Indian Journal of Biochemistry & Biophysics
Vol. 63, March 2026, pp. 311-320
DOI: 10.56042/ijbb.v63i3.19692

Repurposing of statins: An *in silico* approach aimed at inflammation resolution pathways

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Received 14 May 2025; *revised 12 January 2026*

The study was performed to evaluate the *in silico* binding ability of different statins against the enzymes involved in inflammation resolution pathways to enlighten the role of statins in resolution of inflammation and as a goal to repurpose statins as inflammation resolution drugs. The protein structures of four enzymes involved in the synthesis of Specialized Pro Resolving Mediators (SPMs) viz., 12-lipoxygenase (12-LoX), 15-lipoxygenase (15-LoX), 5-lipoxygenase (5-LoX) and Aspirin acetylated cycloxygenase-2 (CoX-2) were retrieved from PDB and were used as receptors. Statins such as Atorvastatin, Simvastatin, Lovastatin, Rosuvastatin, Fluvastatin, Pravastatin and Pitavastatin were used as ligands and their 3D structures were obtained from PubChem database for computational molecular docking. The ligand interaction analysis was performed using AutoDock Vina and Biovia Discovery studio visualizer. The statins showed better binding affinities with 15-LoX and CoX-2 than the other two enzymes, which correlated with the *in vivo* efficacy of statins as reported earlier. Of all the statins, Pitavastatin and Atorvastatin exhibited better binding interactions with the docked enzymes. Statins are well-known for their cholesterol-lowering effects, but findings from this study suggest they may also be repurposed to promote the resolution of inflammation, which might open-up new possibilities for preventing serious chronic diseases.

Keywords: Atorvastatin, LoX and CoX enzymes, Specialized pro-resolving mediators (SPM)

Inflammation is vital for the body's defence and wound healing. The acute inflammatory response when uncontrolled can lead to further tissue damage and results in chronic inflammation which is observed in many commonly occurring diseases, such as cardiovascular disease, neurodegenerative diseases, metabolic syndromes and many other diseases[5,7,11]. Inflammatory responses are like biological cascades and are shaped by a delicate balance between positive and negative feedback loops. In addition to positive and negative checkpoints, the inflammatory cascade boasts an additional checkpoint, comprising of a family of chemicals termed as Specialized Pro-Resolving Mediators (SPMs) that limits body's natural response to inflammation in a self-limiting way and actively promote resolution and tissue repair without compromising host defense[14,20]. The major lipid derived SPMs are lipoxins, resolvins, protectins and maresins. Inflammation resolution is thought to be a passive process, but with the discovery of these resolution mediators it is sought to be an active process, which can be intervened for better tissue repair. SPMs are now identified as agonists with potential to stimulate key cellular resolution events such as limiting polymorphonuclear neutrophil infiltration and efferocytosis-enhancing macrophage clearance of apoptotic cells, which is the key for inflammation resolution[5,18].

SPMs such as lipoxins are biosynthesized from omega-6 poly unsaturated fatty acid (PUFA) derivative-arachidonic acid, whereas resolvins, protectins and maresins are synthesized from omega-3 PUFA derivatives *viz.*, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[10]. SPM biosynthesis typically requires coordinated interactions of infiltrating leukocytes and local cell populations at the site of tissue injury (transcellular biosynthesis). The synthesis of SPMs requires lipoxygenation of substrates which requires the participation of lipoxygenase (LoX) and cyclooxygenase (CoX) enzymes to catalyse the conversion of PUFA

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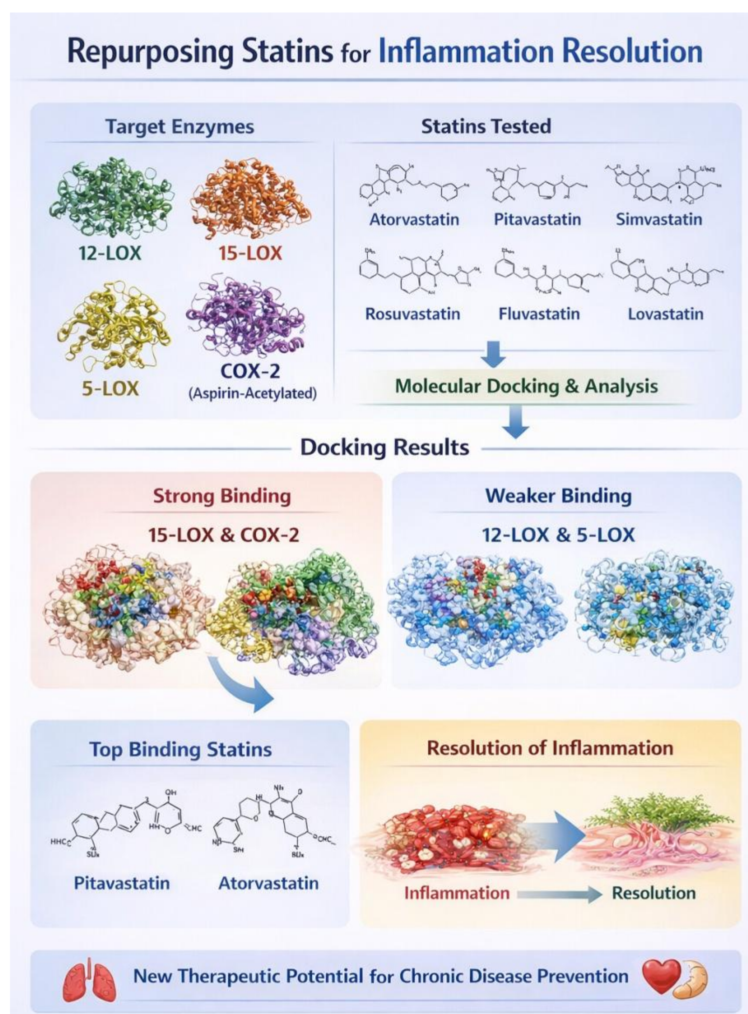

Graphical abstract

substrates to SPMs which further act on specific SPM receptors to produce the beneficial resolution of injury[16]. The major LoX enzymes involved in catalysis of SPM biosynthesis are 5-LoX, 12-LoX, 14-LoX, 15-LoX and 17-LoX[16]. Apart from this, CoX-2 and Cytochrome p-450 enzymes also catalyse the synthesis of SPMs from their PUFA precursors. SPMs work by binding and activating different receptors, including G protein-coupled receptors like FPR2/ALX, GPR32, GPR18, ChemR23, etc[8]. However, the knowledge on the pharmacological aspects of SPMs, including its tissue-specific production, specific receptors and signalling pathways, is currently limited. Further research is essential to explore this growing field of resolution pharmacology, which has significant potential for improving disease management.

Statins are well-established class of cholesterol-lowering drugs, function by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and have been widely prescribed for decades to mitigate cardiovascular (CV) risk and prevent atherosclerotic CV events.[(24)] Beyond its primary role in reducing plasma levels of pro-atherogenic apolipoprotein B-containing lipoproteins, statins also exhibit additional effects, including the modulation of cellular inflammatory mediators that influence both the progression and resolution of inflammation[19]. Moreover, statins contribute to inflammation resolution by downregulating the synthesis of C-reactive protein (CRP) and pro-inflammatory cytokines, complementing the cholesterol-lowering effects[4]. Recent studies suggest that statins may also promote the production of SPMs, potentially attributing to the pleiotropic anti-inflammatory actions[3,19]. However, research examining the direct effects of statins on SPM biosynthesis remains limited. To date, only a small

number of *in vitro* and preclinical studies have explored their role in inflammation resolution and the underlying molecular mechanisms.

In this context and with an objective to repurpose statins as inflammation resolution drugs, an *in silico* attempt was made in this study to understand the binding efficacies of multiple statins on chosen lipoxygenase (LoX-5, LoX-12, LoX-15) and cyclooxygenase (CoX-2) enzymes involved in the synthesis of SPMs.

Materials and Methods

The structures of atorvastatin, lovastatin, simvastatin, rosuvastatin, fluvastatin, pravastatin and pitavastatin were obtained in SDF format from PubChem database. The PubChem identifier of each statin is given in the (Table 1).

The SDF format of each statin was converted and saved in PDB format using Biovia Discovery studio visualizer for further docking. The inflammation resolution pathways centres around the LoX and CoX enzymes. The 3D structures of 5-LoX (3O8Y), 12-LoX (8GHC), 15-LoX (7LAF) and CoX-2 (5F19) were obtained from RCSB-PDB and used as protein targets. Using AutoDock Vina, proteins were prepared by removing water molecule, adding hydrogens and Kollman charges to it. The prepared protein was saved in PDBQT format for further docking[21].

Docking

The PDB format of each statin was converted into PDBQT format using AutoDock Vina. The chosen LoX and CoX protein targets were individually docked with each statin using AutoDock Vina and binding affinity (kcal/mol) for each interaction was recorded. The AutoGrid engine was used to make a grid box around the protein-binding sites and the grid box dimensions (X, Y, Z co-ordinates) were recorded for all the target enzymes [(protein 1: 132.314, 127.343, 131.720), (protein 2: -46.198, 9.661, 530.672), (protein 3: -4.097, 45.508, 3.108) and (protein 4: -22.683, 40.790, 39.382)]. The ligand with best possible binding energy and lowest docking score was further analysed. Biovia Discovery studio visualizer was used to read the interaction between enzymes and different statins and also to visualise the output structures.

Binding pocket coordinates

The binding pockets of the four enzymes were meticulously analyzed using the "Define the Receptor" function in Biovia Discovery Studio visualizer, which identified the most optimal coordinates for potential high-efficacy ligand interactions. This analysis provided crucial insights into the probable binding sites where ligands could exert their biological effects.

Results

The binding interactions of the statins with the target enzymes were meticulously evaluated and tabulated (Table 2). The two top statins with the best binding interaction scores, *i.e.*, high negative

Table 1 — PubChem Ids and Canonical Smiles of Selected Proteins and Ligands

S. No	IDs	Name	Canonical Smiles
1	8GHC	12-lipoxygenase	-
2	7LAF	15-lipoxygenase	-
3	3O8Y	5-Lipoxygenase	-
4	5F19	Aspirin Acetylated Cyclooxygenase-2	-
5	60823	Atorvastatin	CC(C)C1=C(C(=C(N1CCC(CC(CC(=O)O)O)O)C2=CC=C(C=C2)F)C3=CC=CC=C3)C(=O)NC4=CC=CC=C4
6	54454	Simvastatin	CCC(C)(C)C(=O)OC1CC(C=C2C1C(C(C=C2)C)CCC3CC(CC(=O)O3)O)C
7	53232	Lovastatin	CCC(C)C(=O)OC1CC(C=C2C1C(C(C=C2)C)CCC3CC(CC(=O)O3)O)C
8	446157	Rosuvastatin	CC(C)C1=NC(=NC(=C1C=CC(CC(CC(=O)O)O)O)C2=CC=C(C=C2)F)N(C)S(=O)(=O)C
9	446155	Fluvastatin	CC(C)N1C2=CC=CC=C2C(=C1C=CC(CC(CC(=O)O)O)O)C3=CC=C(C=C3)F
10	54687	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
11	5282452	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

Table 2 — The binding affinity of different statins with selected target enzymes

S. No	Ligands	Protein 1 12-lipoxygenase (8GHC)	Protein 2 15-lipoxygenase (7LAF)	Protein 3 5-Lipoxygenase (3O8Y)	Protein 4 - Aspirin Acetylated Cyclooxygenase-2 (5F19)
1	L1 – Atorvastatin	-7.3 ± 0.20	-8.9 ± 0.20	-7.8 ± 0.33	-8.7 ± 0.75
2	L2 – Simvastatin	-6.8 ± 0.18	-9.6 ± 0.79	-7.8 ± 0.26	-8.5 ± 0.91
3	L3 – Lovastatin	-6.7 ± 0.16	-9.6 ± 0.46	-7.7 ± 0.25	-8.4 ± 0.25
4	L4 – Rosuvastatin	-7.1 ± 0.21	-8.4 ± 0.25	-7.2 ± 0.23	-8.0 ± 0.34
5	L5 – Fluvastatin	-6.4 ± 0.18	-9.5 ± 0.29	-8.7 ± 0.45	-8.6 ± 0.53
6	L6 – Pravastatin	-6.4 ± 0.15	-8.1 ± 0.34	-6.8 ± 0.23	-7.9 ± 0.55
7	L7 – Pitavastatin	-7.4 ± 0.19	-9.5 ± 0.39	-8.8 ± 0.58	-8.9 ± 0.50

values against each target enzyme were visualized in (Figs. 1 & 2).

All the statins demonstrated good binding interactions with varying efficacy with both LoX and CoX enzymes. They exhibited the highest binding affinity against 15-LoX, while the lowest was recorded against 12-LoX. This analysis revealed significant variations in binding affinities and also highlighted pitavastatin and atorvastatin as the most potent among statin's in interacting across the tested enzyme targets.

In interactions against 12-LoX, pitavastatin (-7.4) and atorvastatin (-7.3) showed the highest binding affinities among the docked statins. Rosuvastatin, simvastatin, lovastatin, fluvastatin and pravastatin showed binding energies of -7.1, -6.8, -6.7, -6.4 and -6.4 respectively.

All statins demonstrated excellent binding interactions with 15-LOX, with all the scores being equal to or less than -8.4. Among the interactions, lovastatin (-9.6) and simvastatin (-9.6) showed the strongest binding affinity, followed by pitavastatin (-9.5), fluvastatin (-9.5), atorvastatin (-8.9), rosuvastatin (-8.5) and pravastatin (-8.5).

The trend observed in binding interactions with 5-LoX was almost similar to the interactions noticed with 12-LoX. Pitavastatin (-8.8) and fluvastatin (-8.7) exhibited the strongest binding interactions, while rosuvastatin, with -7.2, exhibited the lowest interactive score.

All the statins exhibited excellent interactions against aspirin-acetylated CoX-2, with binding scores in the range of -7.9 to -8.9. Pitavastatin (-8.9) and atorvastatin (-8.7) showed superior interaction when compared to the other statins.

Overall, among the statins, pitavastatin and atorvastatin exhibited the highest binding affinities with inflammation resolution enzymes. This distinguishes them as highly efficacious candidates among the statin class for targeting these resolution pathways.

Binding pocket coordinates

The interaction studies with these ligands facilitated the determination of the binding coordinates for the most relevant ligand-enzyme complexes. These coordinates were documented, compiled and presented in (Table 3).

With regards to protein-1 the intrinsic binding coordinates (X, Y, Z) were identified at 135.42, 126.76, and 150.66. Among the docked ligands, the binding coordinates of rosuvastatin (130.20, 124.17, and 143.84) demonstrated the closest spatial alignment to the natural binding site.

Similarly, for protein-2, the identified native binding coordinates were observed at -31.66, 11.66, and 522.7 and the interaction coordinates of atorvastatin, with -43.50, 5.850, and 519.02, exhibited the closest docking proximity alignment to the natural site.

The intrinsic binding site of protein-3, was demarcated at 4.45, 76.72, and 26.86 along the X, Y, and Z axes. In protein-3 it was noticed that atorvastatin, pravastatin and pitavastatin displayed closest binding affinities in Y-axis, whereas fluvastatin exhibited the closest proximity binding along the Z-axis.

In binding correlation to protein-4, the native binding coordinates were approximated to be at 20.68, 43.27, and 39.71. Whereas the docking metrics indicated that both atorvastatin (22.75, 44.92, 32.87) and pravastatin (25.34, 42.82, 32.01) had closest binding interactions among the evaluated ligands, aligning close to its natural binding site.

Based on the binding affinity analysis against these targeted enzymatic pathways, it is noticed that atorvastatin and pitavastatin were the most favourable ligands having interactions close to the natural binding sites among the docked statins.

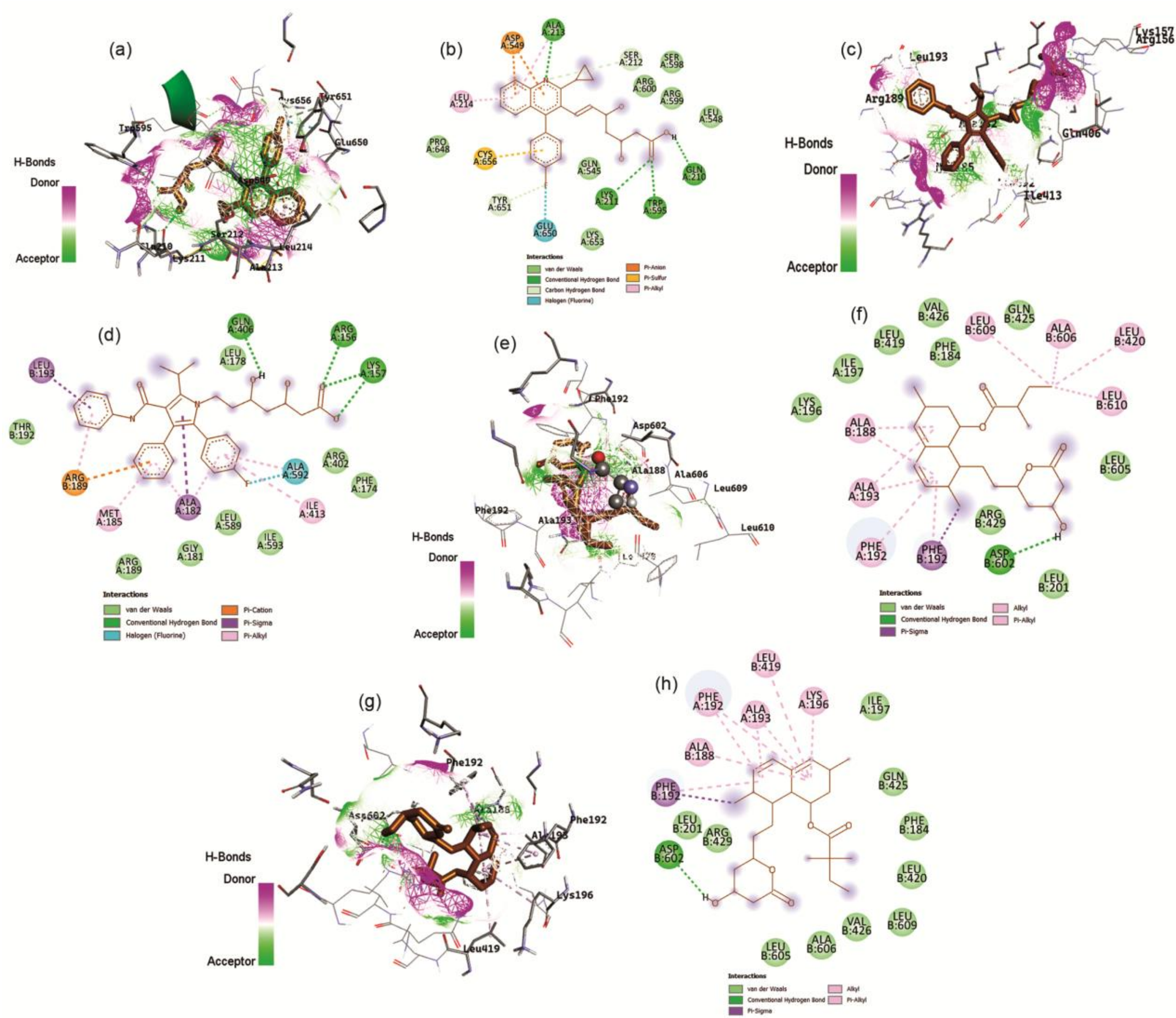

Fig. 1 — (a) Protein 1 *vs* Pitavastatin – 3D; (b) Protein 1 *vs* Pitavastatin – 2D; (c) Protein 1 *vs* Atorvastatin – 3D; (d) Protein 1 *vs* Atorvastatin – 2D; (e) Protein 2 *vs* Lovastatin – 3D; (f) Protein 2 *vs* Lovastatin – 2D; (g) Protein 2 *vs* Simvastatin – 3D; and (h) Protein 2 *vs* Simvastatin – 2D

Table 3 — Binding pocket coordinates of Statins on target enzymes

S.No	Ligands	Protein 1 12-lipoxygenase (8GHC)			Protein 2 15-lipoxygenase (7LAF)			Protein 3 5-Lipoxygenase (3O8Y)			Protein 4 - Aspirin Acetylated Cyclooxygenase-2 (5F19)		
		X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
1	Natural coordinate	135.42[a]	126.76[a]	150.66[a]	-31.66[a]	11.66[a]	522.75[a]	4.45[a]	76.72[a]	26.86[a]	20.68[a]	43.27[a]	39.71[a]
2	Atorvastatin	142.77	140.50	112.0	-43.50[b]	5.850	519.02[b]	7.70	57.13[b]	-7.67	22.75	44.92[b]	32.87[b]
3	Simvastatin	129.15	136.90	123.83	-45.59	8.294	531.72	5.85[b]	53.85	-3.06	26.20	39.90	33.73[b]
4	Lovastatin	129.15	136.90	123.83	-45.32	8.294	531.17	6.77	56.57	-6.75	23.62	37.97	33.73[b]
5	Rosuvastatin	130.20	124.17[b]	143.84[b]	-45.22	8.294	529.00	6.77	56.57	-6.75	26.20	39.90	33.73[b]
6	Fluvastatin	131.86[b]	136.90	124.63	-44.14	11.01[b]	529.00	11.33	30.79	4.3[b]	26.20	39.90	33.73[b]
7	Pravastatin	130.9	130.23	123.83	-44.41	11.01[b]	529.91	7.70	57.13[b]	-7.67	25.34	42.82[b]	32.01[b]
8	Pitavastatin	120.95	131.04	112.01	-45.22	9.20	529.91	7.70	57.13[b]	-7.67	26.20	39.90	33.73[b]

- The values with superscript [a] represent the natural coordinates binding of the enzymes in their pockets as defined by the Biovia Discovery software
- The values with superscript b indicate the closest binding of the ligand in relation to the natural coordinates

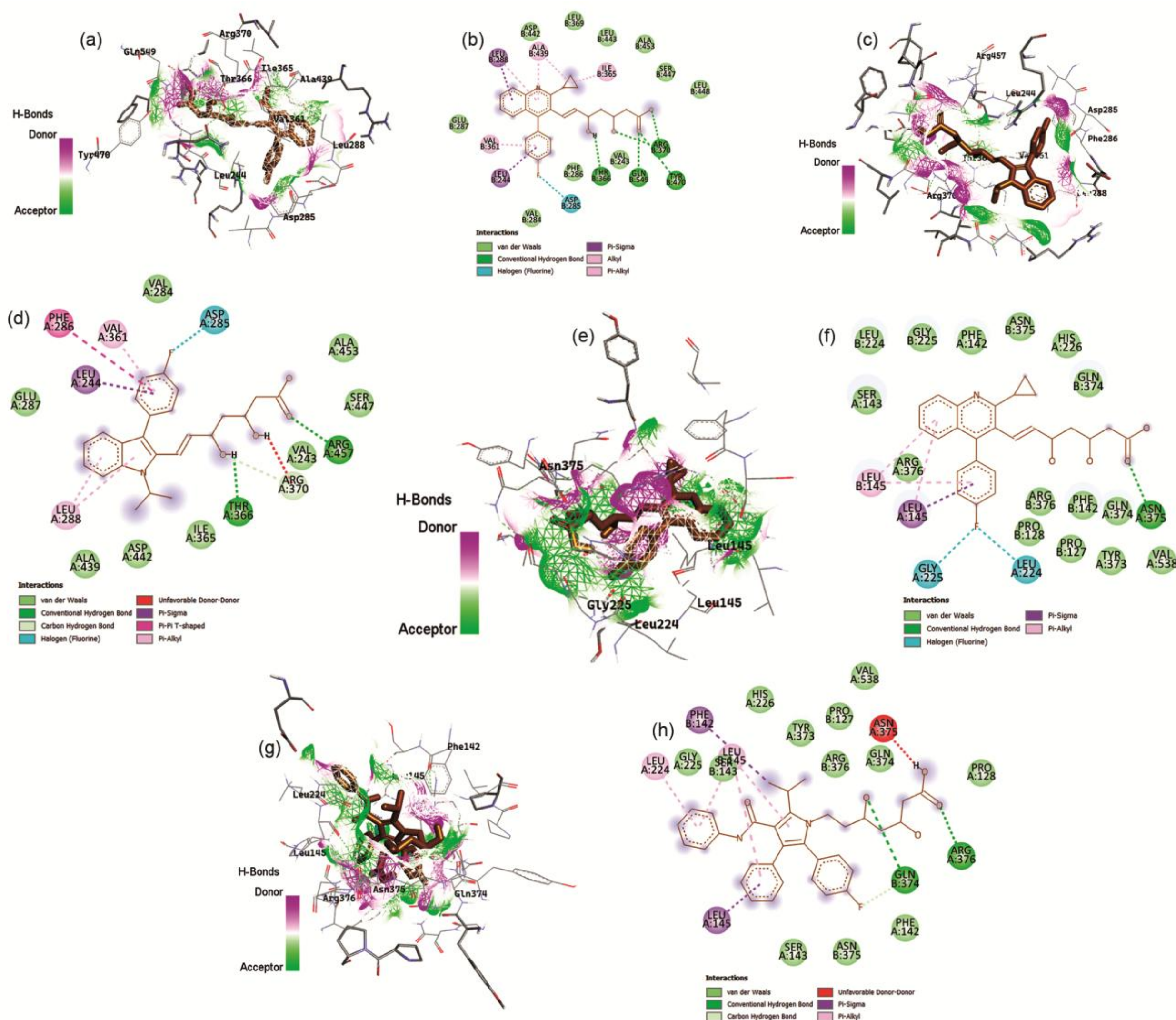

Fig. 2 — (a) Protein 3 *vs* Pitavastatin – 3D; (b) Protein 3 *vs* Pitavastatin – 2D; (c) Protein 3 *vs* Fluvastatin – 3D; (d) Protein 3 *vs* Fluvastatin – 2D; (e) Protein 4 *vs* Pitavastatin – 3D; (f) Protein 4 *vs* Pitavastatin – 2D; (g) Protein 4 *vs* Atorvastatin – 3D; and (h) Protein 4 *vs* Atorvastatin – 2D

Type of binding interactions

The amino acid binding profiles of various statins and the quantitative assessment of its favourable interactions across the four target proteins were analysed and presented (Table 4). This analysis explains the binding tendencies and molecular interactions of various statins and also highlights their affinity for the respective enzymes.

The highest number of favourable interactions against protein-1 was exhibited by atorvastatin (14), exhibiting its strong binding affinity. It was closely followed by pitavastatin (12) and rosuvastatin (11). Lovastatin (8), simvastatin (7) and pravastatin (7) displayed moderate binding interaction affinities. Fluvastatin (3) exhibited weak binding affinity with the least number of favourable interactions.

Against protein-2, pitavastatin was found to be the most potent interactor, having 17 favourable interactions. Lovastatin (13) also demonstrated excellent interaction affinity. Fluvastatin (10), simvastatin (10) and pravastatin (9) showed similar binding tendencies. Atorvastatin (6) and rosuvastatin (5) were found to exhibit relatively lower interaction frequencies against protein-2.

Pitavastatin, with 13 favourable interactions against protein-3, was found to have superior affinity to this enzyme. Notable binding interactions were also exhibited by fluvastatin (9), atorvastatin (8) and rosuvastatin (8). Moderate interactions were displayed by lovastatin (6) and pravastatin (6), while the least interaction affinity was exhibited by simvastatin (4).

Table 4 — Amino acid interactions and number of favourable bonds

S. No	Ligands	Protein 1 12-lipoxygenase (8GHC)		Protein 2 15-lipoxygenase (7LAF)		Protein 3 5-Lipoxygenase (3O8Y)		Protein 4 - Aspirin Acetylated Cyclooxygenase-2 (5F19)	
		Interacting amino acids	No of favourable interactions	Interacting amino acids	No of favourable interactions	Interacting amino acids	No of favourable interactions	Interacting amino acids	No of favourable interactions
1	Atorvastatin	ARG156, LYS157, ALA182, MET185, GLN406, ILE413, ALA592, ARG189, LEU193	14	ALA188, PHE192, ALA606, LEU609	6	VAL243, LEU244, ARG246, VAL361, ASP442, ALA453, ALA456	8	LEU145, LEU224, ASN375, ARG376, PHE142, LEU145, GLN374	11
2	Simvastatin	ALA180, LEU183, LYS211, TRP595, TRP208, GLY209	7	PHE192, ALA193, LYS196, ALA188, HE192, LEU419, ASP602	10	ARG370, ALA456, ARG457, GLN549	4	SER143, ARG376	3
3	Lovastatin	ALA180, LEU183, LYS211, TRP595, TRP208, GLY209	8	PHE192, ALA193, ALA188, PHE192, LEU420, ASP602, ALA606, LEU609	13	VAL243, PHE286, LEU288, VAL361, ARG370, ALA439, ARG457	6	LEU224, ARG376	3
4	Rosuvastatin	ASN544, GLN545, LEU548, ASP549, SER598, GLN601, SER655, CYS656	11	PHE192, LEU419, ARG429, ASP602	5	LEU244, PHE286, LEU288, VAL361, ARG370, ARG438, SER447, ARG457	8	ASN375, PHE142, SER143, LEU145 GLY225, GLN374	8
5	Fluvastatin	GLN205, PHE207	3	GLY189, ALA193, ALA188, PHE192, LEU419, ASP602	10	LEU244, ASP285, PHE286, LEU288, VAL361, THR366, ARG370, ARG457	9	TYR373, GLN374	4
6	Pravastatin	GLU184, LYS188, GLN210, LYS211, TRP595	7	PHE192, ALA193, PHE192, LEU419, GLN425, ARG429, ASP602	9	VAL243, LEU244 ARG246, ASP285, ALA439	6	PHE142, LEU145, LEU224, HIS226, ASN375, ASN375, ARG376	8
7	Pitavastatin	GLN210, LYS211, SER212, ALA213, LEU214, ASP549, TRP595, LU650, TYR651	12	ALA193, LYS196, ILE197, ALA188, PHE192, LYS196, LEU201, LEU419, VAL426, ASP602, LEU605, ALA606	17	LEU244, ASP285, LEU288, VAL361, ILE365, THR366, ARG370, ALA439, TYR470, GLN549	13	LEU145, LEU224, GLY225, ASN375, LEU145	7

Table 5 — Type of Binding interactions

S. No	Ligands	Protein 1 12-lipoxygenase (8GHC)					Protein 2 15-lipoxygenase (7LAF)				
		Conventional Hydrogen Bond	Carbon-hydrogen	Pi-Alkyl	Other bonds	Vander wall forces	Conventional Hydrogen Bond	Carbon-hydrogen	Pi-Alkyl	Other bonds	Vander wall forces
1	Atorvastatin	4	0	6	4	7	0	0	2	4	12
2	Simvastatin	2	1	1	3	7	1	0	3	6	9
3	Lovastatin	3	1	1	3	8	1	0	3	9	5
4	Rosuvastatin	7	0	1	3	9	3	1	0	1	12
5	Fluvastatin	0	0	0	3	10	3	1	1	5	8
6	Pravastatin	5	1	0	1	5	5	1	1	2	10
7	Pitavastatin	4	2	4	2	6	4	1	7	5	4

S.No	Ligands	Protein 3 5-Lipoxygenase (3O8Y)					Protein 4 - Aspirin Acetylated Cyclooxygenase-2 (5F19)				
		Conventional Hydrogen Bond	Carbon-hydrogen	Pi-Alkyl	Other bonds	Vander wall forces	Conventional Hydrogen Bond	Carbon-hydrogen	Pi-Alkyl	Other bonds	Vander wall forces
1	Atorvastatin	1	0	5	2	10	3	1	4	3	12
2	Simvastatin	2	0	0	2	9	1	2	0	0	9
3	Lovastatin	1	1	1	3	6	2	1	0	0	10
4	Rosuvastatin	1	3	2	2	7	3	2	0	3	10
5	Fluvastatin	2	1	3	3	7	1	0	0	3	11
6	Pravastatin	5	0	0	1	8	6	0	1	1	10
7	Pitavastatin	5	0	3	5	7	1	0	3	3	13

Eleven favourable interactions against protein-4 were demonstrated by atorvastatin, showing its excellent binding capacity. Significant interaction tendencies were observed with rosuvastatin (8), pravastatin (8) and pitavastatin (7). The weakest interaction frequencies were displayed by fluvastatin (4), simvastatin (3) and lovastatin (3).

Evaluation of the ligand-protein interactions identified atorvastatin and pitavastatin as the most efficacious ones, showcasing their high-affinity interactions against the selected protein targets. Rosuvastatin and lovastatin also showed significant binding affinities, while relatively weaker interactive tendencies were displayed by fluvastatin, simvastatin and pravastatin.

The ligand-protein interaction data (Table 5) revealed valuable insights on the binding profiles of statins against the selected enzymes. It was observed that among the statins, rosuvastatin exhibited the highest number of conventional hydrogen bond interactions (7) with protein-1, followed by pravastatin (5) and pitavastatin and atorvastatin with each four conventional hydrogen bonds. These statins also executed variety of other interactions, including carbon-hydrogen, pi-alkyl, pi-cation, pi-sigma and van der waals interactions. Interestingly, although fluvastatin exhibited 10 favourable interactions, all were restricted to weak van-der-waals interactions.

Pravastatin formed five conventional hydrogen bonds with protein-2, while pitavastatin had four, whereas fluvastatin and rosuvastatin formed each three hydrogen bonds. Concerning to protein-3 interactions it was noticed that pitavastatin and pravastatin exhibited the highest number of hydrogen bonds (5 each), while the remaining statins, despite having an overall high number of favourable interactions they predominantly formed only van-der-waals bonds.

Pravastatin was identified as the most efficient statin against protein-4 with six conventional hydrogen bond interactions. This was followed by atorvastatin and rosuvastatin, with each three conventional hydrogen bonds. Interestingly, it was observed that all the statins executed a significant number of van-der-waals interactions against protein-4.

Based on the type of bond interactions, it was inferred that among the statins, pravastatin and pitavastatin formed the strongest interactions, primarily driven by the number of conventional hydrogen bonds, followed closely by atorvastatin and rosuvastatin.

Discussion

The role of statins in redefining inflammation and promoting its resolution has assumed significant scientific interest, mainly after the work of Birnbaum *et al*. (2006). They demonstrated that atorvastatin enhances the production of 15-epi-lipoxin A4 (LXA4) in myocardium *via* S-nitrosylation of CoX-2. They observed that the S-nitrosylation of CoX-2 produces 15R-hydroxy eicosatetraenoic acid (HETE) much like the acetylation of CoX-2 by aspirin, which is subsequently transformed by the leukocyte's 5-LoX into 15-epi-LX-A4. Based on these findings, it was hypothesized that statins on interaction with LoX and CoX enzymes, facilitates the biosynthesis of SPM's that not only suppress inflammation but also accelerate the resolution of tissue inflammation. In the present study an *in silico* approach was employed, utilizing the molecular docking to examine the interactions of statins on the enzymes involved in resolution pathways. This computational strategy may facilitate the repurposing of statins as potential therapeutic agents for active resolution of inflammation. As there were no comprehensive correlation analyses available to evaluate the relationship between inflammation resolution and statins, these molecular docking studies will be highly instrumental in bridging the critical knowledge gap in exploring resolution pathways.

The results of the current study indicated that pitavastatin exhibited superior interaction with the resolution mediating enzymes, closely followed by atorvastatin and rosuvastatin. Interestingly, this finding disagrees the findings of Banach *et. al.,* (2023), where they reported that pitavastatin exhibited a slightly lower potency when compared to rosuvastatin and atorvastatin.

In this study, it was found that all the statins exhibited good binding efficacies against the selected enzymatic targets. The most pronounced binding affinity was observed against CoX-2 and 15-LoX. This aligns with the *in vivo* findings reported in the studies of Birnbaum *et al*. (2006) and Ye *et al*. (2008). They observed that high-dose statin therapy upregulates cytosolic phospholipase-A2 and CoX-2, thereby subsequently enhance the synthesis of prostacyclin and 15-deoxy-PGJ2. Furthermore, statins were also reported to activate protein kinase A, leading to the phosphorylation of 5-LoX. This cascade effect results in a decreased production of pro-inflammatory leukotrienes with alternative increase in the synthesis of 15-epi-lipoxin A4, an eicosanoid renowned for its potent anti-inflammatory and inflammation-resolution properties[3].

In addition to augmenting the levels of 15-epi-lipoxin A4 as discussed above, the statins also promote the biosynthesis of t-series resolvins (RvTs) or 13-series resolvins through the interaction of DHA on 15-LoX. Walker *et al.* (2017) reported that the administration of pravastatin (0.2 mg/kg) or atorvastatin (0.2 mg/kg) to inflammatory arthritic mice evinced a significant increase in the systemic and tissue concentrations of RvTs. They also noticed that this increase was associated with a marked reduction in joint disease severity. A similar effect was observed by Dalli *et. al.* (2015) in the *in vitro* studies, where statins were shown to potentiate the production of SPMs through their action on PUFAs. It is evident from the results of the present study that all the statins have exhibited robust binding interactions with 15-LoX enzyme. This reinforces the findings of both *in vitro* and *in vivo* investigations as discussed earlier. These results further substantiate the role of statins in modulating lipid mediator pathways, which might further contribute to the resolution of inflammation and restoration of tissue homeostasis.

The most critical bonding between a ligand and a protein is the hydrogen bond interaction, as it enhances protein-ligand stability[12]. It was noticed in this study that both pitavastatin and pravastatin formed the highest number of hydrogen bond interactions with the target enzymes, followed by rosuvastatin and atorvastatin. This observation is in agreement with the findings of Reiner *et al*. (2020), who investigated the binding efficacy of statins against the main protease enzyme of COVID-19. They reported that pitavastatin exhibited the strongest hydrogen bonding and superior binding interactions, followed by rosuvastatin. The significant role of hydrogen bonding in statin-protein interactions is highlighted by these results.

The lipid mediator class switching is a critical biological process in inflammation resolution. The transition of pro-inflammatory prostaglandins to anti-inflammatory, pro-resolving mediators is majorly orchestrated by this process. The essential enzymes which play a vital role in lipid mediator class switching are 15-LoX and CoX-2. This class switch leads to the formation of Prostaglandin-J series, which further gives rise to the formation of endogenous SPMs[10]. These mediators establish a crucial temporal

and spatial checkpoints within the inflammatory cascade, ensuring a controlled resolution of inflammation. Upregulation of the production of anti-inflammatory prostanoids, such as Prostaglandin D_2 (PGD_2) and Prostaglandin E_2 (PGE_2) by their enzymatic activity, not only curtail the un-controlled inflammation, but also contributes to the active tissue remodelling and repair.

Conclusion

To evaluate the binding potency of various statins on resolution pathway enzymes to their putative active sites, docking technology was used in this study. Based on their effectiveness in interacting with different enzymes, the statins were categorised. A slight limitation of this study, is that the inflammation-resolving efficacy of statins at the inflammatory site is majorly dependent on the bioavailability of statins at the target sites. This information is beyond the scope of the current technology. Also, an additional variable which is not accounted for in this model is the metabolism of individual statins which influences their ability to reach these sites. Despite these limitations, this study employs a novel approach that helps us assess the efficacy of interaction of molecules with the resolution pathway enzymes, offering insights into their prospective repurposing as drugs for the resolution of inflammation.

Acknowledgement

We are thankful to the Tamil Nadu Veterinary and Animal Sciences University, Chennai for providing technical and financial support

Conflict of interests

All authors declare no conflict of interests.

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