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Preliminary hypothetical assessment and *in silico* molecular docking of statin to VEGFR2 and VEGFR3 protein complex associated with angiogenesis and lymphangiogenesis in diabetic wounds

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ABSTRACT

Vascular endothelial growth factor (VEGF) receptors are the critical drivers for blood vessel formation and lymphangiogenesis in diabetic wounds. However, due to the high blood glucose levels, advanced glycation end products release inflammatory mediators, which lead to hypoxic conditions that decrease the synthesis of essential growth factors and degeneration of the blood vessels necessary for the recovery of the diabetic wound. Hence, repurposing statins and identifying the binding potential to the active amino acid residues on the vascular endothelial growth factor receptor (VEGFR 2) and VEGFR 3 receptor could be a preliminary analysis and hypothetical computational challenge that statins can bind to VEGFs. That initiates the VEGF levels and reduces the inflammation associated with the conversion of M2 macrophages. Hence VEGFR 2 and VEGFR 3 are promising therapeutic targets for starting angiogenesis and lymphangiogenesis in diabetic wounds. Furthermore, the statins (Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin, and Simvastatin) subjected to computational molecular docking (Lib docking), and the best statins are selected based on the LibDock score and hydrogen and hydrophobic bond interactions. The results revealed that Atorvastatin (125.90 and 126.0), Fluvastatin (126.37 and 120.0), Pravastatin (129.90 and 128.6), and Rosuvastatin (130.01 and 130.62) had shown a significant binding potential to VEGFR2 and VEGFR3 protein complex and more excellent LibDock score with less variation when compared with the remaining statin molecules. Furthermore, the selected molecules were subjected to computational pharmacophore modeling and toxicity studies. The pharmacophore modeling was carried out to identify the feature set amino acids between the ligand and protein complex, followed by hypothetical confirmation of toxicity when the statin molecules interact with the skin tissue to determine carcinogenicity and skin sensitivity. The toxicity studies revealed that the four statins molecules showed moderate to less skin sensitivity and non-carcinogenicity. In addition, they have demonstrated different solubility parameters computationally. The preliminary hypothetical analysis can be a computational proof for further targeting statins (Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin) to VEGFR2 and VEGFR3 for promoting angiogenesis and lymphangiogenesis in diabetic wounds. Taking these evidence-based docking results could provide a future pathway to carry out further research characterization and evaluation of statins for treating and managing diabetic wounds.

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INTRODUCTION

The diabetic wound is a lifetime complication that increases the risk of peripheral neuropathy, osteoarthropathy, ischemic foot ulcers, and gangrene, leading to amputation. Till now, 30% of the world's diabetic population has experienced

lifetime complications, bearing high costs, due to diabetic wound (Aldana and Khachemoune, 2020). The influence of diabetes mellitus substantially reduces the healing process associated with wound repair. Numerous mechanisms involving the epidermis, connective tissue, and vasculature have been proposed. Among them, impaired diabetic wound healing is still one of the most incapacitating diabetes consequences, resulting in amputations, morbidity, and mortality because no single molecular target has been translated into a successful treatment. Diabetes-related defects include angiogenesis and lymphangiogenesis in the granulation tissue, which is crucial for regenerating tissue integrity. One classic scenario where the damaging outcomes of the microvasculature of diabetic wound are evident in the marked endothelial dysfunction in the wound region (Piaggesi and Apelqvist, 2017). Thus, remodeling the expanding microvasculature is a crucial criterion for angiogenesis and lymphangiogenic processes in diabetic wounds, which includes endothelial cell proliferation, vascularization, and stable pericyte signaling (Jussila and Alitalo, 2022). Angiogenesis is the development of the pre-existing damaged blood vessels in the wound region, and lymphangiogenesis is the formation of lymphatic vessels from the pre-existing lymph vessels located near the capillary beds of the damaged tissue (Benest *et al*., 2008).

Where angiogenesis and lymphangiogenesis frequently coexist in the healing process of tissue development in the wound region, the interactions between many cell types, including monocytes and macrophages, become crucial in such a complex and dynamic wound environment. Impaired angiogenesis has been linked to the onset by its impact on the endocrine system and adipose tissue functionalization (Hadrian *et al*., 2021). The effects of high glucose exposure on angiogenic processes include poor responses to hypoxia and proangiogenic stimuli, reduced availability of nitric oxide (NO), lack of proangiogenic activators, and pericyte loss (Benest *et al*., 2008). Thus, selecting a potent vascular endothelial growth factors receptor (VEGFRs) for initiating angiogenesis and lymphogenic signaling is the initial target for the diabetic wound. Vascular endothelial growth factors (VEGFs) are cell-specific mitogens and potent inducers of vascular permeability, among several distinct variables implicated in regulating the angiogenic and lymphogenic response in diabetic wounds. They are the family of tyrosine kinases consisting of VEGFR1, VEGFR2, and VEGFR3 (Melincovici *et al*., 2018). These receptor tyrosine kinases have been distinguished into seven extracellular immunoglobulin homology domains and a split tyrosine kinase intracellular domain that activates angiogenesis in diabetic wounds (Nosrati *et al*., 2021a,b). The VEGFR 3 indirectly helps VEGFR 2 capillary sprouting, migration proliferation, and lymphomagenesis by signaling the VEGFA, C, and D growth factor and other growth factors responsible for the regeneration of the damaged tissue (Leppänen *et al*., 2013). Therefore, to upregulate the tyrosine kinases statin molecules are repurposed for targeting VEGFR2 and VEGFR3 for promoting angiogenesis and lymphangiogenesis in diabetic wounds. Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors used for treating cardiovascular atherosclerosis) are known as potent ligands for developing new blood vessels in ischemic heart tissues (Cui *et al*., 2020). They have shown significant outgrowth of endothelial progenitor cells and capillary density in the ischemic heart (Ko *et al*., 2018). Thus, repurposing the statin molecules can be a novel strategic target for VEGFRs that initiate wound healing and tissue regeneration in diabetic wounds. Therefore, targeting these tyrosine kinase receptors, VEGFR 2 and VEGFR 3, by statins activate the major pathway for the transduction of angiogenesis and lymphangiogenesis in diabetic wounds.

The VEGFR2 and VEGFR3 are linked with the PI3K/AMPK/AKT that induces the Nitric oxide synthetase (eNos)/NO pathway which stimulates the VEGF cell signaling in the wound area. The VEGF is one of the main initiators for vascularization of the blood vessels and conversion of M2 macrophages that inhibits the inflammation and boosts the lymphangiogenesis in diabetic wounds. By repurposing and targeting the statin molecules to VEGFR2 and VEGFR3 initiates the PI3K phosphorylation and nitric oxide production in the diabetic wounds that the stabilizes hypoxic condition and activates the ERK signalling for VEGF that stimulates the endothelial cell migration and proliferation. The endothelial cells further improvises angiogenesis, lymphangiogenesis and tissue remodelling in diabetic wounds (Zhang *et al*., 2017).

In the present study, the statin molecules were analyzed for their binding affinity with VEGFR 2 and VEGFR 3 using computational molecular docking studies. The statins molecules are ranked based on the higher LibDock score and hydrogen and hydrophobic interactions with the active amino acid residues of the VEGFR2 and VEGFR3 protein complex. Selected molecules are further subjected to the computational pharmacophore feature set to VEGFR 2 and VEGFR 3 complexes and toxicity studies for carcinogenicity, skin sensitization, and irritancy on the rat skin. In the computational analyses, Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin showed significant binding potential to VEGFR2 and VEGFR3 protein complex and more promising LibDock score with less variation when compared with the remaining statin molecules. Thus, considering this preliminary computational analysis of the study, we have hypothesized that repurposing the statins enhances the VEGFR2 and VEGFR3 activity that activates angiogenesis and lymphangiogenesis in diabetic wounds.

Hypothesis

In the current hypothesis, we have selected statins molecules and VEGFR 2/VEGFR 3 protein complexes for the computational-based molecular docking studies, where the VEGFs are the key drivers for the angiogenesis and lymphangiogenesis in diabetic wounds. However, the critical drawback is incomplete wound recovery due to excess inflammation and improper blood supply leading to the degeneration of blood vessels in the wound bed region. The increase in the inflammatory mediators in diabetic wounds is due to hyperglycemic conditions that release advanced glycation end-products that inhibit the conversion of M1 to M2 macrophages, finally leading to hypoxia and a decrease in the signaling of angiogenesis and lymphangiogenesis in the wound region. Hence, in the present hypothesis, we have repurposed the statin molecules that are HMG-CoA reductase inhibitors used for treating cardiovascular atherosclerosis. These statins are targeted to the VEGFR 2 and VEGFR 3 protein complex and analyzed for their LibDock score and binding affinity to the amino acid residues in the protein complex. Based on the LibDock score and hydrogen and hydrophobic bond interactions, the selected statins are subjected to computational pharmacophore modeling

and toxicity and solubility studies to identify the feature and check the sensitivity and carcinogenicity on the skin. In this study, the selected statins (Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin) binding to VEGFR2 and VEGFR3 protein complex can be estimated to promoting angiogenesis and lymphangiogenesis in diabetic wounds. Considering this preliminary work of evidence, we hypothesize that statin molecules have the binding ability to the VEGFR 2 and VEGFR 3 protein complex that can influence angiogenesis and lymphangiogenesis in diabetic wounds. Hence, Statins could be a choice of drug delivery that can improve angiogenesis, anti-inflammatory, lymphangiogenesis, and tissue remodeling during early diabetic wounds (Ko *et al*., 2018), and topical delivery of statins may be beneficial. Furthermore, it can overcome adverse effects associated with oral administration. Thus, considering and targeting statin molecules topically to VEGFR2 and VEGFR3 using a well-designed wound dressing biomaterial could be a new strategy to deliver the drug in a controlled manner for the treatment and management of diabetic wounds. The diagrammatic illustration of the hypothesis is represented in Figure 1.

MATERIAL AND METHOD

The computational tools for molecular docking were performed in Discovery Studio 4.1 Chemistry at Harvard macromolecular mechanics (CHARMM) Software. The lib docking was used to analyze the data dock score. The Pub Chem ID for the ligands Atorvastatin (60823), Fluvastatin (446155), Lovastatin (53232), Pitavastatin (5282452), Pravastatin (81093), Rosuvastatin (446157), and Simvastatin (54454), and all 2D and 3D structures were downloaded from Pub Chem. The polar hydrogen charges were added before docking. In addition, the VEGFR 2 (PDBI: 2X1W) and VEGFR3 (PDBI: 4BSJ) were downloaded from the protein database.

Protein preparation

Computational studies were performed using the Discovery Studio 4.1 client. First, the three-dimensional structures were chosen based on different parameters like resolution, completeness, expression system, source organism, co-crystal ligands, etc. Next, the structures of VEGFR2 (Resolution: 2.7Å and PDBI: 2X1W) and VEGFR3 (Resolution: 2.5Å and PDBI: 4BSJ) were downloaded from the protein data bank (Gulcan *et al*., 2007). Then, the VEGFR2 and VEGFR3 structures were prepared using CHARMM minimization and used for further studies (Vanommeslaeghe and MacKerell, 2015).

Ligand preparation

The statins, Simvastatin, Atorvastatin, Pitavastatin, Pravastatin, Fluvastatin, Lovastatin, and Rosuvastatin, were selected for the current hypothesis study. The 2D and 3D structures were downloaded from Pub Chem and Hydrogen was added and optimized using the CHARMM force field (Pal *et al*., 2019).

Binding site prediction

The binding site identification of VEGFR2 and VEGFR3 was performed using the discovery studio 4.1 version (Yele *et al*., 2019).

Molecular docking

The LibDock module of DS 4.1 performed molecular docking. The LibDock docking program uses a set of pre-generated ligand conformations and a receptor with a specified binding site. LibDock is a high-throughput algorithm for docking ligands into an active receptor site. Ligand conformations are pre-calculated and generated by DS 4.1. The minimization was done by smart minimization. The LibDock score is the docking score that confirms the binding affinity. The highest LibDock score indicates favorable binding. The docking score, relative energy, and absolute energy were calculated for the seven statin drug molecules (Mollahosseini *et al*., 2022; Yele *et al*., 2019).

Toxicity prediction

Toxicity prediction by computer-assisted technology (TOPKAT) assessment is used to confirm the probable value of the toxicity of statins in Discovery Studio absorption, distribution, metabolism, excretion, and toxicity calculations. The prediction was determined using the quantitative structure toxicity relationship (QSTR). Six mathematical models, such as plasma protein binding, human intestinal absorption, blood-brain barrier penetration, Cytochrome P450 2D6 inhibition, aqueous solubility, and hepatotoxicity, were used to screen the compounds. The toxicity model consists of solubility, carcinogenicity, skin sensitization, and irritancy (Pal *et al*., 2019; Yele *et al*., 2019).

Generation of pharmacophore models

The receptor–ligand pharmacophore generation is performed for statin-VEGFR2 and VEGFR3 interactions. The pharmacophore features like hydrogen bond donor/acceptor, hydrophobic, positive/negative ionization, and aromatic ring are obtained. The pharmacophore ligand–protein mapping method was used to determine the pharmacophoric features in statin-VEGFR2 and VEGFR3 complexes. The top, binding features, and selectivity score are calculated (Sangande *et al*., 2020; Yang *et al*., 2010).

RESULTS

Protein preparation and stereological value of the receptor

The receptors' stereological aspects were generated using the Ramachandran plots. VEGFR2 exhibited 90.7% residues in the allowed regions, 7.1% in the marginal region, and 2.2% in the disallowed region. VEGFR3 showed 97.3% of residues in the allowed region and 0.00% in the disallowed region, remaining in marginal region. The water molecules were removed, and structures were minimized using CHARMM minimization.

Binding site identification

The binding site identification of VEGFR2 and VEGFR3 was performed using the discovery studio 4.1 version. The protein complex consists of D2 and D3 domains and stimulates angiogenesis (Yang *et al*., 2010). So, the binding sites are explicitly selected from this site of the protein complex. Dimerization of the extracellular domains D2 and D3 is followed by tyrosine autophosphorylation of intracellular kinase domains to generate downstream regulation (Kisko *et al*., 2011). In the binding site prediction, the VEGFR2 grid contains the following amino

acid residues SER 252, ASN 253, VAL 218, GLY 220, SER 193, VAL 219, ILE 138, TYR 190, THR 131, GLU 140, SER 189, SER 193, LYS 144, TYR 142, ARG 222, THR 221, ASN 141, LEU 252, GLU 284, VAL 218, ILE 138, GLU 251, THR 139, TYR 22, GLU 251, GLU 284, ASN 141, LYS 142, GLY 220, THR 139, ILE 138, LYS 142.

The residues present in the VEGFR3 grid box are ILE 424, ASP 543, HIS 425, GLU 428, GLN 423, ARG 476, GLN 477, ARG 427, GLU 544, TYR 548, ARG 545, ILE 547, SER 430, SER 431, ALA 429, GLU 426, GLN 478, ILE 424, ARG 477, ARG 545, TYR 548, LEU 546, TYR 435, ALA 429, ILE 547, SER 433, ILE 547, TYR 435.

The binding sites of VEGFR2 and VEGFR3 protein complexes are shown in Figure 2.

Molecular docking

The molecular docking was performed using the LibDock of DS 4.1. The statin molecules were selected for further study based on the LibDock score ranking and the hydrogen bond and hydrophobic bond interactions with the active amino acid residues. The VEGFR2 and VEGFR 3-complexes with a statin, showing high LibDock scores, are considered significant molecules with an excellent affinity towards active amino acid residues on the receptors. Among the seven statin molecules-Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin and Simvastatin, Four statin molecules Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin have shown excellent LibDock score with the VEGFR 2 and VEGFR 3 with potential for less variation. Comparing the docking scores with both the receptors, Pravastatin (129.90 and 128.6) and Rosuvastatin (130.01 and 130.62) demonstrated a greater binding affinity and LibDock score to the VEGFR 2 and VEGFR 3 complex. The results are shown in Tables 1 and 2, and the structural interactions between the four statins and the VEGFR2 and VEGFR3 protein complex are shown in Figures 3 and 4.

Interaction of ligands with receptor

Statins are selected based on hydrogen bonding and hydrophobic bond interactions with the active sites of the amino acid residues of VEGFR2 and VEGFR3. THR139, GLU140, ASN141, ARG 222, GLU 284, SER 189, TYR 190, ASN 253, and LYS142 of VEGFR 2. The VEGFR 2 binding site residues are shown in Figure 2. Pravastatin, Fluvastatin, Atorvastatin, and Rosuvastatin exhibit hydrogen bonding mainly in the C-terminal region and involve active amino acid residues. The hydrogen and hydrophobic bond interactions between the VEGFR 2 and the statin molecules binding to the active amino acid residues and their distance (Å) are mentioned in Table 1 and represented in Figure 3 in (i) 3D (ii) 2D structure format in the image (a–d).

The statins interacting with VEGFR 3 amino acid residues are SER 431, ARG 477, GLN 478, GLU 428, ILE 424, ILE 547, LYS 427, SER 430, SER 431, TYR 435, TYR 548, and LEU 546. The hydrogen and hydrophobic bond interactions between the VEGFR 3 and the statin molecules binding to the active amino acid residues and their distance (Å) are mentioned in Table 2 and represented in Figure 4 in (i) 3D (ii) 2D structure format in the image (a–d).

Pharmacophore modeling

The essential features between statin-VEGFR2 and VEGFR3 complexes are analyzed using receptor-ligand pharmacophore generation. These features of VEFGR2 and

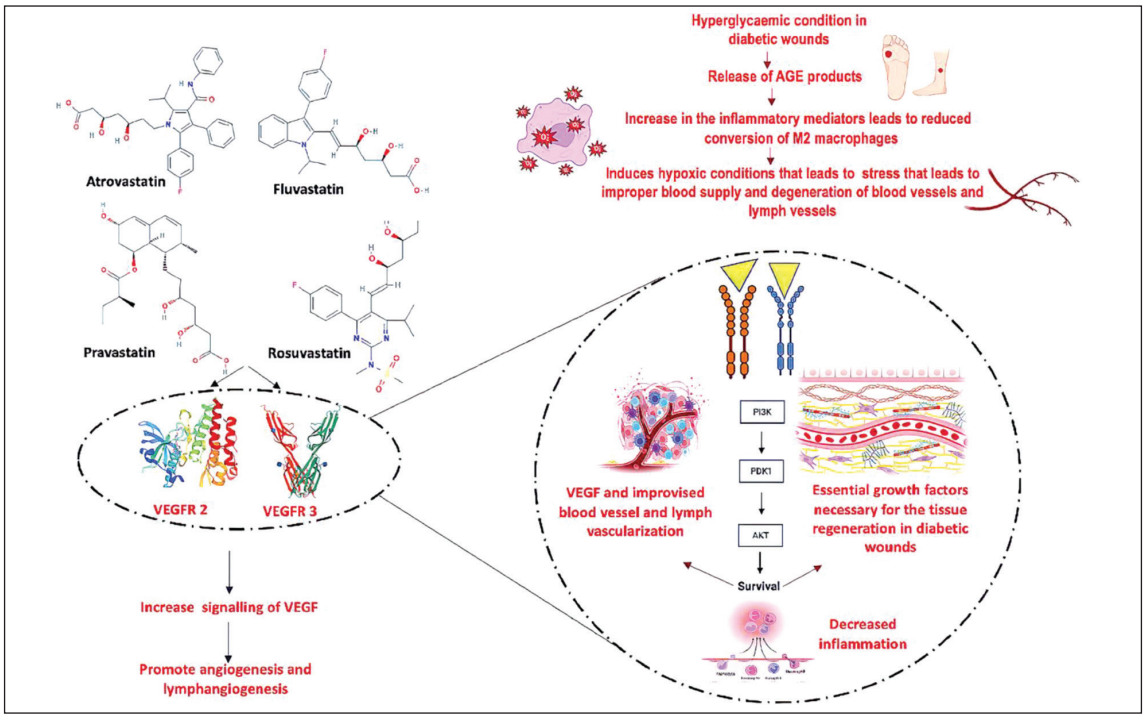

Figure 1. Hypothetical diagrammatic representation of targeting statin molecules to VEGFR2 and VEGFR3 to initiate angiogenesis and lymphangiogenesis in diabetic wound.

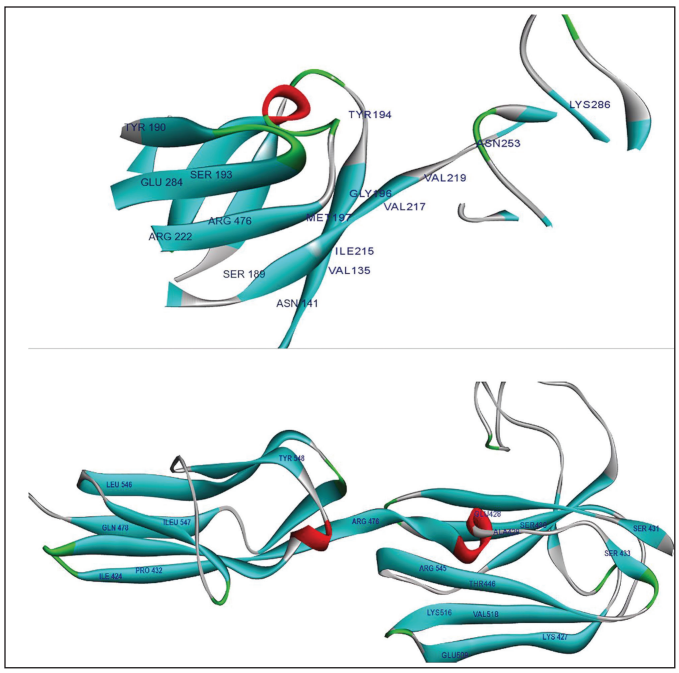

Figure 2. Active amino acid key residues of VEGFR2 and VEGFR3. (a) structure of VEGFR2 binding pocket residues are TYR 194 GLY 196 MET 197 ILE 215 VAL 135 ASN 141 ASN 253 LYS 286 SER 189 ARG 222 ARG 476 GLU 284 SER 193 TYR 190 and (b) structure of VEGFR3 binding pocket residues in receptors are ARG 476 ARG 545 THR 446 LYS 516 VAL 518 GLU 509 LYS 427 SER 433 SER 431 TYR 548 LEU 546 GLN 478 ILE 547 PRO 432 ILE 424.

VEGFR3 protein active binding sites represent the pharmacophoric structural model for the active protein sites. The features are chosen based on their hydrogen bond donors and acceptors observed between the ligands and the protein complex of VEGFR2 and VEGFR3. The pharmacophore features and the selective score of other statins are reported in Tables 3 and 4. In the VEGFR2 complex, the feature set for Atorvastatin ILE 138 GLU 140 SER 189 GLU 284 GLU 251 VAL 210 TYR 221, Fluvastatin GLU 140 ASN 141 LYS 142 SER 189 TYR 190 LEU 252, Pravastatin GLU 140 SER 189 TYR 190 GLU 251 GLU 284 VAL 219, and Rosuvastatin GLU 251 VAL 218 ILE 138 GLU 140 ASN 141 are structurally represented in Figure 5. The feature set for VEGFR3 complex to Atorvastatin ASP 543 GLU 545 ARG 477 ARG 545 GLN 479 GLU 428 SER 430 SER 431 LEU 546 LYS 427, Fluvastatin ARG 477 GLN 478 ILE 424 LYS 427, Pravastatin GLN 478 LYS 427 SER 430 SER 431 PRO 432 LEU 546, and Rosuvastatin ARG 545 ILE 547 TYR 548 TYR 435 SER 431 LYS 427 is structurally represented in Figure 6.

Toxicity

The toxicity studies for the drug molecules were performed using the TOPKAT module. Solubility, irritancy, carcinogenicity, and skin sensitization have been calculated using the QSTR model. The drug molecules showed mild-to-moderate irritancy and non-carcinogenicity. Pravastatin and Rosuvastatin have shown good solubility when compared with other molecules. Considering the above factors, statins can be made into better

Table 1. Computational molecular docking (Lib docking) and results of the selected four statin molecules based on LibDock score and hydrogen and hydrophobic bond interactions between the VEGFR 2 and the statin molecules binding to the active amino acid residues and their distance in (Å).

Ligands	Amino acid residues and distance (Å)	Absolute energy	Relative energy	LibDock score
Atorvastatin	SER 189 (2.93) TYR 190 (2.96) SER 193 (1.96) ASN 141 (1.22) ARG 222 (2.44)	67.111	7.684	125.908
Fluvastatin	GLU 289 (1.89) GLU 251 (2.89) VAL 219 (2.72) ASN 141 (1.71) SER 189 (1.78)	64.118	7.892	126.379
Pravastatin	SER 189 (1.18) ASN 253 (2.20) TYR 190 (2.22) SER 193 (2.71) ASN 141 (1.94) LYS 142 (2.20)	50.578	9.809	129.960
Rosuvastatin	GLU 284 (2.74) LEU 252 (2.85) GLU 251 (2.42) ASN 253 (2.72) VAL 219 (1.81) GLU 140 (2.33) ASN 141 (1.23) SER 189 (1.81)	64.574	9.357	130.970

Table 2. Computational molecular docking (Lib docking) and results of the selected four statin molecules based on LibDock score and hydrogen and hydrophobic bond interactions between the VEGFR 3 and the statin molecules binding to the active amino acid residues their distance (Å).

Ligands	Amino acid residues and distance (Å)	Absolute energy	Relative energy	LibDock score
Atorvastatin	SER 431 (2.91) SER 430 (2.92) GLU 428 (1.71) LYS 427 (1.98) ARG 545 (2.43) ILE 477 (2.48)	65.8	6.5	126.0
Fluvastatins	SER 430 (2.80) SER 431 (2.81) LYS 427 (1.23) ARG 545 (2.78) ILE 547 (2.94) TYR 435 (2.94)	66.3	2.9	120.0
Pravastatin	GLU 426 (2.99) ARG 545 (1.18) LEU 546 (1.19) TRY 548 (13.65) SER 430 (2.98)	60.3	2.9	128.68
Rosuvastatin	ILE 547 (2.45) ARG 545 (2.78) GLN 478 (2.99) LYS 427 (1.89) ALA 429 (2.66) SER 430 (2.76) SER 431 (1.371) PRO 431 (2.56)	64.3	5.4	130.62

topical biomaterial for treating diabetic wounds. The toxicity report of the four statin molecules is shown in Table 5.

DISCUSSION

In the *in silico* molecular docking studies performed, the statins (Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin, and Simvastatin) were subjected to computational molecular docking (Lib docking), and the best statins are selected based on the LibDock score and hydrogen and hydrophobic bond interactions. Further, the chosen statin molecules (Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin) are estimated for pharmacophore modeling and toxicity prediction for skin sensitivity, irritancy, and carcinogenicity. The results indicate that the statins have demonstrated significant feature set identification with the protein complex and were found to be nontoxic to the skin tissue computationally; that could be an excellent value because no study has ever demonstrated the molecular interaction of the statins to the tyrosine kinase protein complex (VEGFR2 and VEGFR3) and identified the active amino acid residues that could be effective target approach for angiogenesis and lymphangiogenesis diabetic wounds. Although statins are hyperlipidemic drugs used in treating cardiovascular blood flow in atherosclerosis, they could be a novel repurposing approach for targeting angiogenesis and lymphangiogenesis in diabetic wounds (Fong, 2014; Tessier *et al*., 2021). Structurally, the statins consist of active groups that are Phenyl (Atorvastatin), Isopropyl (Fluvastatin), Naphthalene ring (Pravastatin), and Fluorophenyl (Rosuvastatin) are bound to the potential binding sites available in the VEGFR2, and VEGFR3 protein complex that enhances the wound healing process by influencing active amino acid residues SER 193, SER 189, TYR190, ASN 141, VAL 219, GLU 284, ARG 222 GLU 140 for VEGR2 complex, and SER 431, SER 430, GLU 438, LYS 427, ARG 545, LYS 427 for VEGFR 3 complex, which is most commonly found in all the four statins (Jere *et al*., 2019).Thus, the rationale behind this experiment was that VEGFR2 and VEGR3 are associated with angiogenesis and lymphangiogenesis that initiate the further mechanisms of essential cellular activities in diabetic wounds. Therefore, VEGFR2 and VEGR3 are the most promising targets for managing and treating diabetic wounds. In the current hypothesis, the VEGFR2 protein activates the angiogenic process, and VEGFR3 indirectly activates the signaling of angiogenesis and lymphangiogenesis in the affected wound region (Yasunami *et al*., 2015). In the findings, it was reported that the tyrosine kinase derivates are most likely to be in the activation of the phosphatidyl-inositol-3kinase (P13K)-Akt-dependent and activation of the P13K-Akt-eNOs signaling pathway involved in the transduction of the proatherosclerotic endogenous compound factors that influence the endothelial cell sprouting and their functional capacity to promote neovascularization and tissue regeneration (Toker *et al*., 2009). These are interlinked with the mechanistic influence of the angiogenesis and lymphangiogenesis in diabetic wounds. In many studies, statins were reported for their wound-healing efficacy for angiogenesis and lymphangiogenesis (Wang, 2013). Likewise, in one of the works, they have taken one of the phenyl group-bound statins (Atorvastatin) helpful in treating diabetic foot ulcers. Upon topical application, the statin has shown improved endothelial progenitor cell proliferation in the Streptozotocin-induced diabetic rat histopathology and reduced oxidative stress, which can promote further microvascular function in diabetic wounds (Wang *et al*., 2020). In one of the findings, it was reported that Atorvastatin accelerates the healing

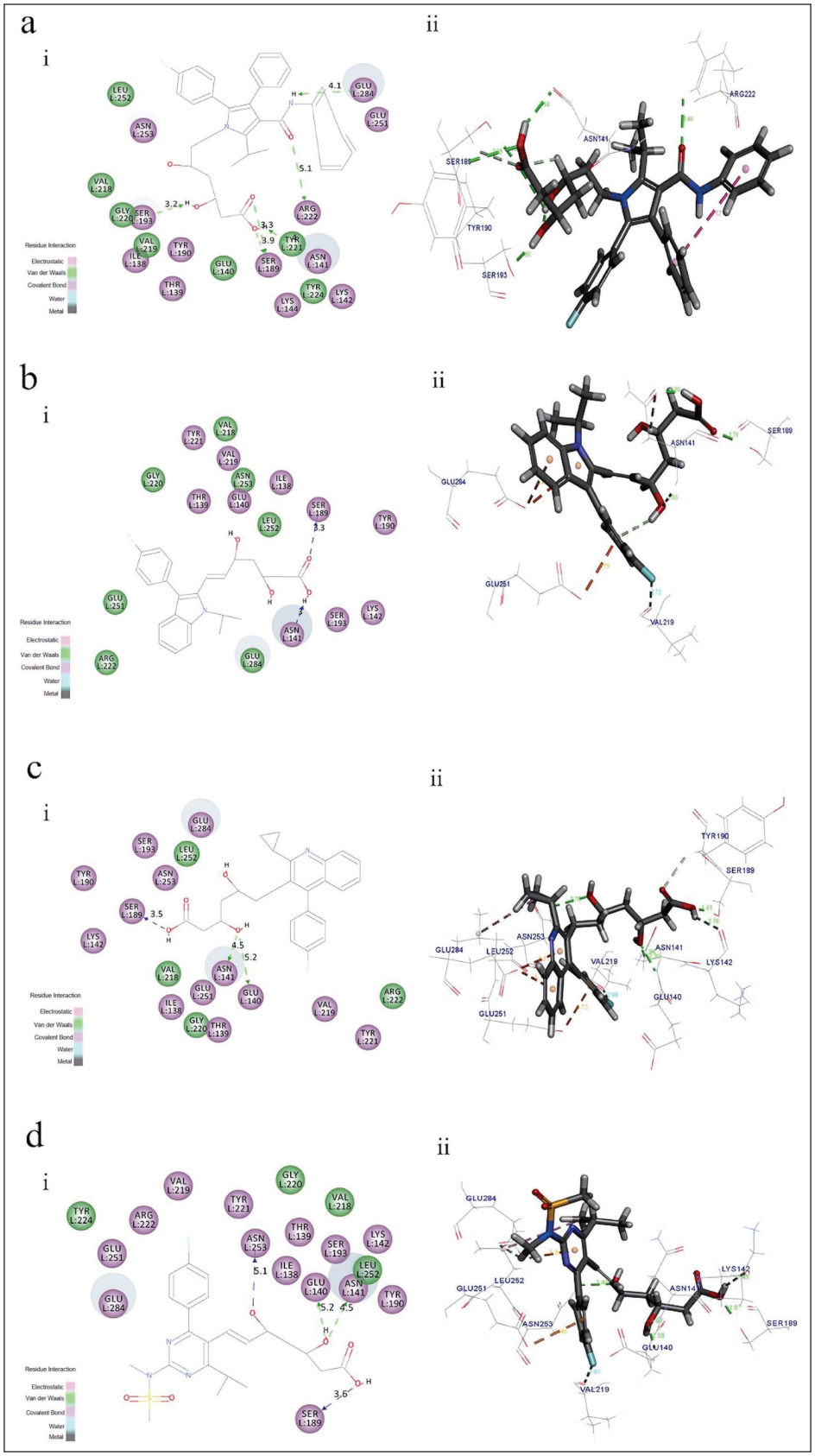

Figure 3. Structural (i) 2D and (ii) 3D representation of docked confirmation statin molecules (a). Atrovastatin, (b). Fluvastatin, (c). Pravastatin and (d). Rosuavastatin active amino active residues of VEGFR 2 protein complex. Purple lines represent ligand bonds. Green dotted lines represents hydrogen bond interactions. Residues involved in hydrophobic interactions are indicated by red lines surrounded with corresponding atoms by grey lines.

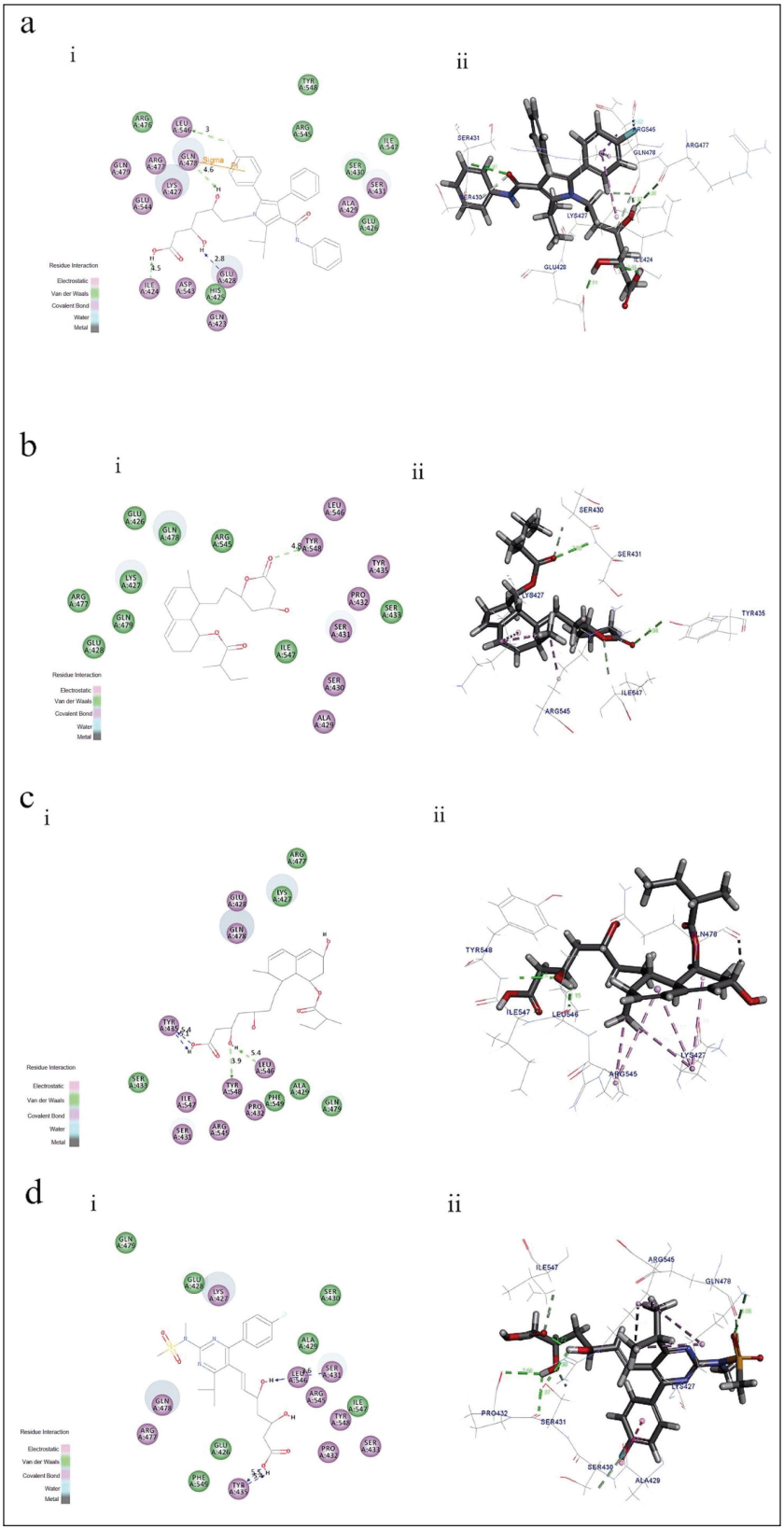

Figure 4. Structural (i) 2D and (ii) 3D representation of docked confirmation statin molecules (a). Atrovastatin, (b). Fluvastatin, (c). Pravastatin and (d). Rosuavastatin active amino active residues of VEGFR 3 protein complex. Purple lines represent ligand bonds. Green dotted lines represents hydrogen bond interactions. Residues involved in hydrophobic interactions are indicated by red lines surrounded with corresponding atoms by grey lines.

Table 3. Identification of the feature set and selective score by computational pharmacophore modeling for the selected four statin molecules to VEGFR 2 complex.

Drugs	Numbers of features	Features set	Selective score
Pravastatin	6	AADDHH AAADHH ADHH	11.2
Atorvastatin	4	ADDH AADH AAAH	8.1
Rosuvastatin	3	AADH AAAD	5.4
Fluvastatin	3	AAD AAA	5.4

A- Hydrogen bond acceptor; D- Hydrogen bond donor; H- Hydrophobic.

Table 4. Identification of the feature set and selective score by computational pharmacophore modeling for the selected four statin molecules to VEGFR 3 complex.

Drugs	Numbers of features	Features set	Selective score
Rosuvastatin	4	DHHN ADHH	9.3
Pravastatin	3	DDN ADN ADD AAD	8.8
Atorvastatin	4	AAAH	6.0
Fluvastatin	3	AAD	5.2

A- Hydrogen bond acceptor; D- Hydrogen bond donor; H- Hydrophobic.

in full thickness wounds. Faster resolution of the myofibroblast enhanced the angiogenesis and vascular maturation. It also showed a greater effect in diminishing scar formation and dampening the inflammatory response in the full thickness wounds (Akershoek *et al*., 2017). Similarly, Atorvastatin has been suggested an alternative drug therapy for treating cutaneous wounds. Atorvastatin treated group has demonstrated tissue restoration of fibroblast cells in the early proliferation phase in the excised wounds of the albino rats (Tejaswini *et al*., 2020). In recent findings, rosuvastatin was found to be promising carrier for deep chronic wounds. At the end of second week the connective tissue underneath continued to heal and almost found to be completely normal by the end of the third week (Zaki *et al*., 2022). Similarly, Rosuvastatin has demonstrated anti-inflammatory effects and cell viability of the endothelial cells and blood platelets in the injured myocardial tissue (Potenza *et al*., 2009). Likewise, Pravastatin has stimulated the activity of collagen synthesis and fibroblast cell proliferation, where they are the secondary mediators for the NOs protein expression in wounds that are indirectly linked to the improvement of endothelial function in diabetic wounds that have shown improved healing (Asai *et al*., 2012). Another finding has shown that statins have the potential toward macrophage infiltration of macrophages and exerted antiapoptotic effect which directly affects the lymphatic, angiogenic, and granulation tissue in diabetic wounds (Tessier *et al*., 2021). Likewise, pravastatin has accumulation of hydro proline that expressed upregulation of eNos and NO expression in partially healed wounds (Laing *et al*., 2010). Subsequently, there are less finding reported for fluvastatin in wound healing studies still research has to be performed in the field of tissue regeneration (Furuhashi *et al*., 2022). In one recent findings it was suggested that fluvastatin has potential towards bone tissue regeneration. It has shown augmentation of new bone formation, infection control and soft tissue wound closure in rat calvaria. Similarly, in one of the findings, statins could decrease the farnesyl pyrophosphate facilitating vascular relaxation, promoting neovascularization, and postnatal neovascularization of ischemic tissue after myocardial infarction in animal models, and revealing an increase in endothelial cell proliferation in the injured region (Wang *et al*., 2020).

CONCLUSION

In diabetic wound conditions the oxidative stress and downregulation of essential growth is created in wound area that leads to the dysfunctionality in production of essential and non-essential amino acids that are necessary for wound recovery in diabetic wounds. Where, Atorvastatin, Fluvastatin, Pravastatin and Rosuvastatin have demonstrated an excellent binding affinity towards the essential amino acids like ILE, LEU, LYS, VAL, and non-essential amino acids such as ASP, GLU, GLY, SER, and TRY. The non-essential amino acids are synthesized within the body. In diabetic wound area deficiency of amino acids occurs which leads to delayed tissue regeneration with impaired angiogenesis and lymphangiogenesis in diabetic wounds. Hence, in the present work, we have hypothesized that repurposing the statins enhance the VEGFR2 and VEGFR3 activity that activates angiogenesis in diabetic wounds. Based on the LibDock score, hydrogen and hydrophobic bond interactions with the active amino acid residues, four statins, Pravastatin, Fluvastatin, Atorvastatin, and Rosuvastatin, are selected for further studies. Additionally, the pharmacophore modeling between statins and the protein complex was computationally determined to identify the features set necessary for statins-VEGFR2 and VEGFR3 protein complex. Finally, the toxicity studies for the drug molecules are performed using TOPKAT. Solubility, irritancy, carcinogenicity, and skin sensitization have been calculated using the QSTR model, and the statin molecules showed mild to moderate irritancy hypothetically. The hypothetical estimation could be proof of evidence that the four statins can address (Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin) the complete wound healing in diabetic conditions by activating the VEGFR2 and VEGFR3 initiates the PI3K phosphorylation and NO production in the diabetic wounds that stabilizes hypoxic condition and initiates the ERK signalling for VEGF that stimulates the endothelial cell migration and proliferation. The endothelial cells further activates angiogenesis, lymphangiogenesis and tissue remodelling in diabetic wounds. Hence, we conclude by taking the support of the literature prediction, statins can be a repurposing tool for targeting VEGFR2 and VEGFR3 in diabetic wounds topically and considering this hypothetical data, the selected four statins can be subjected to further studies to transform the four (Atorvastatin, Fluvastatin, Pravastatin, and Rosuvastatin) statins into better biomaterials for treating diabetic wounds.

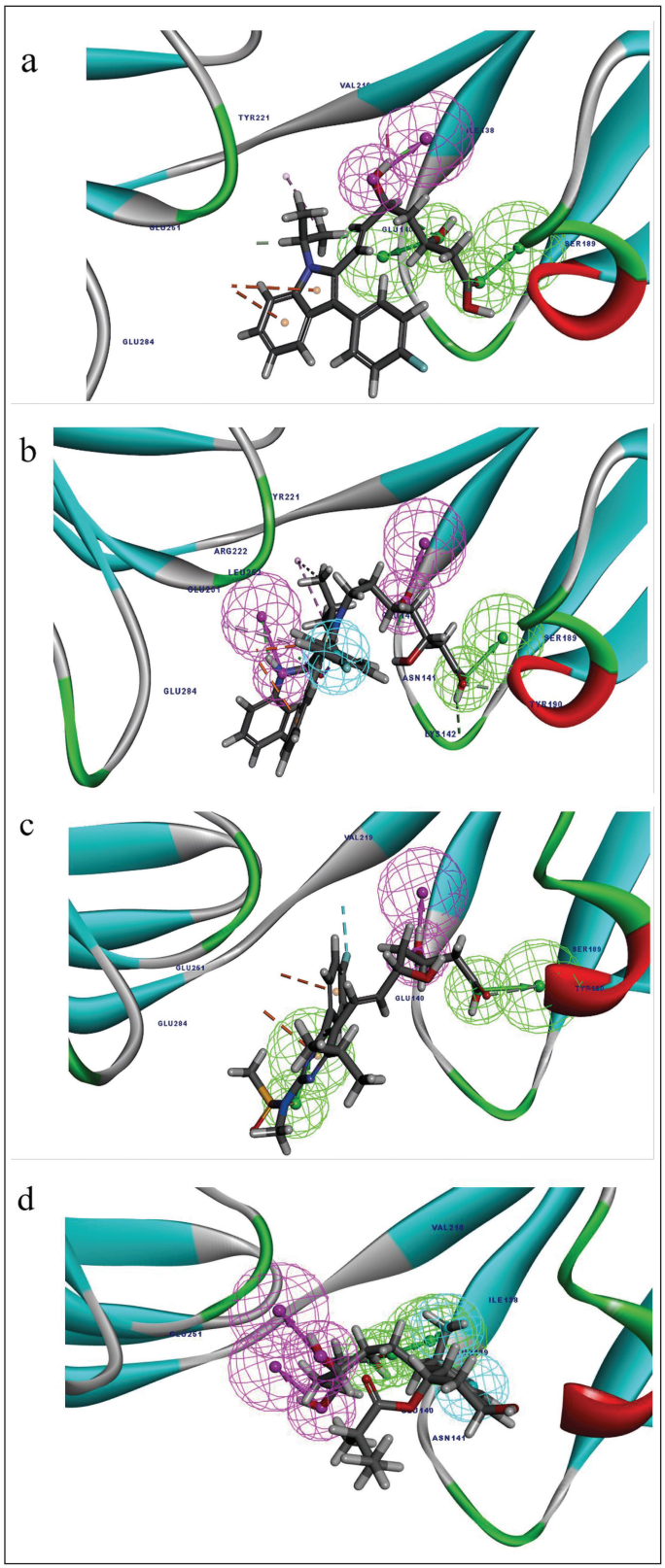

Figure 5. Phamacophore model of the statin molecules (a). Atrovastatin, (b). Fluvastatin, (c). Pravastatin and (d). Rosuavastatin with VEGFR2 protein complex feature set amino acid residues. Green circle indicates hydrogen bond donor from atorvastatin, pink circled feature indicates the hydrogen bond acceptor feature and blue indicates hydrophobic feature.

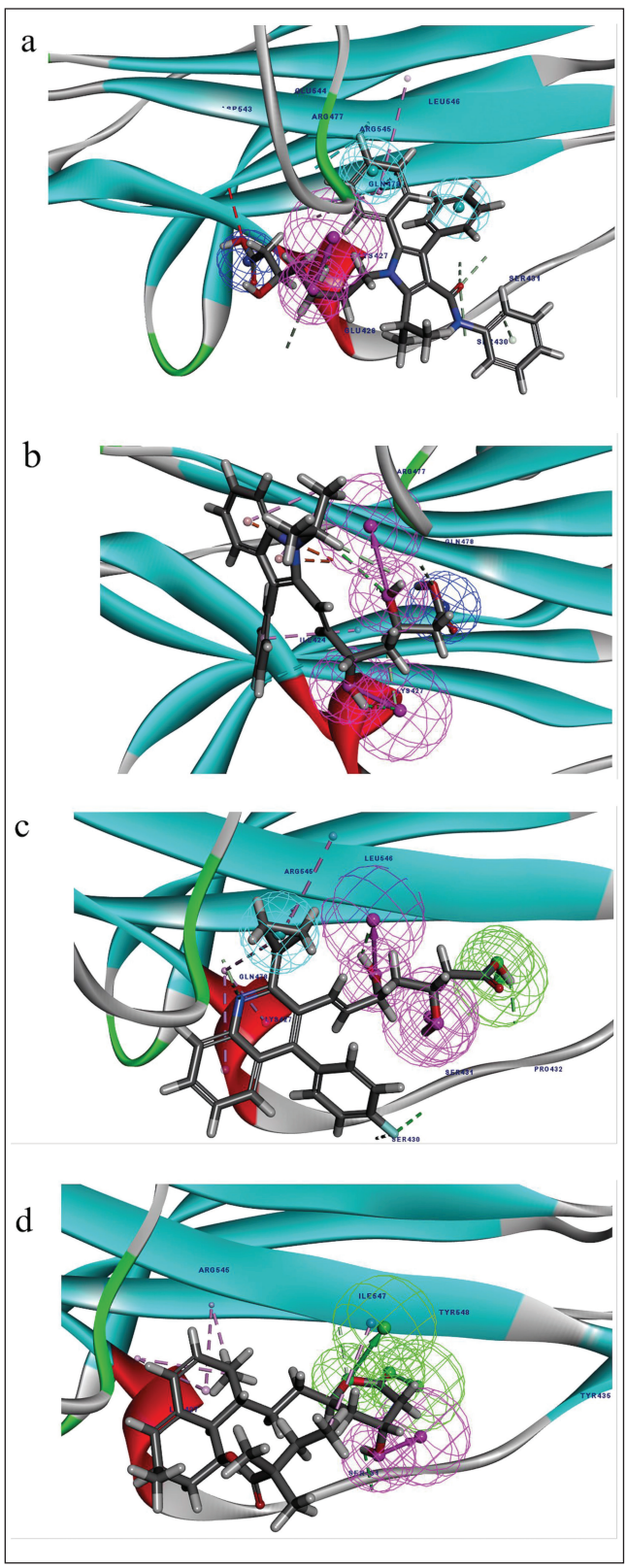

Figure 6. Phamacophore model of the statin molecules (a). Atrovastatin, (b). Fluvastatin, (c). Pravastatin and (d). Rosuavastatin with VEGFR3 protein complex feature set amino acid residues. Green circle indicates hydrogen bond donor from atorvastatin, pink circled feature indicates the hydrogen bond acceptor feature and blue indicates hydrophobic feature.

Table 5. Computerized TOPKAT report of the drug molecules for skin sensitization and irritancy for the selected four statin molecules to the rat skin.

Drugs	Skin irritancy	Carcinogenicity	Skin sensitization	Solubility	Absorption
Pravastatin	None	Noncarcinogenic	Weak	Low	Very good
Rosuvastatin	Mild	Noncarcinogenic	Weak	Good	Very good
Atorvastatin	None	Noncarcinogenic	Strong	Good	Moderate
Fluvastatin	None	Noncarcinogenic	Weak	Low	Good

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LIST OF ABBREVIATIONS

CHARMM, Chemistry at harvard macromolecular mechanics; eNos, Nitric oxide synthetase; ERK s, Extra cellular signal regulated kinase; NO, Nitric oxide; QSTR, Quantitative structure toxicity relationship; TOPKAT, Toxicity prediction by computer-assisted technology; VEGF, Vascular endothelial growth factor; VEGFR, Vascular endothelial growth factor receptor.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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