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Drug Repurposing of Statins for MMP-2 Modulation: Insights from Docking and Structural Dynamics

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A B S T R A C T

MMP-2 is a vital endopeptidase implicated in extracellular matrix remodeling, tumor invasion, and metastasis in ovarian cancer; its targeting thus represents a promising therapeutic approach. Statins are widely prescribed HMG-CoA reductase inhibitors which have shown pleiotropic anticancer effects, while carboplatin remains one of the standard chemotherapeutic agents in ovarian cancer therapy. This paper evaluates the potential of selected statins as allosteric inhibitors of MMP-2 through an in silico drug repurposing strategy. The three-dimensional structure of the hemopexin-like domain of MMP-2 was retrieved from the Protein Data Bank (PDB ID: 1RTG) and refined using GalaxyRefine. Molecular docking studies were performed using Schrödinger Glide to assess binding affinities of eight statins and carboplatin with MMP-2. Further characterizations of the molecular interactions were done through hydrogen bonding, hydrophobic contacts, and Pi–alkyl interactions. Protein–ligand complex stability and dynamics were assessed using NMA along with ENM via iMODS. Among the statins, atorvastatin showed the strongest binding affinity ($\Delta G = -7.9$ kcal/mol) with MMP-2, outperforming carboplatin ($\Delta G = -6.6$ kcal/mol). The docking analysis revealed critical interaction of atorvastatin with GLY215, TRP212, and HIS42. Stability and flexibility of the atorvastatin–MMP-2 complex were observed through NMA and ENM. The obtained results provide evidence of an allosteric inhibition mechanism. Other statins showed moderate binding affinities, while carboplatin was primarily found to interact via its conventional DNA-targeting mode. This study suggests that atorvastatin may act as an allosteric inhibitor of MMP-2, hereby presenting a novel repurposing opportunity for ovarian cancer therapy. Predictions based on computational analysis indicate favourable binding as well as structural stabilization of the MMP-2 regulatory domain; further in vitro and in vivo validation is required. Used in combination with standard chemotherapeutics like carboplatin, it may improve therapeutic efficacy with minimum off-target effects.

Keywords: *Ovarian cancer, MMP-2, Statins, Atorvastatin, Carboplatin, Molecular docking, In silico drug repurposing*.

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INTRODUCTION

Ovarian cancer is the third most common and lethal malignancy of the female reproductive system, which presents a great clinical challenge due to the late diagnosis and high metastatic potential of this cancer (Siegel et al., 2024). One of the main driving factors for OC development and progression is the degradation of ECM, mainly by matrix metalloproteinases, among which the role of Matrix Metalloproteinase-2 (MMP-2) is crucial for promoting the invasiveness and metastasis capabilities of tumor cells (Kessenbrock et al., 2010). MMP-2 belongs to the group of zinc-dependent endopeptidases and degrades types IV and V collagen of the basement membrane; this process promotes the migration of cancer cells and angiogenesis (Visse & Nagase, 2003). High levels of MMP-2 expression in advanced stages of ovarian cancer indicate a poor prognosis, increased metastatic dissemination, and neovascularization (Davidson et al., 1999; Schmalfeldt et al., 2001). Hence, selective inhibition of MMP-2 emerged as a promising therapeutic strategy for controlling the progress of the tumor.

These improvements in computational methodologies, including molecular docking, virtual screening, and drug repurposing frameworks, now revolutionize early-stage inhibitor discovery and offer rapid, inexpensive yet highly predictive alternatives to classic, traditional pipelines of drug development. It finally puts investigators in a position where they can, through computation, interrogate huge compound libraries, optimize candidate molecules, and predict interaction profiles with target proteins before lab validation. This diminishes developmental risks and quickens translational progress. Especially, molecular docking studies have been instrumental in understanding atomic-level interactions between drugs and their targets, thus supporting the rational design and repurposing of compounds with improved specificity and efficacy.

Beyond their classic lipid-lowering role, statins have gained considerable interest due to their pleiotropic anticancer properties. Indeed, an increasing body of evidence shows that statins can induce apoptosis, repress tumor proliferation, inhibit angiogenesis, and modulate oncogenic signaling through various cancers (Graaf et al., 2004; Gbelcová et al., 2017). Anticancer properties of simvastatin, atorvastatin, and lovastatin have been documented in several reports by inhibition of the mevalonate pathway, Ras/Rho signaling, and upregulation of pro-apoptotic gene expression (Demierre et al., 2005). Of note, statins have also been associated with suppressing metastatic events through reducing ECM remodeling, inhibiting cellular motility, and downregulating MMP expression (Kusama et al., 2001). Recent studies further point to the potential of statins as inhibitors of the MMP family of enzymes. Some statins have been shown, through in silico and experimental analyses, to bind with the catalytic and noncatalytic domains of MMPs, disrupting their enzymatic activities and inhibiting cancer cell invasion (Husain et al., 2022). Complementary in silico screening analyses underlined a number of statin molecules showing promising binding properties toward MMP-2, with emphasis placed on the broadening role of repurposed lipid-lowering agents in ovarian cancer therapies (Pooja et al., 2021). All these collectively demonstrate that integrating computational predictions with knowledge of the mechanistic pharmacology of statins has the potential to accelerate the discovery of novel MMP-2 inhibitors. This possibility is extended, in the present study, with eight clinically approved statins that were tested for repurposing as allosteric inhibitors of MMP-2 in ovarian cancer. Based on the strategic focus on the noncatalytic binding region for improved selectivity and fewer off-target effects, this study characterizes the binding affinities and structural interactions of statins with MMP-2 through molecular docking and molecular dynamics simulations. This is done with the aim of identifying promising statin candidates and supporting cost-effective therapeutic strategies that could improve the clinical management and outcome in ovarian cancer patients.

MATERIALS AND METHODS

Protein Sequence Retrieval

The primary amino acid sequence of human MMP-2 was retrieved from UniProt, which is a comprehensive and curated resource for protein sequence and functional annotation (https://www.uniprot.org/. Downloads were made for the FASTA sequence corresponding to MMP-2 (UniProt ID: P08253) for downstream computational analyses. UniProt Consortium (2023).

Physicochemical Property Analysis

The SWISS ADME tool was utilized developed by the Swiss Institute of Bioinformatics to evaluate the physicochemical properties ligands. ProTox was utilized to evaluate the toxicity of ligands. ProtParam computes various essential parameters, including the molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and GRAVY score, which are useful for understanding protein stability and structural behavior (Gasteiger et al., 2005).

Secondary Structure Prediction

Secondary structure of MMP-2 was predicted by using the PSIPRED server. http://bioinf.cs.ucl.ac.uk/psipred/

PSIPRED uses evolutionary information, position-specific scoring matrices, and a neural network–based algorithm to predict the secondary structure elements like α-helices, β-sheets, and coils. It was shown to be quite accurate and reliable for prediction of secondary structures in diverse protein families (Buchan & Jones 2019).

Tertiary Structure Retrieval

The three-dimensional structure of human MMP-2 was retrieved from the Protein Data Bank (PDB) at https://www.rcsb.org/ using the crystallographic structure with PDB ID: 1RTG. This is the hemopexin-like regulatory domain of MMP-2, which lacks the zinc-containing catalytic region. The PDB represents the single, global archive for experimentally determined macromolecular structures obtained via X-ray crystallography, cryo-EM, and NMR spectroscopy (Berman et al., 2000).

Protein Structure Refinement

ERRAT is a web-based tool, which especially examines the quality of crystallographically determined protein models by analyzing the non-bonded interactions of the models. Structural validation was firstly made by using ERRAT (Colovos & Yeates, 1993). Then, the structure of MMP-2 was refined on the GalaxyRefine server, https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE which uses molecular dynamics-based relaxation to enhance side-chain geometry, overall structural accuracy, and stereochemical quality.

Validation of 3D Structure

Further verification of the structure was done using a Ramachandran plot generated through the SWISS-MODEL Ramachandran assessment tool, https://swift.cmbi.umcn.nl/servers/html/ramaplot.html. The Ramachandran plot assesses the backbone dihedral angles (φ and ψ) allowing for the verification of sterically feasible conformation in the protein model (Lovell et al., 2003).

Ligand Retrieval

Eight clinically approved statins were selected for evaluation as potential MMP-2 inhibitors and retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/. These included atorvastatin, simvastatin, pravastatin, rosuvastatin, fluvastatin, lovastatin, pitavastatin, and cerivastatin. All ligand structures were downloaded in SDF format for subsequent molecular docking and simulation analyses (Kim et al., 2021).

Table 1: Name of Statins selected as Ligands and Carboplatin (Reference Drug).

S no.	Ligand Name	SMILES
1	Simvastatin	CCC(C)(C)C(=O)O[C@H]1C[C@H](C=C2[C@H]1[C@H]([C@H](C=C2)C)CC[C@@H]3C[C@H](CC(=O)O3)O)C
2	Atorvastatin	CC(C)C1=C(C(=C(N1CC[C@H](C[C@H](CC(=O)O)O)O)C2=CC=C(C=C2)F)C3=CC=CC=C3)C(=O)NC4=CC=CC=C4
3	Rosuvastatin	CC(C)C1=NC(=NC(=C1/C=C/[C@H](C[C@H](CC(=O)O)O)O)C2=CC=C(C=C2)F)N(C)S(=O)(=O)C
4	Pravastatin	CC[C@H](C)C(=O)O[C@H]1C[C@@H](C=C2[C@H]1[C@H]([C@H](C=C2)C)CC[C@H](C[C@H](CC(=O)O)O)O)O
5	Fluvastatin	CC(C)N1C2=CC=CC=C2C(=C1/C=C/[C@@H](C[C@@H](CC(=O)O)O)O)C3=CC=C(C=C3)F
6	Pitavastatin	C1CC1C2=NC3=CC=CC=C3C(=C2/C=C/[C@H](C[C@H](CC(=O)O)O)O)C4=CC=C(C=C4)F
7	Lovastatin	CC[C@H](C)C(=O)O[C@H]1C[C@H](C=C2[C@H]1[C@H]([C@H](C=C2)C)CC[C@@H]3C[C@H](CC(=O)O3)O)C
8	Cerivastatin	CC(C)C1=C(C(=C(C(=N1)C(C)C)COC)C2=CC=C(C=C2)F)/C=C/[C@H](C[C@H](CC(=O)O)O)O
9	Carboplatin	C1CC(C1)(C(=O)[O-])C(=O)[O-].N.N.[Pt+2]

Ligand Preparation

The chemical structures of selected statins (including simvastatin, atorvastatin, rosuvastatin, pravastatin, fluvastatin, pitavastatin, lovastatin, and cerivastatin) were drawn by using ChemDraw Ultra 12.0 saved in SDF format. These compounds were then converted to PDB format and energy-minimized using Open Babel-an open-source cheminformatics toolkit developed for structural optimization and molecular file conversion (O'Boyle et al., 2011). Statins have been selected based on their well-documented pleiotropic effects, including anti-metastatic and anti-inflammatory properties (Liao & Laufs, 2005; Schachter, 2005).

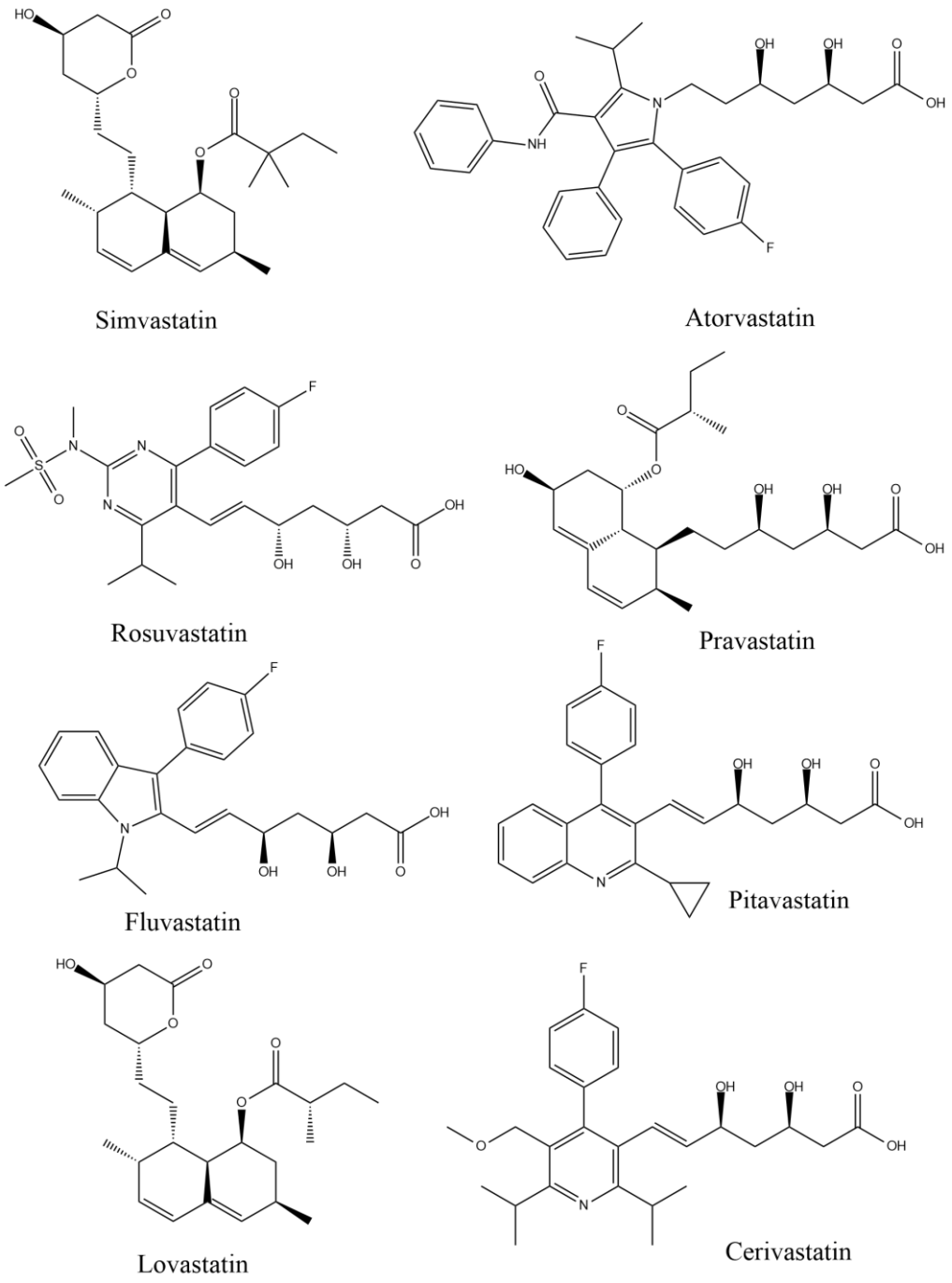

Figure 1: Chemical structure of marketed statins.

Ligand Optimization

Geometry optimization for all statin ligands was initially done in PyRx with the MMFF94 force field, which provides fast, reliable energy minimization for small-molecule conformers (Dallakyan & Olson, 2015). The minimized ligands were subjected to a second stage of optimization in the Schrödinger Suite using the LigPrep module. LigPrep performs OPLS4 force-field minimization, corrects abnormal bond geometries, enumerates low-energy tautomers, ionization states, and stereochemical variants, and produces output suitable for conformational analysis and subsequent docking workflows (Schrödinger, 2020). After optimization, Gasteiger charges were added and rotatable bonds were defined using AutoDockTools in order to prepare the molecules for the AutoDock Vina docking protocol.

Binding Pocket Identification - Schrödinger-based workflow

Potential allosteric binding pockets within the hemopexin-

like regulatory domain of MMP-2 (PDB ID: 1RTG) were analyzed for the identification of suitable noncatalytic binding regions known to mediate substrate recognition and protein-protein interactions (Morgunova et al., 1999; Visse & Nagase, 2003). The protein structure was prepared using Schrödinger's Protein Preparation Wizard, which performs automatic bond order correction, side-chain optimization, hydrogen addition, and restrained minimization using the OPLS4 force field. Binding pocket identification was performed with Schrödinger's SiteMap, which predicts druggable pockets based on hydrophobic/hydrophilic balance, pocket volume, enclosure, and donor/acceptor properties. The metrics used for ranking pockets according to their suitability for allosteric ligand binding included SiteScore and DScore. Final verification and structural visualization were performed using the molecular graphics platform Maestro (Schrödinger, 2020).

Molecular Docking (Glide Docking in Schrödinger)

Next, molecular docking of all statin ligands against the selected allosteric pocket of MMP-2 was made with Glide in Schrödinger Maestro, which allows highly accurate ligand–receptor binding predictions based on its advanced empirical scoring function and pose prediction algorithm (Friesner et al., 2004). From the predicted allosteric pocket in SiteMap, the receptor grid was prepared to ensure appropriate positioning for the docking box toward the noncatalytic domain. Docking was made in Glide Standard Precision (SP) mode, and then Extra Precision (XP) docking was followed for top-ranked ligands to improve the accuracy of the binding. Estimates of binding free energy (GlideScore) were noted for all statins. With the help of Maestro's interaction diagram tools, protein–ligand interactions such as hydrogen bonding, hydrophobic contacts, salt bridges, and π–π interactions were performed and further verified with LigPlot+ (Laskowski & Swindells, 2011). Statins acted through hydrophobic ring systems and a flexible side chain compatible with favorable interactions within MMP-2's allosteric hydrophobic cavities, supporting their potential as allosteric modulators.

iMODS

The iMODS server https://imods.iqf.csic.es/. In this respect, molecular dynamics simulations were carried out, which provides detailed insights into molecular motion with a minimum of computational resources. This method calculates normal modes in the input structure using internal coordinates (like torsion angles) to represent collective functional movements of biological macromolecules (López-Blanco et al., 2014).

RESULTS

Sequence Retrieval and Protein Analysis

The amino acid sequence of MMP-2 in FASTA format was retrieved from the UniProt database, UniProt ID: P08253. The three-dimensional structure of the C-terminal hemopexin-like regulatory domain was recovered from the Protein Data Bank at a resolution of 2.60 Å, under PDB ID: 1RTG. In order to understand the general architecture of the protein, structural features such as α-helices, β-sheets, and coil regions were further analyzed and visualized using Discovery Studio, as shown in Figure 2.

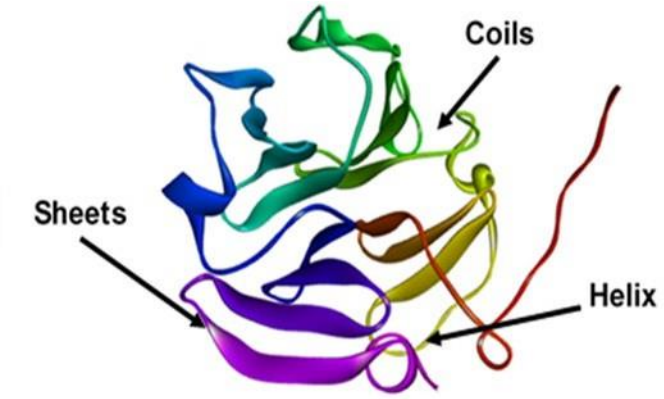

Figure 2: 3D Structure of Hemopexin Like Domain of MMP-2 (PDB ID:1RTG) Showing Coils, Sheets and Helix Region.

Analysis of Physiochemical Properties of Ligands

Physicochemical parameters like MW, numbers of HBA and HBD, MolLogP, MolPSA, and Lipinski's Rule violation give important information about the drug-likeness and oral bioavailability of the tested compounds. According to Lipinski's Rule of Five, molecules with MW ≤ 500 g/mol, HBA ≤ 10, HBD ≤ 5, LogP ≤ 5, and PSA ≤ 140 Å² are likely to have useful permeability and oral absorption. In general, most statins are within acceptable limits of drug-likeness, as a few violations

may occur in respect to higher polarity or molecular weight. Carboplatin, though not typical for a small-molecule drug-like structure, follows Lipinski's criteria except for an atypical LogP value of 0, showing its hydrophilic nature.

Statins with no rule violations include Simvastatin, Fluvastatin, Pitavastatin, Cerivastatin, which are predicted to exhibit favorable oral absorption. Molecules with PSA > 140 Å² are more hydrophilic; the passive permeability of such compounds is reduced, e.g., Pravastatin, Rosuvastatin; these drugs depend on transporter-mediated uptake. Atorvastatin possesses one MW violation but is still adequately absorbed because of its strong lipophilicity and active transport mechanisms. Although numerically compliant, carboplatin behaves differently due to its platinum-based coordination chemistry and also is not orally bioavailable. Physiochemical Properties of Ligands and Carboplatin is shown in Table 2.

Table 2: Physiochemical Properties of Ligands and Carboplatin.

Compounds	Mol. Weight	# Of HBA	# Of HBD	Mol Logp	Molpsa	Lipinski's Rule Violation
Simvastatin	418.57	5	1	3.74	72.83	0
Atorvastatin	558.64	6	4	3.58	111.79	1
Rosuvastatin	481.54	9	3	2.32	149.30	1
Pravastatin	424.53	7	4	2.99	124.29	0
Fluvastatin	411.47	5	3	2.93	82.69	0
Pitavastatin	421.46	6	3	2.79	90.65	0
Lovastatin	404.54	5	1	3.86	72.83	0
Cerivastatin	459.55	7	3	3.56	99.88	0
Carboplatin	371.25	6	2	0.00	86.74	0

Secondary Structure Prediction

The PSIPRED server was used to predict the secondary structure elements of the MMP-2 protein. From the analysis, it can be observed that α-helices and β-sheets are distributed throughout the protein, represented by Figure 3. Indeed, the pink regions correspond to 14 residues forming α-helices, while the yellow regions represent 75 residues constituting β-sheets. The remaining grey segments indicate loop and coil regions that contribute to structural flexibility. Overall, the results demonstrate a balanced distribution of helices and sheets, supporting the structural stability and well-defined functional architecture of the MMP-2 protein.

Figure 3: Secondary Structure of MMP-2 Obtained Through PSIPRED.

Analysis of Protein Quality

The overall quality factor of the MMP-2 model, as calculated by the ERRAT validation tool, was 86.772 (Figure 4). A score this high indicates that the model is dependable enough for the next steps of analysis, such as molecular docking, but with some areas that could still be refined. Usually, ERRAT values of ≥90 show that a protein structure is of good quality. This suggests that the MMP-2 model is acceptable but still has room for further optimization in terms of structural accuracy.

The Y-axis in the ERRAT plot indicates the error value, and the X-axis corresponds to amino acid sequence position. Red-colored bars indicate residues whose error values exceed the 99% confidence threshold, while yellow-colored bars are regions that surpass the 95% confidence limit. White and grey bars indicate regions within acceptable limits of error and thus represent structurally reliable parts of the protein model.

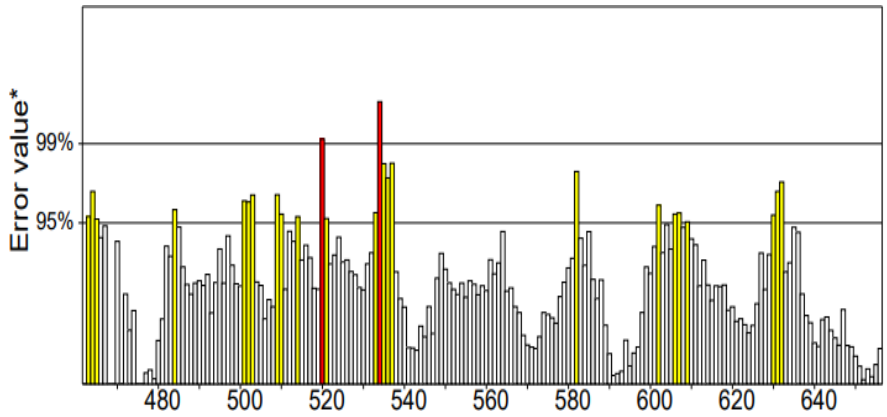

Figure 4: Results obtained through ERRAT.

Refinement of the Protein

Further refinement of the MMP-2 model for better structural quality was performed with the GalaxyRefine server. This tool increases protein accuracy through improved backbone flexibility, side-chain optimization, and reduction in steric clashes. It gave a model with a 97.5% Ramachandran favored score after refinement, meaning that the geometric reliability was increased along with overall structural stability. This refined model, of high quality, is hence suitable for use in downstream analyses such as molecular docking (Table 3).

Table 3: Galaxy Refine Index of MMP-2

Model	GDT-HA	RMSD	Mol Probity	Clash score	Poor rotamers	Rama favored
MODEL 1	0.9692	0.387	1.713	12.7	0.0	97.5

Ramachandran Plot

A Ramachandran plot was generated to assess the distribution of phi (φ) and psi (ψ) dihedral angles within the protein structure. The results showed that 92.9% of the residues were located in the most favored regions, while 5.9% and 1.2% of residues fell within the additionally allowed and generously allowed regions, respectively. Importantly, no residues appeared in disallowed regions, indicating excellent stereo-chemical quality and confirming the structural reliability of the modeled protein (Figure 5).

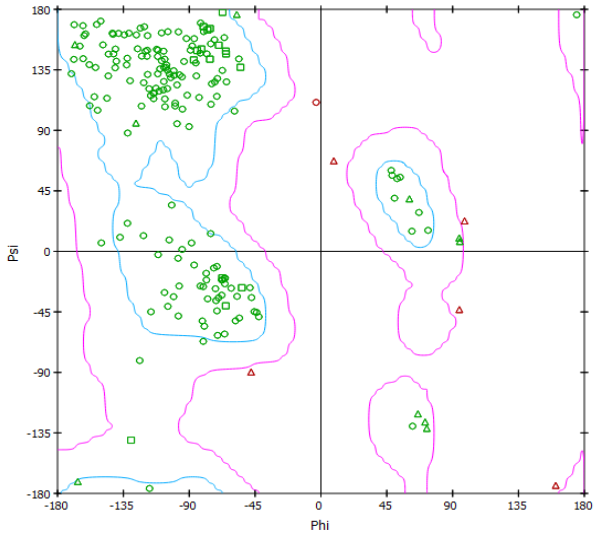

Figure 5: The Ramachandran Plot Findings Reveal That the Most Favorable Zone Contains 92.9% of the Residues.

Binding Affinity of the Cephalosporins to MMP-2

The calculated binding affinity of atorvastatin against the MMP-2 protein was found to be −7.9 kcal/mol among the listed eight statins, whereas the reference drug carboplatin has shown relatively lower binding affinity, −6.6 kcal/mol. The remaining statins showed relatively weak molecular interactions with a binding energy range of −5.3 to −6.8 kcal/mol (Table 4). Further, detailed analysis of the atorvastatin-MMP-2 complex has revealed that GLY213, GLY 215 is involved in hydrogen bonding, TRP212 in carbon-hydrogen interactions, and HIS42 in Pi-alkyl interactions (Table 5). Three-dimensional and two-dimensional representations depicting molecular interactions of atorvastatin and carboplatin into the active site of MMP-2 are shown in Figure 6A-D, showing key residues that play an important role in the stabilization process of these complexes.

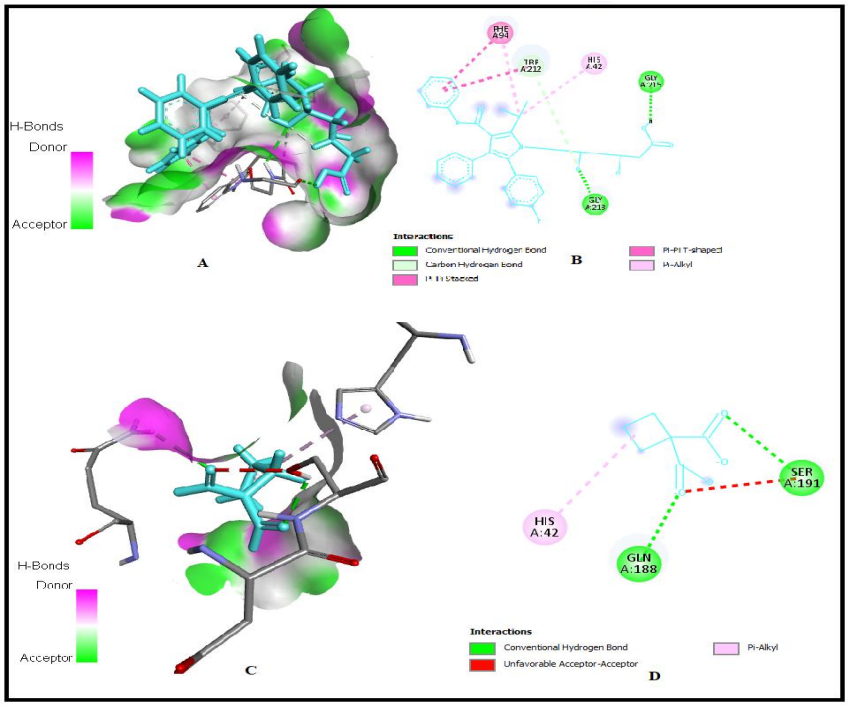

Figure 6: Interaction of atorvastatin and carboplatin with MMP-2. (A) Three-dimensional representation showing atorvastatin bound to MMP-2 (surface representation). (B) Two-dimensional diagram of important interactions between atorvastatin and MMP-2 (C) Three-dimensional view of carboplatin interacting with MMP-2 (surface form). (D) Two-dimensional map showing the interactions of carboplatin with MMP-2.

Table 4: Docking Interaction Energies of Ligands with MMP-2.

S no.	Ligand Name	Interactions Energies (Kcal/mol)
1	Simvastatin	-6.1
2	Atorvastatin	-7.9
3	Rosuvastatin	-5.4
4	Pravastatin	-6.8
5	Fluvastatin	-5.3
6	Pitavastatin	-6.2
7	Lovastatin	-5.9
8	Cerivastatin	-6.0
9	Carboplatin	-6.6

Table 5: MMP-2 Amino Acids Involved in Different Types of Interactions with Atorvastatin.

Type of interactions	Amino acids
Carbon Hydrogen bonding	TRP212
Hydrogen bonding	GLY213, GLY 215
Pi-Alkyl bonding	HIS 42

Molecular Dynamic Simulations

Flexibility is one of the intrinsic properties of biological macromolecules, which enables protein–ligand interactions and substrate recognition. In order to assess the dynamic behavior of the docked complexes, iMODS was used to conduct Normal Mode Analysis (NMA), combining molecular motion with the coordinates of the docked atorvastatin–MMP-2. The plot of deformability showed that the structure flexibility generally depended on the local distortions for the individual residues hinged across the chain, represented in peaks-green to show maximum mobility. Figure 7A: The B-factor values in the apo and liganded forms showed the uncertainty of each atom, and the graph constructed thereof was a clear representation of the stability of the docked complexes with respect to their structural coordinates. We calculated the eigenvalue of the complex as 1.513588×10^{-4}, with very minor fluctuation, pointing to a stabilized hemopexin domain upon ligand binding and supporting the proposed allosteric inhibition mechanism (Figure 7D). An inverse relationship between eigenvalues and variance was observed for each normal mode, as illustrated in Figure 7C. Analysis of the covariance matrix revealed the correlated, uncorrelated, and anti-correlated motions colored in red, white, and blue, respectively (Figure 7E). Finally, the ENM depicted Cα atoms of the protein connected through springs with different strengths. Lighter grey regions corresponded to flexible regions, while dark grey represented more rigid connectivity, thus providing insight into the induced rigidity and flexibility within the structure by the binding of atorvastatin.

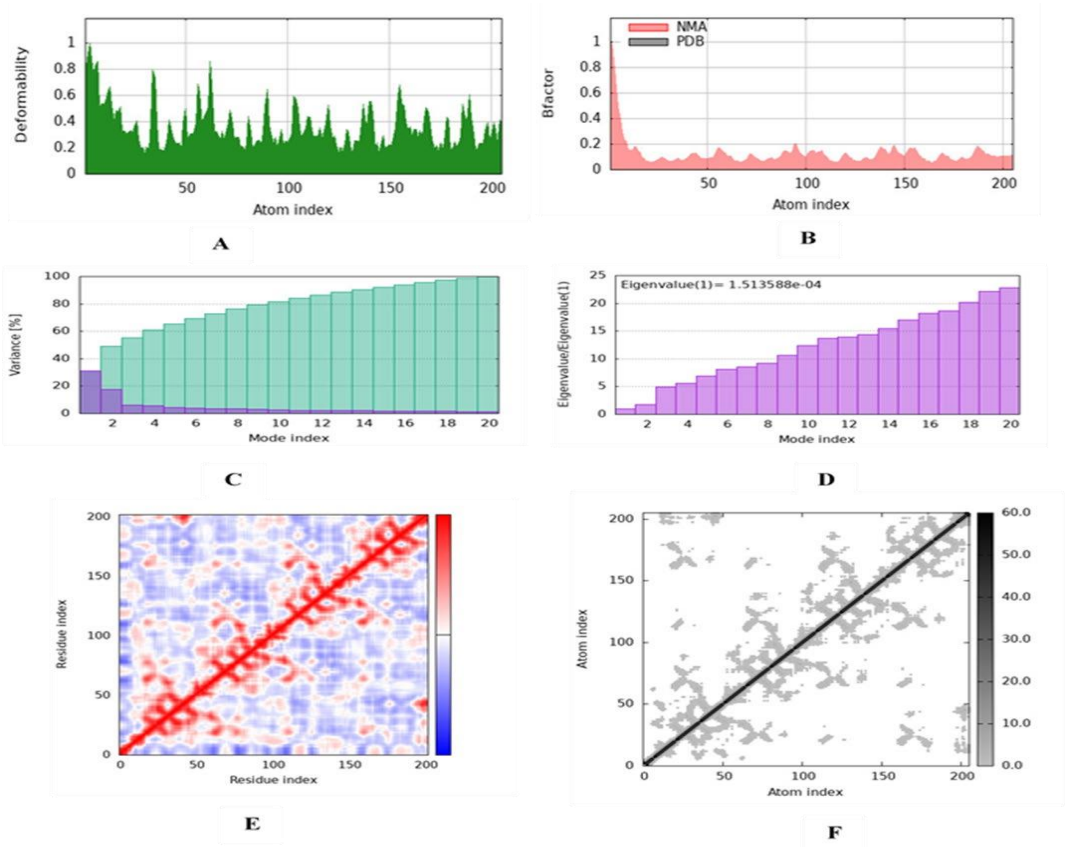

Figure 7: iMODS Normal Mode Analysis (NMA) of atorvastatin bound to MMP-2. Graphical representations of the docked complex are shown below: (A) Deformation plot highlighting the residue-wise flexibility, where peaks represent hinge regions. (B) B-factor plot showing the relative mobility of each atom in the complex. (C) Variance plot showing

the relationship between normal mode variance and corresponding eigenvalues. (D) Eigenvalues indicating overall structural stiffness of the complex. (E) Covariance matrix displaying correlated (red), uncorrelated (white), and anticorrelated (blue) atomic motions. (F) Elastic network model (ENM) of the connectivity of Cα atoms via springs. Lighter grey represents more flexible regions, and dark grey represents more rigid interactions.

DISCUSSION

Matrix metalloproteinase-2 is an enzyme that has been implicated in a number of physiological and pathological processes, including ovarian cancer. Physiologically, MMP-2 is involved in the remodeling of the extracellular matrix that occurs during wound healing, angiogenesis, and tissue repair. When dysregulated, however, MMP-2 can take part in tumor progression, invasion, and metastasis (Visse & Nagase, 2003). Overexpression of MMP-2 has been linked to advanced stages of ovarian cancers, high histological grades, and poor prognosis, indicating its value as a potential therapeutic target (Kamat et al., 2006; Marchenko et al., 2018; Yu et al., 2019).

This study investigated the potential of statins-a class of HMG-CoA reductase inhibitors-for the modulation of MMP-2 in ovarian cancer. Statins have well-documented cholesterol-lowering effects and excellent safety profiles in humans (Istvan & Deisenhofer, 2001). Aside from their cardiovascular benefits, statins demonstrate pleiotropic effects, such as anti-proliferative, pro-apoptotic, and anti-angiogenic activities in a wide range of cancers (Ben Sahra et al., 2011; Kodach et al., 2011). Alongside this, carboplatin, a platinum-based chemotherapeutic, served as a reference compound due to its established cytotoxic effects in ovarian cancer therapy.

The structural and functional analysis of MMP-2 revealed that it was a stable, hydrophilic protein with well-balanced secondary structure features, including α-helices and β-sheets. Model refinement by GalaxyRefine enhanced ERRAT quality scores from 86.772 to 94.086, while the analysis of the Ramachandran plot confirmed that 97.5% of residues were in favorable regions, hence confirming the reliability of docking simulations. This is an important step, because small errors in protein structure can strongly influence the outcome of a docking and binding predictions (Laskowski et al., 1993; Colovos & Yeates, 1993).

The molecular docking study showed that, among the selected statins, atorvastatin exhibited the highest binding affinity with a ΔG of -7.9 kcal/mol, much higher than that of the reference compound carboplatin, which is -6.6 kcal/mol. Other statins showed less binding affinity, between -5.3 and -6.8 kcal/mol, indicating variable potential toward MMP-2 interaction. Further interaction analysis of the docked complex showed that atorvastatin interacts via hydrogen bonding with GLY215, carbon-hydrogen interaction with TRP212, and Pi-alkyl interaction with HIS42 in order to stabilize the complex. Carboplatin interacts with MMP-2, though with low binding energies, as supported by a small number of noncovalent contacts, reflecting its primary DNA-targeting cytotoxic mode of action. The negative value for ΔG ascertains the thermodynamic stability of the two complexes, underlining atorvastatin as a promising allosteric inhibitor of MMP-2.

NMA by iMODS was used to assess the dynamic behavior of the complexes. The low eigenvalue for the atorvastatin–MMP-2 complex evidenced a low fluctuation that attested to high stability of the hemopexin domain. Deformability plots and B-factor analysis supported the fact that, while the protein was structurally stable, it retained flexibility in all key regions, thereby supporting the potential allosteric inhibition mechanism. Analysis of the covariance matrix resulted in good correlation amongst residues, and the elastic network model indicated regions of both rigidity and flexibility, emphasizing the robustness of the docked complex. Results obtained suggest that atorvastatin stabilizes the hemopexin-like regulatory domain of MMP-2, thereby potentially restricting substrate recognition and activity. Clearly, allosteric inhibition of MMP-2 has several advantages compared with typical Zn^{2+}-chelating inhibitors due to enhanced selectivity and reduced off-target effects. Among these, statins offer added value by creating pleiotropic anticancer effects which could synergize with MMP-2 inhibition, thereby limiting tumor invasion, angiogenesis, and metastasis. Carboplatin exerts its effects via DNA crosslinking and cytotoxicity but does not directly modulate the activities of MMP-2 and thus may further underscore the potential value of repurposing statins as adjuvant therapeutics targeting the tumor microenvironment. Despite promising in silico findings on statins, their dosage, pharmacokinetics, and potential side effects will have to be considered in clinical applications for cancer therapy. Combination strategies, such as co-

administration with standard chemotherapeutics or encapsulation in nanocarriers, could favor tumor-specific delivery while reducing systemic toxicity. *In-vivo* and *in-vitro* validations will be necessary, considering the investigation of dual inhibitory potential toward both MMP-2 and other oncogenic pathways. Zhao et al. (2020) present evidence that atorvastatin binds strongly and stabilizes the MMP-2 hemopexin domain, acting as a potential allosteric inhibitor in ovarian cancer therapy, using carboplatin as a reference standard to confirm that statins may provide benefits complementary to modulating the tumor microenvironment and thus support the repurposing of these drugs as adjuvant anticancer agents.

CONCLUSION

In the present study, atorvastatin showed the highest binding affinity with the MMP-2 protein among the statins tested, whereas carboplatin was used as a reference compound with relatively lower interaction energy. The negative ΔG value for the atorvastatin–MMP-2 complex (−7.9 kcal/mol) suggests a thermodynamically stable interaction, indicating that atorvastatin may act as a potential allosteric inhibitor of MMP-2 in ovarian cancer. This observation opens a window of opportunity for repositioning atorvastatin, an FDA-approved drug with adequate safety records, for new therapeutic approaches targeting MMP-2-mediated tumor progression.

Although exciting, these results from in silico studies merely represent an exploratory step. Experimental validation, through both in vitro and in vivo studies, will be required to confirm the inhibiting activity of atorvastatin on MMP-2 and its role in growth and metastasis in ovarian cancer. Further studies will be needed to explore pharmacokinetics, appropriate dosing schedules in an oncology setting, and possible synergism when combined with conventional chemotherapeutic agents such as carboplatin in order to establish safe and effective adjunct therapies.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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