioactive form as long as possible for the desired biological events to happen. The early assessments of ADME properties of top-hit molecules essentially show a vital role in drug development by predicting the absorption, distribution, metabolism, and excretion of complexes. The ADME properties of the top-hit and standard drugs were estimated by SwissADME to predict the drug-likeness properties and to evaluate pharmacokinetics properties (Daina et al., 2017). This server takes SMILES structural scripts of the ligands as input, providing theoretical values and utilizing numerous algorithms based on different model parameters for physiological properties, pharmacokinetics, and drug-likeness; amongst these in-house practised methods includes BOILED-Egg, iLOGP, and Bioavailability Radar. The drug-like properties of these compounds were assessed via 'Lipinski's Rule of Five' calculation, central nervous system (CNS), and blood-brain barrier (BBB) permeability. More than 40% of drug candidates fail due to poor ADME qualities in clinical trials phase '0′ and '1'. Enhancing and forestalling the ADME characteristics allows poor-drug molecules to be recognized in the development process as soon as possible while escaping last-stage failures. It fundamentally progresses in spending less time, money, and assets (Daina et al., 2014).

2.7. Result and discussion

Computational approaches deliver a massive value for the drug discovery and drug development processes. Computer models derived *in silico* virtual screening analyses reveal the possible interactions between proteins and ligands (Atiya et al., 2023; Murgueitio et al., 2012; Tiwari and Singh, 2022). Edible mushrooms and their diversified content of triterpenoids have been examined continuously for their potential as HMG-CoA reductase inhibitors with considerable results compared to standard statin drugs (Gil-Ramírez et al., 2013; Gil-Ramírez et al., 2013, 2013; Wang et al., 2015, 2017; Wongkhieo et al., 2023; Zhang et al., 2018). Numerous review works also highlighted the cardioprotective and anti-hyperlipidemic activities of edible mushrooms, which act via different mechanisms, including the targeting of HMG-CoA reductase (Guillamón et al., 2010; Mustafa et al., 2022; Rauf et al., 2023; Zhao et al., 2023). Herein, we have forwarded the binding interaction affinities of the tetracyclic triterpenes retrieved from mushrooms against the critical targets of HMG-CoA reductase, employing computational methods.

To identify the best HMG-CoA reductase inhibitor, a virtual screening of 86 tetracyclic triterpenes derived from mushrooms was performed against 1HW8 and 1HW9 (Fig. S1). Structure diversity and top score hits with the best SP Glide scores are considered potential HMG-CoA reductase inhibitors and are listed in Table 1.

The docking study showed that the selected hits also have similar types of interactions, whereas three (03) hits were identified as potent out of 86 selected compounds based on SP Glide score ranging from −7.790 to −8.478 kcal/mol for both the target proteins 1HW8 and 1HW9. The compound **M_02(c)** showed the best docking score compared to others under study. The SP Glide scores of the tetracyclic terpene compound **M_02(c)** were observed to be −8.478 and −8.291 kcal/mol for 1HW8 and 1HW9, respectively. Additionally, all the selected hits exhibited better SP Glide scores compared to standard drugs compactin (−6.651 kcal/mol: 1HW8; −5.794 kcal/mol: 1HW9), lovastatin (−5.762 kcal/mol: 1HW8; −4.936 kcal/mol: 1HW9), and simvastatin (−6.041 kcal/mol: 1HW8; −5.108 kcal/mol: 1HW9). Among all the standard drugs under study, only pravastatin showed the best SP Glide scores of −8.560 and – 8.921 kcal/mol for 1HW8 and 1HW9, respectively, and was found to be marginally more significant than SP Glide scores of **M_02(c)** for both the target proteins. However, it was observed that HMG-CoA reductase inhibition activity of pravastatin, simvastatin, and lovastatin resulted as IC_{50} values of 95, 18 and 61 nM, respectively for HepG2 cell homogenates. Further, simvastatin and pravastatin exhibited inhibitory activity in cholesterol synthesis resulting in IC_{50} values of 23 and 105 nM, respectively while tested in human hepatocytes (Cohen et al., 1993). However, certain tetracyclic terpenes derived from mushrooms were reported to have promising IC_{50} values ranging from 8 to 60 μM against the activity of HMG-CoA reductase (Wang et al., 2015; Zhang et al., 2018). Few more studies have also shown evidence of edible mushrooms being evaluated as potent HMG-CoA reductase inhibitors with anti-hyperlipidemic activities (Abidin et al., 2018; Bobek et al., 1995; Hossain et al., 2003).

The number of interactions showed that known inhibitors and the selected hits interact mainly with ASN-755, GLU-559, ARG-590, LYS-691, and ASP-690 amino acid residues at the active site, as shown in Table 2 & Fig. 3. The majority of the hits interact with active sites of both target proteins 1HW8 and 1HW9, were found to have interacted via hydrogen donors and acceptors. The validation of the SP docking protocol was performed utilizing the co-crystallized HMG-CoA reductase inhibitors to the grid of HMG-CoA reductase of 1HW8 and 1HW9 and

Table 1
Binding score of selected hits and Standard drugs with 1HW8 and 1HW9 protein.

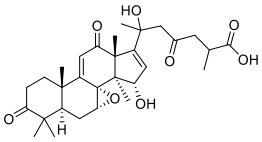
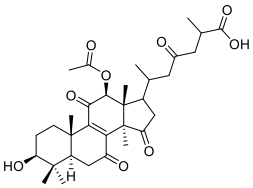
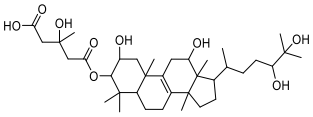
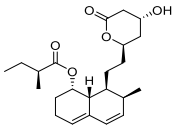
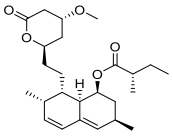
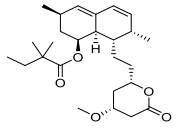
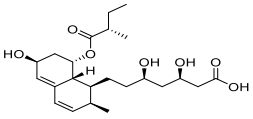
Selected Hits & Standards	Compound Structure	SP Glide Score (Kcal/mol) 1HW8	SP Glide Score (Kcal/mol) 1HW9
M_02(c)		**−8.478**	**−8.291**
M_08(h)		−8.058	−7.942
MB_25		−7.967	−7.799
Compactin		−6.651	−5.794
Lovastatin		−5.762	−4.936
Simvastatin		−6.041	−5.108
Pravastatin		**−8.560**	**−8.921**

Table 2
Interactions of selected hits with 1HW8 and 1HW9.

Selected Hits & Standards	Molecular interactions with 1HW8	Molecular interactions with 1HW9
M_02(c)	ASN-658, SER-684, ASN-686, CYS-688, LYS-735, LYS-692, ASN-755, ARG-590, GLU-559	ASN-658, ARG-590, LYS-735, SER-684, ASP-690, LYS-692, CLU-559, LYS-691, ASN-755
M_08(h)	ASN-755, ARG-590, ASP-690, SER-684, LYS-691, LYS-692, LYS-735	ASN-755, LYS-692, LYS-691, SER-684, ASP-690, ARG-590, LYS-735
MB_25	LYS-692, CYS-688, ASN-686, LYS-735, SER-684, ARG-590, GLU-665	ASP-690, LYS-692, LYS-735, SER-684, ARG-590, GLU-665
Compactin	ASN-755, GLU-559, ARG-590	ARG-590, GLU-559
Lovastatin	ARG-590, GLU-559, ASN-755, LYS-691	ARG-590, ASP-690, ASN-755, LYS-691
Simvastatin	ASN-755, ASP-690, ARG-590	ASN-755, LYS-691, GLU-559, ARG-590
Pravastatin	GLU-599, ARG-590, LYS-691, LYS-692, ASP-690, LYS-735, ASN-755,SER-684	GLU-599, ASN-755, LYS-691, LYS-692, ASP-690, ARG-590, HIE-752, LYS-735, SER-684

compared the RMSD value to the actual pose of the co-ligand. The co-crystallized ligand was superposed over the docked co-crystal conformation (Fig. 2). The RMSD values of the co-ligand of 1HW8 and 1HW9 were 0.77 Å and 1.23 Å, respectively, indicated that the used docking method was effective in predicting the binding mode of the ligands as it lay lesser than 2.0 Å.

The MD simulation findings for both apo-protein and protein-ligand complexes of the standard drug (1HW8_Pravastatin and 1HW9_Pravastatin) and best hit [1HW8_M_02(c) and 1HW9_M_02(c)] were explored over a time scale of 100 ns to understand the complex's dynamic behavior and stability. Proteins are expected to be folded when the RMSD is lower and unfolded when the RMSD is higher (Costa et al., 2022). The RMSD protein value in 1HW8_M_02(c) complex was higher than the RMSD value of apo-protein (1HW8) up to 100 ns. Initially, the ligand's RMSD slightly fluctuated until 20 ns, gradually increased, and remained stable until the simulation was completed. The highest RMSD protein value in protein-ligand complex was less than 2.4 Å. The RMSDs of apo-protein and protein-ligand complex are shown in Fig. 4 (A & C).

In the protein-ligand complex viz. 1HW8_Pravastatin, the RMSD value of protein was higher than the RMSD value of apo-protein. The RMSD value of the ligand in the 1HW8_Pravastatin complex was found to be lesser than the RMSD value of the complex protein. The highest value of RMSD of the protein-ligand complex was 2.8 Å, under the permissible range, as illustrated in Fig. 4 (A & D). In 1HW9_M_02(c), the protein-ligand complex RMSD value of protein was lesser than RMSD of apo-protein. Further, the RMSD value of the ligand in the protein-ligand complex slightly fluctuated near 30 ns; beyond that, the ligand remained stable until 100 ns. Apart from that, the RMSD value of the ligand was less than the RMSD value of the protein from the 1HW9_M_02(c) complex. The highest value of RMSD of the protein-ligand complex was observed to be 2.8 Å. In 1HW9_Pravastatin protein-ligand complex, the RMSD of protein was lesser than the apo-protein. The RMSD value of ligand in protein-ligand complex was initially higher up to 25 ns, but it remained stable until the simulation was completed. The highest value of RMSD of protein from the protein-ligand complex was found to be 2.7 Å. Subsequently, MMGBSA energy was calculated from the MD simulation trajectory to calculate the free-energy of the binding of ligands to proteins. The MMGBSA binding energy for 1HW8_M_02(c) and 1HW9_M_02(c) protein-ligand complex was calculated as −27.230 and −34.930 kcal/mol, respectively; whereas, MMGBSA energy was calculated as −33.948 and −36.314 kcal/mol for the complex of 1HW8_Pravastain and 1HW9_Pravastatin, respectively.

In order to reveal the stability of the complex with the ligand, the existing amino acid residues of the protein play an important role. The variability of specific amino acid residues may be analysed using the RMSF (Root Mean Square Fluctuation) parameter. The RMSF is a functional analysis describing the protein chain's local fluctuation and binding site. Vertical green bars illustrated in the plot specify the protein residues that interact with ligands. The RMSF values of complex model 1HW8_M_02(c), protein backbone residues were found in the limit of 0.4–3.7 Å, and for 1HW8_Pravastatin, protein backbone residues were in the limit of 0.5–4.5 Å. However, the RMSF of 1HW8 apo-protein backbone residues was observed to be 0.5–4.0 Å. Subsequently, for 1HW9 apo-protein backbone residues, the RMSF ranges were found between 0.5 and 4.5 Å; whereas, 1HW9_M_02(c) complex RMSF lay in between 0.5 and 4.3 Å and for 1HW9_pravastatin RMSF ranges were observed in between 0.5 and 4.5 Å, these results were displayed in Fig. 5. The fluctuations of protein-ligand complexes were found to be under the acceptable range. The elevated value of RMSF indicates superior flexibility of the protein and contrariwise. However, the limited deviations between RMSF values of apo-protein and protein-ligand complex protein backbone residues suggest that protein fluctuation was minimized after binding. This finding implies that the active residual sites within the binding pocket strongly interact with the tested compounds. The RMSD and RMSF values of 1HW8_M_02(c) and 1HW9_M_02(c) suggest that there were fewer structural rearrangements, fewer conformational changes, as well as fewer internal movements around the binding residues, and therefore, these are found to be as good binders.

The binding site residues involved in bonding interactions of

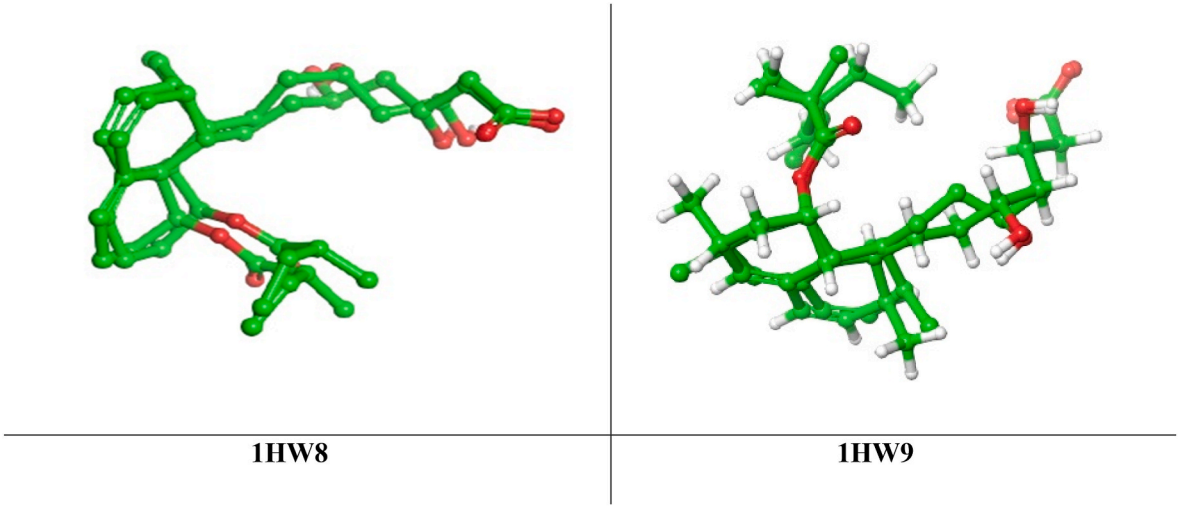


Fig. 2. Superposition of the docked co-ligands on its crystallographic bound conformation of HMG-CoA reductase proteins 1HW8 and 1HW9.

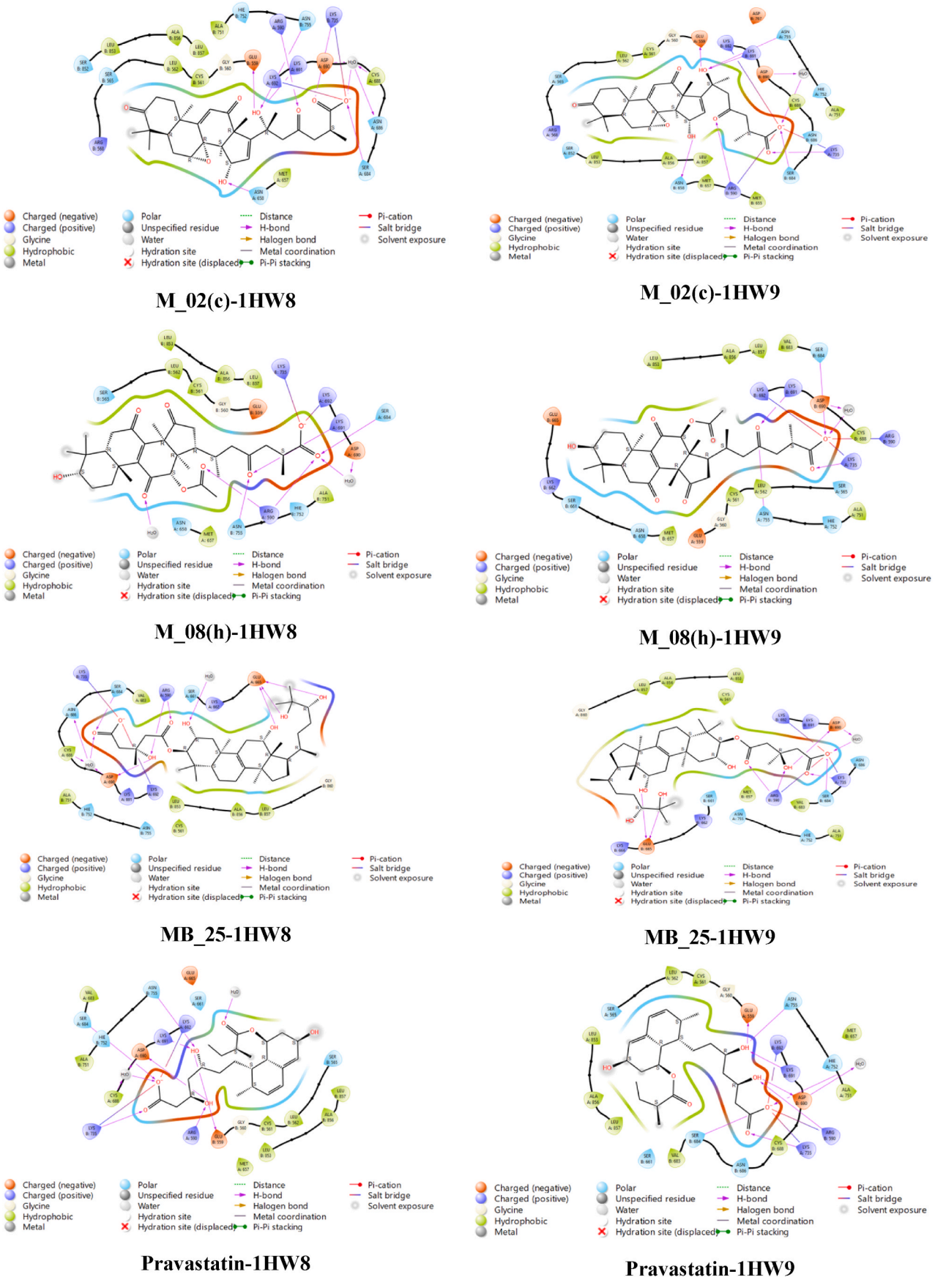


Fig. 3. 2D interactions of best-selected hits and standard reference pravastatin with 1HW8 and 1HW9.

1HW9_M_02(c) showed stabilised form in most of the trajectories, and encountered complexes were obtained to be as more consistent, as shown in Fig. 6 with residues LYS-735 (66%), ASN-755 (67%), SER-684 (46%), HIS-752 (32%) through hydrogen bonds, whereas, ARG-590 (62%), ASP-690 (47%), GLU-559 (30%) through water-bridge and ARG-590 (63%) through a salt bridge.

Additionally, ADME studies are essential in determining the drug-likeness attributes of compounds. "Lipinski rule of 5" is one of these, which helps to distinguish between drug-like and non-drug like molecules. The rule illustrates molecular properties predominant for a drug's pharmacokinetics in the human body, though it does not predict the compound's pharmacological potential. All potential inhibitors in this study were found to have drug-like properties according to 'Lipinski's Rule of Five'(Lipinski, 2016). The parameters observed in 'Lipinski's Rule of Five' influence the drug-like properties of molecules in the form of several rotatable bonds, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), molecular weight (MW), molar refractivity (MR), and iLOGp value. Moreover, the HBD and HBA must fall between 5 and 10 for an excellent drug-like molecule, whereas molecular weight must be below 500 a.m.u., iLOGp value must be less than 5, and molar refractivity range must be between 40 and 130 cm^3. The reference standard drug, pravastatin was observed to be stuck to Lipinski's rule.

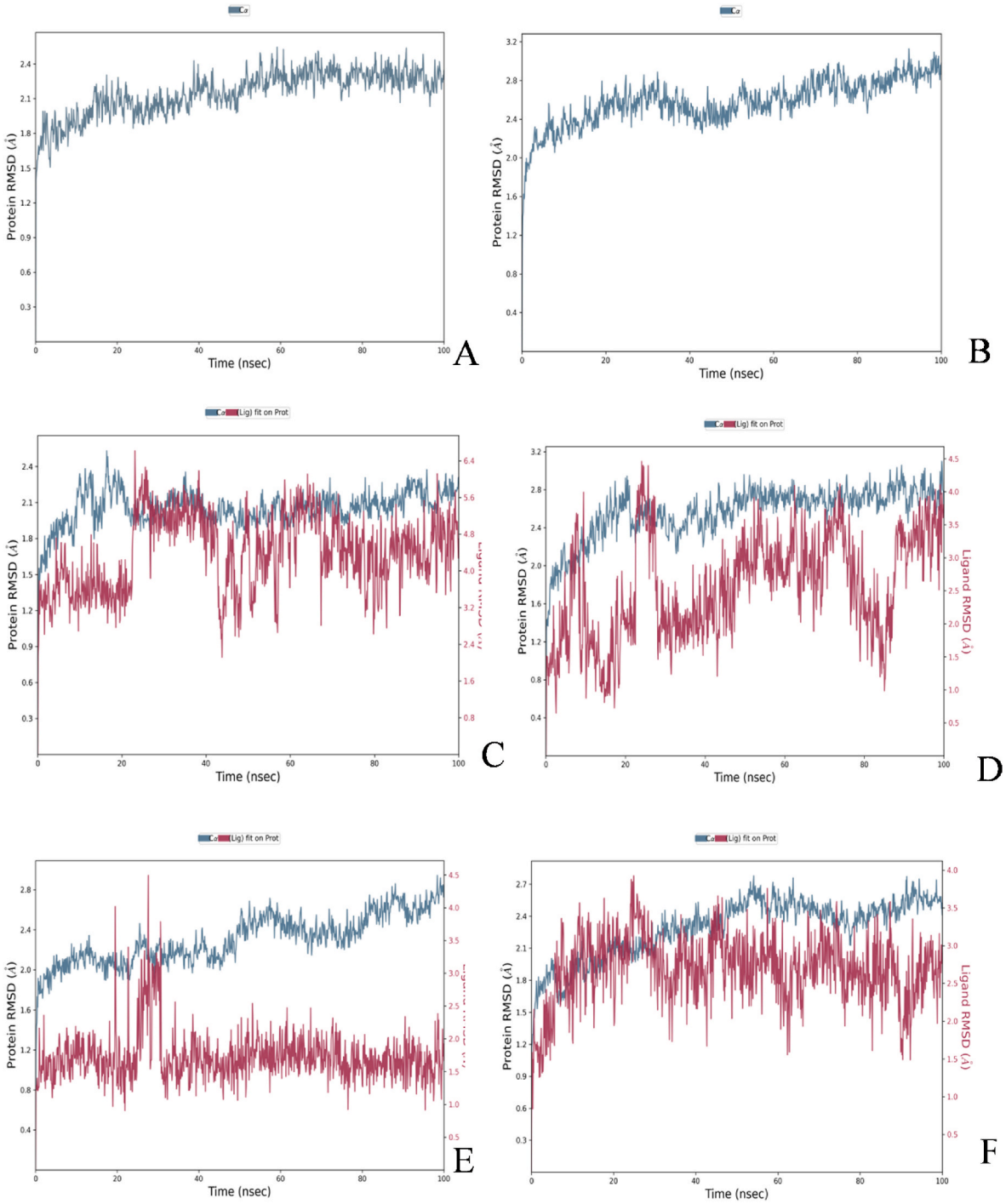


Fig. 4. RMSD plot of **A:** 1HW8 apo-protein; **B:** 1HW9 Apo-protein; **C:** 1HW8_M_02(c) complex; **D:** Pravastatin_1HW8 complex; **E:** 1HW9_ M_02(c) complex; **F:** Pravastatin_1HW9 complex.

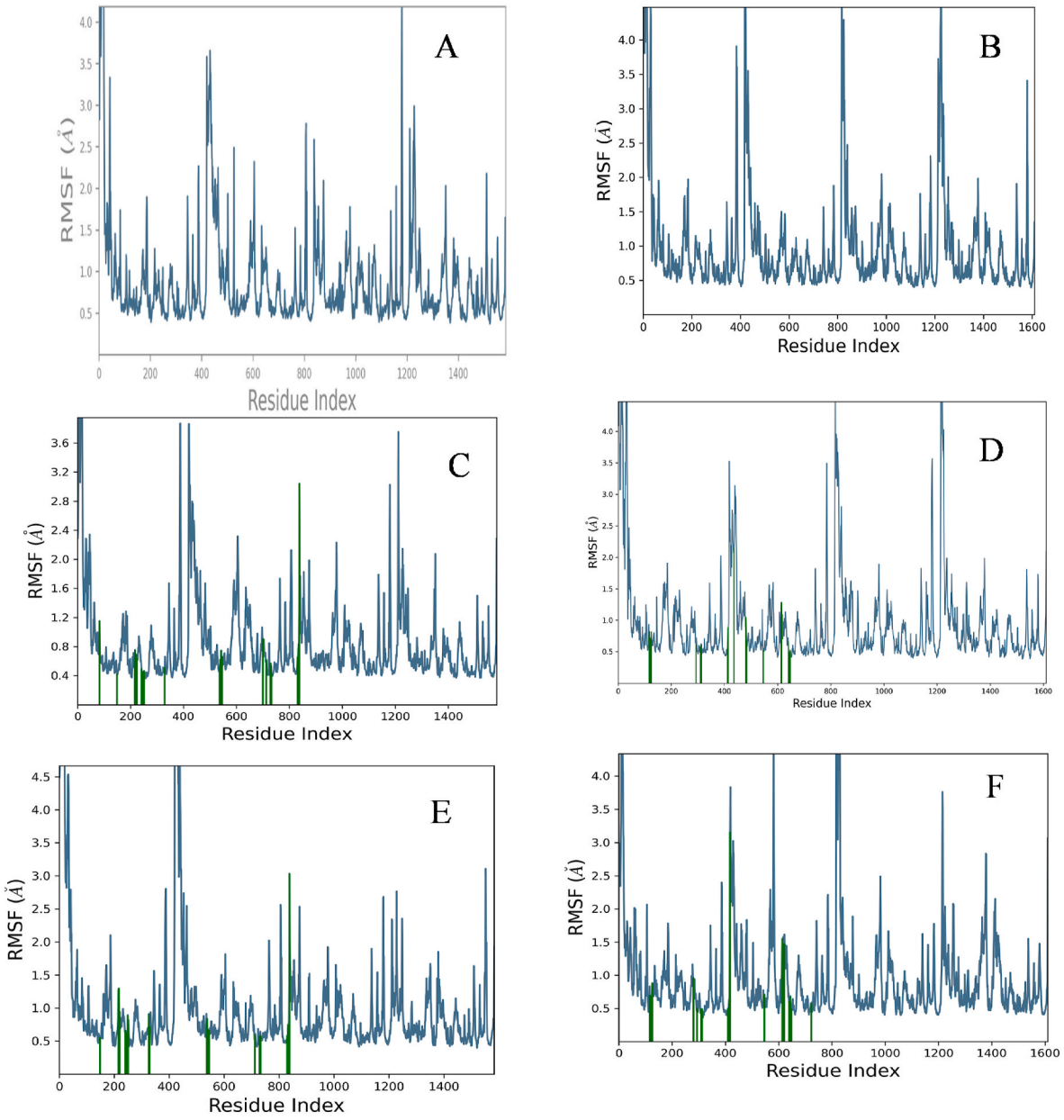


Fig. 5. RMSF plot of **A:** 1HW8 apo-protein; **B:** 1HW9 Apo-protein; **C:** 1HW8_M_02(c) complex; **D:** Pravastatin_1HW8 complex; **E:** 1HW9_M_02(c) complex; **F:** Pravastatin_1HW9 complex.

Simultaneously, unlike pravastatin, the compound **M_02(c)** passed Lipinski's criteria with one violation in form of its molecular weight [MW of **M_02(c)** surpasses 500 g/mol]. The results of physicochemical and ADME properties of **M_02(c)** and pravastatin indicated that **M_02(c)** may have suitable ADME properties like pravastatin, as shown in Table 3. Unlike SwissADME, bioavailability radar also offers a graphical representation of the drug-likeness properties of an oral bioactive molecule. As depicted in Fig. 7, six primary physicochemical characteristics are considered: lipophilicity, size, polarity, solubility, flexibility, and saturation. A compound, in order to be "drug-like," has to fall into the pink area attributed to the properties on each axis. It is defined by the following parameters such as, oral bioavailability, involving lipophilicity in the range of −0.7 and + 5.0, molecular weight between 150 and 500 g/mol, flexibility with not more than nine rotatable bonds, polarity defining TPSA between 20 $Å^2$ to 130 $Å^2$, insolubility within Log S ranging between −6 and 0, unsaturation having carbon fraction in sp^3-hybridization between 0.25 and 1.0. Herein, pravastatin is predicted to be less orally bioavailable due to its flexible nature with 11 rotatable bonds. However, **M_02(c)** is better in this regard, falling into the pink area with only one violation of MW being slightly above 500 g/mol.

For these reasons, this *in silico* study differs from studies specially performed on HMG-CoA reductase to the best of our knowledge. The present work provides a wide-open opportunity for the need to validate its findings via *in vitro* and *in vivo* studies of the edible mushroom extracts or their isolated tetracyclic triterpenes aimed at targeting HMG-CoA reductase. Our recent findings can provide future research support in the form of natural anti-hyperlipidemic and cardioprotective therapeutics to researchers.

3. Conclusion

In the current investigation, molecular docking and molecular dynamics simulations were performed to predict the cardioprotective potential of tetracyclic bioactive molecules targeting HMG-CoA reductase

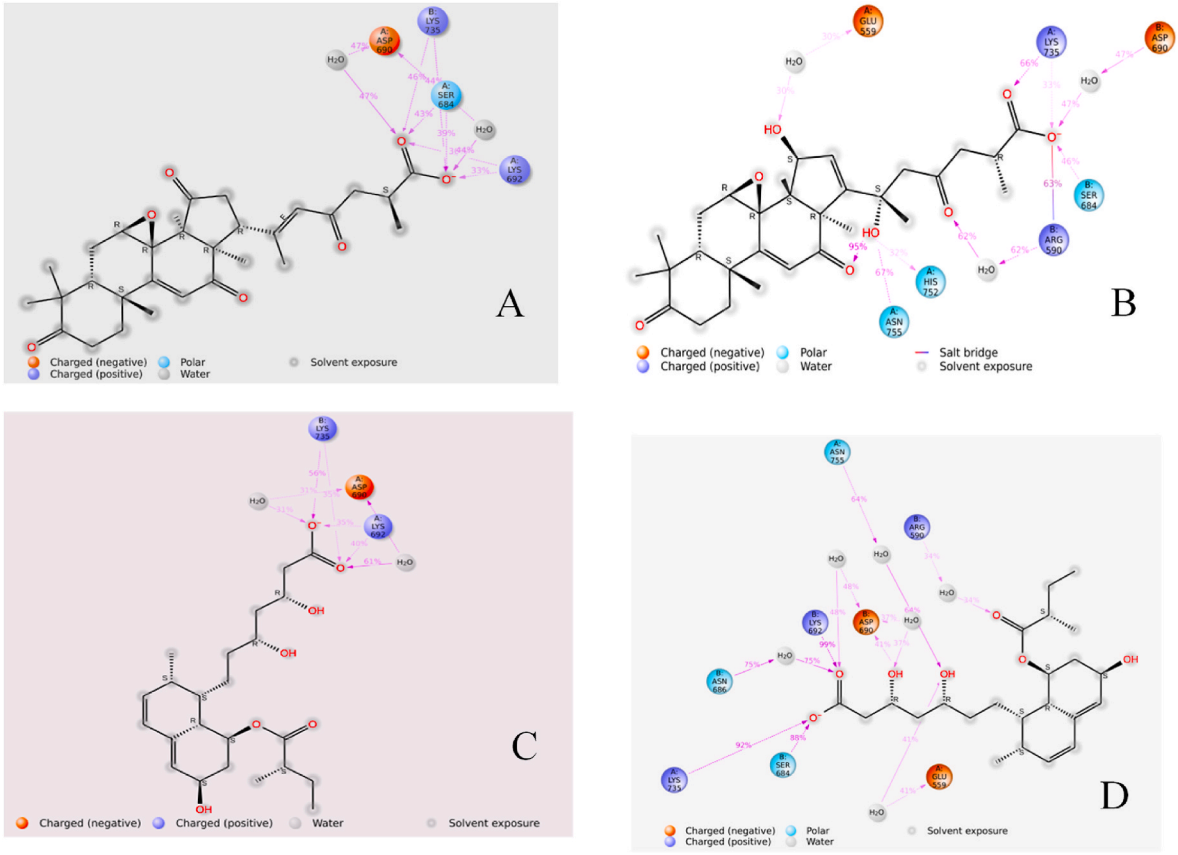


Fig. 6. Hydrogen bonding analysis for the ligand **M_02(c)** and Pravastatin with Protein 1HW8 and 1HW9; **A:** 1HW8_M_02(c), **B:** 1HW9_M_02(c), **C:** 1HW8_Pravastatin, **D:** 1HW9_Pravastatin.

Table 3
Physiochemical properties of **M_02(c)** and pravastatin.

Parameters	M_02(c)	Pravastatin(Std.)
Molecular weight	528.63	424.53
NHA	38	30
NAHA	0	0
NHBA	8	7
NHBD	3	4
NRB	6	11
MR	139.62	114.04
TPSA (Å^2)	141.5	124.29
iLOGp	3.18	2.99
LogS	−2.77	−4.75
SC	Soluble	Moderately Soluble
GI	Low	High
BBB	No	No
vROF	1	0
vGhose	3	0
vVeber	1	1
BS	0.56	0.56
SA	7.26	5.88

MW: Molecular weight, **NHA:** No. of heavy atoms, **NAHA:** No. of aromatic heavy atoms, **NHBA**: No. of hydrogen bond acceptors, **NHBD**: No. of hydrogen bond donors, **NRB:** No. of rotatable bonds, **MR:** Molar refractivity, **TPSA:** Topological polar surface area, **LogS:** Solubility, **SC:** Solubility class, **GI:** Gastrointestinal absorption, **BBB:** Blood Brain, Barrier Penetraton, **vROF:** Violation of Lipinski's rule of five, **vGhose**: Violation of Ghose rule, **vVeber:** Violation of Veber rule, **BS:** Bioavailability score, SA: Synthetic accessibility; **S:** Soluble, **MS:** Moderately soluble.

from an in-house database containing myco-constituents reported for mushrooms. Based on *in-silico* results, the tetracyclic triterpenes contained in mushrooms were detected to have a comparable binding affinity for the HMG-CoA reductase to the statin drugs, pravastatin, compactin, lovastatin, and simvastatin. The hit **M_02(c)** showed an almost equivalent SP Glide score with the standard statin drug pravastatin against both target proteins, 1HW8 and 1HW9. Further analysis through MD simulations up to 100 ns on HMG-CoA reductase specified the durable stability of the M_02(c)_1HW8 and M_02(c)_1HW9 complexes throughout the simulation period. The MMGBSA binding energies of **M_02(c)** against 1HW8 and 1HW9 protein indicated a significant interaction between the Protein-Ligand complex. Most importantly, ADME properties of selected hit **M_02(c)** and standard drug pravastatin were found within acceptable limits, indicating the drug-likeness potential of **M_02(c)**. In this context, further investigation and validation can be performed using *in-vivo* and *in-vitro* assay methods to evaluate the potential inhibitory activity of the compound **M_02(c)**, which was the most potent hit in this study. Subsequently, this study can provide valuable groundwork for the prospective exploration of tetracyclic triterpenoids, which may serve as innovative HMG-CoA reductase inhibitors for treating hyperlipidemia and other cardiovascular disorders.

CRediT authorship contribution statement

Rishav Mazumder: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Deijy Choudhury:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Alekhya Sarkar:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Ashmita Ghosh:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Conceptualization. **Sudhan Debnath:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision,

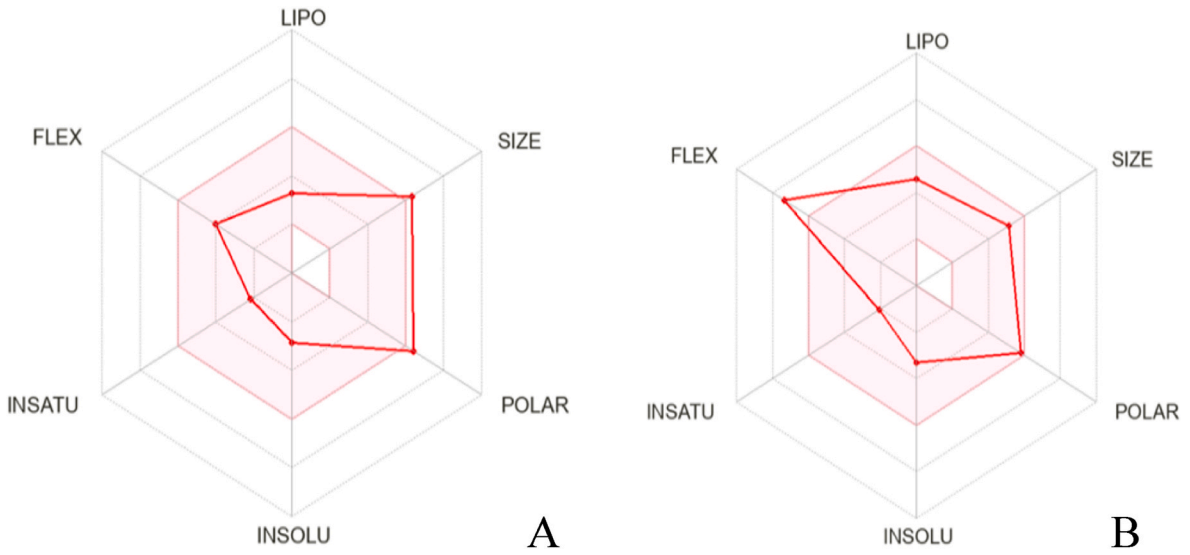


Fig. 7. The bioavailability radar plot of **A: M_02(c)** and **B:** Pravastatin is depicted schematically (**INSTU:** unsaturation; **INSOLU:** Insolubility, **LIPO:** Hydrophobicity, **FLEXI**: Rotatable bonds, **SIZE**: Molecular weight; **POLAR:** Polar surface area).

Software, Methodology, Conceptualization. **Bimal Debnath:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Conceptualization. **Rajat Ghosh:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amolm.2024.100053.

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