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Original Research Article

The Exploration of 6-Gingerol and 6-Shogaol using Network Pharmacology and Molecular Docking as Potential Inhibitors of Atherogenesis

Diana K Jasaputra[1], Aldrich Christiandy[2], Ardo Sanjaya[3,4,*]

[1] *Department of Pharmacology, Faculty of Medicine, Maranatha Christian University, Bandung, 40164, Indonesia.*
[2] *Undergraduate Program in Medicine, Faculty of Medicine, Maranatha Christian University, Bandung, 40164, Indonesia.*
[3] *Department of Anatomy, Faculty of Medicine, Maranatha Christian University, Bandung, 40164, Indonesia.*
[4] *Biomedical Research Laboratory, Faculty of Medicine, Maranatha Christian University, Bandung, 40164, Indonesia.*

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ABSTRACT

Atherosclerosis is the narrowing of blood vessels due to fatty plaque buildup in the intimal layer. Preventing atherosclerosis is a promising treatment avenue. Research shows that ginger (*Zingiber officinale*), particularly its active compounds 6-gingerol and 6-shogaol, can lower total cholesterol and LDL (low-density lipoprotein) levels, although the mechanisms remain unclear. The Comparative Toxigenomics Database was used to identify genes interacting with 6-gingerol and 6-shogaol, followed by enrichment analysis via ShinyGO and mapping onto KEGG pathways to identify upstream protein regulators. Docking studies were conducted using AutoDock 4.2, with visualization in Discovery Studio. Five proteins emerged as potential targets: PPAR-*γ* (peroxisome proliferator-activated receptor gamma), PPAR-*δ* (peroxisome proliferator-activated receptor delta), LOX-1 (lectin-like oxidized low-density lipoprotein receptor 1), ACAT1 (acetyl-CoA acetyltransferase 1), and PI3K (phosphoinositide 3-kinase gamma). 6-gingerol and 6-shogaol inhibited PI3K, PPAR-*δ*, and LOX-1 similarly to their co-crystallized ligands. However, the docking protocols could not account for LOX-1 tetramerization or ACAT1 steric hindrance, highlighting the need for further investigation into these interactions. Regarding PPAR-*γ*, the compounds did not show compatible interaction patterns, making it an unlikely target. The experiment provides insight into how 6-gingerol and 6-shogaol may affect lipid metabolism and atherosclerosis, mainly interacting with PI3K, PPAR-*δ*, and LOX-1. No supporting evidence was found for their interaction with the other tested proteins.

Keywords: Gingerol, Shogaol, *Zingiber officinale*, Atherosclerosis.

Introduction

Atherosclerosis is defined as narrowing blood vessels caused by the buildup of fatty plaque, fibrosis, and calcification in the intimal layer.[1,2] It is influenced by factors such as genetics, obesity, dyslipidemia, hypertension, smoking, and chronic inflammation.[3,4] While initially asymptomatic, atherosclerosis progresses to atheroma, which can rupture, causing thrombosis and significant morbidity. Prevalence begins early, affecting up to 17% of individuals under 20 years and increasing to 85% in those over 50.[5] Atherosclerosis remains a leading cause of vascular disease worldwide, with an increasing incidence over the last decade.[6,7] Key contributors to atherogenesis include high LDL (low-density lipoprotein) levels and endothelial dysfunction, often linked to hypertension, smoking, and diabetes.[8–10] Dysfunctional endothelium facilitates LDL accumulation in the arterial intima, where it oxidizes into oxLDL (oxidized low-density lipoprotein).[11] OxLDL attracts macrophages, forming foam cells that secrete cytokines, promoting inflammation and fibrous cap formation (Figure 1).[1,12]

*Corresponding author. Email: ardo.sanjaya@med.maranatha.edu
Tel: +00359877115255

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Over time, this process results in necrotic core formation and a vulnerable fibrous cap, increasing the risk of thrombosis.[9–11] Current pharmacological treatments primarily target hypertension and high cholesterol using antihypertensives and statins. Emerging approaches include targeting inflammatory processes and foam cell formation, which are critical in atherosclerosis progression. Ginger (*Zingiber officinale*) is a widely recognized medicinal plant[13,14] with documented benefits in traditional medicine systems such as Chinese and Ayurveda.[15] Global interest in ginger has grown significantly over the past two decades, reflected in increasing production and consumption.[16] Ginger exhibits anti-inflammatory, antioxidant, and cardiovascular protective properties. Its active compounds, 6-gingerol and 6-shogaol, demonstrate antihypertensive, anti-inflammatory, and anti-atherosclerotic effects. For instance, 6-shogaol reduces shear stress on endothelial cells and inhibits arterial calcification through Akt/ROS and NLRP3 inflammasome pathways, while both compounds downregulate pro-inflammatory cytokines like IL-1β, NF-κB, and TNF-α.[20–23] However, the exact proteins or pathways influenced by 6-gingerol and 6-shogaol remain unknown. Network pharmacology offers a promising approach to drug discovery, moving beyond the traditional "one drug-one target-one disease" model. This method maps complex drug-disease interactions, enabling the exploration of multiple targets simultaneously.[24] Networks reflect the complex signaling pathways associated with specific diseases and allow a thorough exploration of multiple targets during drug discovery. Network pharmacology has successfully investigated 6-gingerol and 6-shogaol for anti-obesity,[25] anti-tumor,[26] and anti-emetic effects,[27] with findings highlighting the PI3K/Akt pathway as a key mechanism. However, the effects of ginger's bioactive compounds on pathways involved in atherogenesis remain unclear. This study aims to evaluate the inhibitory potential of 6-gingerol and 6-shogaol from ginger (*Zingiber officinale*) on atherogenesis by identifying gene interactions and pathways using network pharmacology and assessing their binding affinity to key

atherosclerosis-related proteins through molecular docking. The novelty of this research lies in its use of network pharmacology combined with molecular docking to identify and explore the specific interactions of 6-gingerol and 6-shogaol with multiple molecular targets involved in atherogenesis, highlighting their potential therapeutic effects in cardiovascular disease.

Materials and Methods

Identification of Interacting Genes and Enrichment Analysis

Potential gene targets of 6-gingerol and 6-shogaol were identified using the Comparative Toxigenomics Database (https://ctdbase.org/) [28] for interacting genes. Duplicate gene entries were merged before enrichment analysis using ShinyGO (http://bioinformatics.sdstate.edu/go/).[29] KEGG pathway[30] mapping was performed to identify upstream protein regulators for molecular docking.

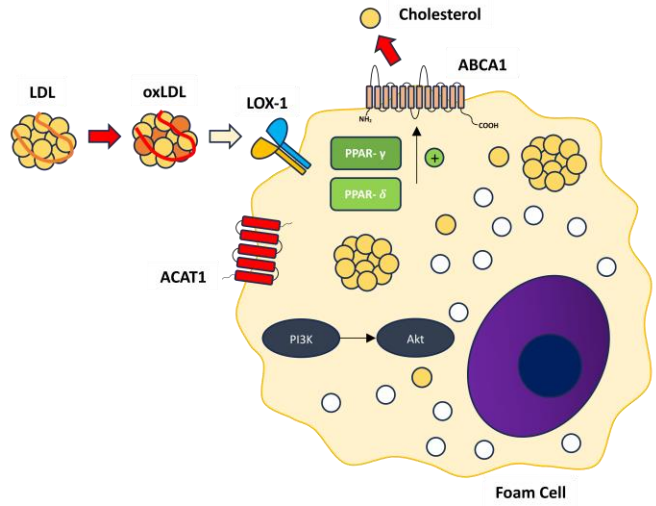

Figure 1: Regulation of Foam Cell Formation: Cholesterol and oxLDL Transport in Foam Cells

LOX-1 uptakes oxLDL into the foam cell. PPAR-γ and PPAR-δ upregulate ABCA1 expression on foam cell that causes cholesterol efflux. Esterification of cholesterol by ACAT1 leads to lipid droplet accumulation. PI3K/Akt pathway exerts various pathways such as lipid droplet accumulation, reduction of lipid transporter, and increased pro-inflammatory cytokines (not shown in the figure). Figure adapted from Wang et al.[10] (LDL = low-density lipoprotein; oxLDL = oxidized low-density lipoprotein; LOX-1 = lectin-like oxidized low-density lipoprotein receptor 1; ACAT1 = acetyl-CoA acetyltransferase 1; PI3K = phosphoinositide 3-kinase gamma; Akt = protein kinase B; PPAR-γ = peroxisome proliferator-activated receptor gamma; PPAR-δ = peroxisome proliferator-activated receptor delta; ABCA1 = ATP binding cassette transporter A1)

Ligand Preparation

Coordinate files for 6-gingerol (CID 442793) and 6-shogaol (CID 5281794) were obtained from PubChem[31] (https://pubchem.ncbi.nlm.nih.gov/) in *.sdf format and were converted to *.pdb format using the Online SMILES Translator (https://cactus.nci.nih.gov/translate/). PDBQT files for docking were prepared in AutoDock 4.2[32,33] following the protocol by Forli et al. [34]

Receptor Preparation, Docking, and Visualization

Crystal structures for PPAR-γ (peroxisome proliferator-activated receptor gamma) (5YCN), PPAR-δ (peroxisome proliferator-activated receptor delta) (7WGL), LOX-1 (lectin-like oxidized low-density lipoprotein receptor 1) (6TL9), ACAT1 (lectin-like oxidized low-density lipoprotein receptor 1) (6VUM), and PI3K (phosphoinositide 3-kinase gamma) (5JHB) were retrieved from the RCSB Protein Data Bank[35] (https://www.rcsb.org/). Water, ligands, ions, and solvent molecules were removed using Discovery Studio,[36] except oleic acid and coenzyme A, retained in ACAT1 for their functional relevance.[37] Co-crystallized ligands served as positive controls and validated the docking protocol (RMSD < 2 Å). Docking was performed in AutoDock 4.2 per Forli et al.[34] Docking results were visualized using Discovery Studio,[36] displaying 2D diagrams of protein-ligand interactions. Binding energy, inhibition constants, and ligand efficiency were reported.

Results and Discussion

The search in the Comparative Toxigenomics Database yielded 236 and 183 entries for Gingerol and shogaol, respectively (Supplementary Table 1). After merging duplicate entries, identified 86 and 56 genes were identified for enrichment analysis using ShinyGO. Both compounds were significantly enriched for the lipid and atherosclerosis pathway,[38] which is the focus of this research (Figure 2). Detailed enrichment results with enriched genes involved in the lipid and atherosclerosis pathway are provided in Supplementary Table 2. The top 10 significant pathways, ranked by the false discovery rate, are presented (Figure 2A, 2B). Gingerol's interacting genes were notably enriched for pathways in cancer, which may present future research opportunities, but lipid and atherosclerosis ranked fifth with a moderate ~30-fold enrichment. In contrast, shogaol's genes showed the highest enrichment for lipid and atherosclerosis, with up to ~40-fold enrichment. These findings highlight Gingerol and shogaol as potential modulators of lipid and atherosclerosis processes.

Table 1: Validation Results of the Docking Protocol Used in this Experiment

Protein	Ligand	Estimated Free Energy of Binding (kcal/mol)	Ligand Efficiency	Reference RMSD
PI3K	PIKin3	-7.49	-0.24	1.281 Å
PPAR-γ	Lobeglitazone	-9.78	-0.29	0.559 Å
LOX-1	BI-0115	-7.90	-0.40	0.683 Å
ACAT1	Nevanimibe	-10.66	-0.34	1.080 Å
PPAR-δ	Bezafibrate	-7.90	-0.32	1.390 Å

PI3K: phosphoinositide 3-kinase gamma; PPAR-γ: peroxisome proliferator-activated receptor gamma; LOX-1: lectin-like oxidized low-density lipoprotein receptor 1; ACAT1 = acetyl-CoA acetyltransferase 1; PPAR-δ = peroxisome proliferator-activated receptor delta; RMSD = Root Mean Square Deviation.

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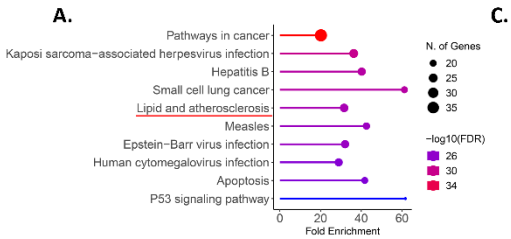
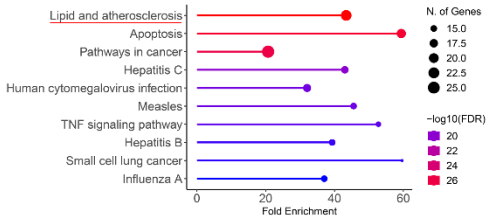


C.

Gene Symbol	Gene Description	Gene Symbol	Gene Description
BID	BH3 interacting domain death agonist	TNFRSF10B	TNF receptor superfamily member 10b
FAS	Fas cell surface death receptor	TNFSF10	TNF superfamily member 10
CASP8	caspase 8	IL1B	interleukin 1 beta
MAP2K4	mitogen-activated protein kinase kinase 4	PPARG	peroxisome proliferator activated receptor gamma
GSK3B	glycogen synthase kinase 3 beta	CASP9	caspase 9
BAX	BCL2 associated X, apoptosis regulator	IL6	interleukin 6
NFKBIA	NFKB inhibitor alpha	TP53	tumor protein p53
MMP9	matrix metallopeptidase 9	AKT1	AKT serine/threonine kinase 1
NFKB1	nuclear factor kappa B subunit 1	CASP3	caspase 3
SOD2	superoxide dismutase 2	BCL2	BCL2 apoptosis regulator
NFE2L2	nuclear factor, erythroid 2 like 2	RELA	RELA proto-oncogene, NF-kB subunit
APAF1	apoptotic peptidase activating factor 1	JUN	Jun proto-oncogene, AP-1 transcription factor subunit



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Gene Symbol	Gene Description	Gene Symbol	Gene Description
FAS	Fas cell surface death receptor	FASLG	Fas ligand
GSK3B	glycogen synthase kinase 3 beta	TNFSF10	TNF superfamily member 10
BAX	BCL2 associated X, apoptosis regulator	IL1B	interleukin 1 beta
MAPK1	mitogen-activated protein kinase 1	PPARG	peroxisome proliferator activated receptor gamma
NFKBIA	NFKB inhibitor alpha	CASP9	caspase 9
MMP9	matrix metallopeptidase 9	AKT1	AKT serine/threonine kinase 1
MAPK3	mitogen-activated protein kinase 3	CASP3	caspase 3
IKBKB	inhibitor of nuclear factor kappa B kinase subunit beta	BCL2L1	BCL2 like 1
NFKB1	nuclear factor kappa B subunit 1	BCL2	BCL2 apoptosis regulator
SOD2	superoxide dismutase 2	RELA	RELA proto-oncogene, NF-kB subunit
NFE2L2	nuclear factor, erythroid 2 like 2	CHUK	component of inhibitor of nuclear factor kappa B kinase complex

Figure 2: Gene Enrichment Results for Gingerol and Shogaol

A, B. Whole gene enrichment results for genes interacting with Gingerol and shogaol, respectively shown, are the enriched pathway sorted by the level of fold enrichment. The Lipid and Atherosclerosis pathway is marked with a red underline. The lollipop plot is sorted according to the False Discovery Rate (FDR). The color depicts the level of significance as measured by the FDR. The size of the dot reflects the number of genes in each pathway. **C, D.** Genes involved in the Lipid and Atherosclerosis pathway for Gingerol and shogaol, respectively. The gene symbols with a short gene description are shown.

Using ShinyGO, interacting genes of gingerol and shogaol were mapped (Figure 2C, 2D) to the Lipid and Atherosclerosis pathway (Figure 3). Key upstream proteins identified as potential targets included LOX-1, PPAR-γ, and PI3K. Literature research also highlighted ACAT1 and PPAR-δ as additional targets related to cholesterol efflux. These pathways suggest a multifaceted mechanism for preventing foam cell formation, which was further explored through docking experiments. The docking protocol was validated by re-docking co-crystallized ligands with the respective receptors.

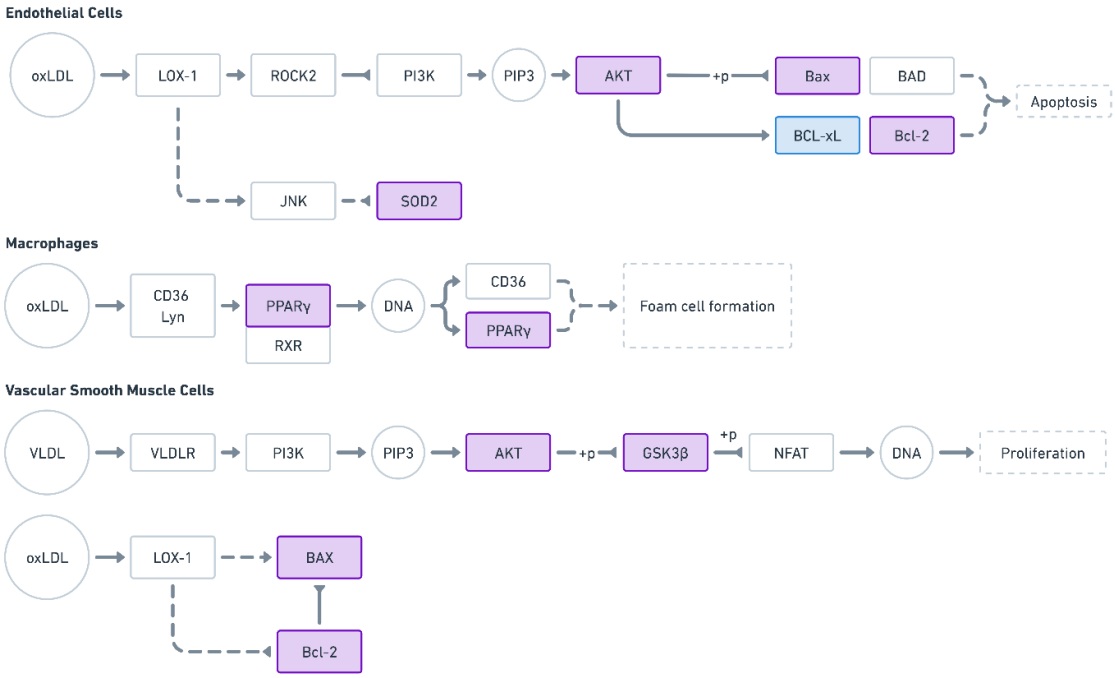

Figure 3: Modified KEGG Pathway of Lipid and Atherosclerosis

This figure showed a snippet of the Lipid and Atherosclerosis KEGG pathway [38] redrawn with permission, focusing on the components with genes interacting with either Gingerol or shogaol. Purple box: interacting genes of both Gingerol and shogaol. Blue box: interacting genes of shogaol. Dashed lines: indirect or unknown interaction. Solid lines: direct molecular interaction. Dashed boxes: cellular function or phenotype.

A standard grid box size of 40x40x40 Å and a resolution of 0.375 Å were used and the protocol achieved RMSD values < 2 Å for all proteins (Table 1). Validated parameters were then applied to dock 6-gingerol and 6-shogaol with the identified receptors. For all target proteins except PI3K, the binding energy of 6-gingerol and 6-shogaol was lower than that of their respective co-crystallized ligands (Table 2). Notably, both compounds demonstrated binding energy comparable to the PI3K inhibitor (PIKin3, the co-crystallized ligand of PI3K). Predicted binding energies for all protein targets were < -6 kcal/mol, a widely accepted threshold for drug candidate screening.[39] To further evaluate these interactions, compared the amino acid residues involved in binding between the ligands and their respective co-crystallized ligands were compared. Particular attention was given to crucial residues identified as significant for binding energy or interaction stabilization.[40] These key residues were determined from the original structural studies of the crystallized proteins. This comparison provides insights into how 6-gingerol and 6-shogaol interact with their target proteins and highlights their potential as drug development candidates. Detailed interaction data are provided in Supplementary Table 3.

Table 2: Molecular Docking Results of 6-Gingerol and 6-Shogaol

Protein	Estimated Free Energy of Binding (kcal/mol)		Estimated Inhibition Constant, Ki (μM)		Ligand Efficiency	
	6-Gingerol	6-Shogaol	6-Gingerol	6-Shogaol	6-Gingerol	6-Shogaol
PI3K	-7.43	-7.14	3.59	5.82	-0.35	-0.36
PPAR-*γ*	-6.82	-6.73	10.07	11.68	-0.28	-0.34
LOX-1	-6.56	-6.96	15.62	7.91	-0.31	-0.35
ACAT1	-6.39	-6.62	20.79	14.06	-0.30	-0.33
PPAR-*δ*	-6.77	-7.07	13.49	6.57	-0.32	-0.35

PI3K: phosphoinositide 3-kinase gamma; **PPAR-*γ***: peroxisome proliferator-activated receptor gamma; **LOX-1**: lectin-like oxidized low-density lipoprotein receptor 1; **ACAT1** = acetyl-CoA acetyltransferase 1; **PPAR-*δ*** = peroxisome proliferator-activated receptor delta

Interaction of 6-Gingerol and 6-Shogaol with PI3K

Previous studies have analyzed the interaction of 6-gingerol and 6-shogaol with PI3K using similar approaches but for different purposes.[25–27] The interaction of these compounds with PI3K has been well-documented. This study further compares their interaction with crucial amino acids in PI3K. The 2D visualization reveals eight hydrophobic interactions for 6-gingerol and 13 hydrophobic interactions for 6-shogaol (**Figure 4B-C**). Hydrophobic interactions account for over 50% of bonds in high-efficiency ligands[41,42] and contribute to the comparable binding energy of these ligands with PIKin3, a potent PI3K inhibitor.[43] The 2D visualizations also showed that 6-gingerol interacts with Asp836, Asp841, Tyr867, Val882, and Asp964 through van der Waals forces, hydrogen bonds, π-π T-shaped, and alkyl/π-alkyl interactions. In contrast, 6-shogaol interacts with all these residues except Asp836 and exhibits more hydrophobic interactions, suggesting it may bind PI3K more effectively than 6-gingerol. Along the carbon chains of both ligands, van der Waals interactions further stabilize binding. Despite these findings, the interaction with Asn951, which requires water molecules, could not be simulated using molecular docking.[43]

Predicted Interaction of 6-Gingerol and 6-Shogaol with PPAR-δ

The 2D interaction analysis revealed eight hydrophobic interactions between 6-gingerol and PPAR-*δ* and 13 interactions between 6-shogaol and PPAR-*δ* **(Figure 4E–F)**. Both ligands interacted with the crucial residues Thr253, His287, His413, and Tyr417, previously identified as key for bezafibrate binding,[44] through van der Waals forces, hydrogen bonds, and alkyl/π-alkyl interactions. The evenly distributed van der Waals interactions likely enhance anchoring to the active site. The similar binding energies (-7.90 kcal/mol for bezafibrate, -6.77 kcal/mol for 6-gingerol, and -7.07 kcal/mol for 6-shogaol) further support their potential as PPAR-*δ* ligands. These findings suggest that 6-gingerol and 6-shogaol could interact effectively with PPAR-*δ*, warranting further lead optimization and experimental validation.

Predicted Interaction of 6-Gingerol and 6-Shogaol with LOX-1

The 2D visualization showed that 6-gingerol forms six hydrophobic interactions with LOX-1, while 6-shogaol forms 11 hydrophobic interactions (Figure 4H–I). Key residues Pro201, Trp203, Tyr245, Leu258, Ala259, and Ala260 interact with 6-gingerol via van der Waals and hydrogen bonds, and with 6-shogaol via van der Waals and alkyl/π-alkyl interactions. These residues also interact with BI-0115, the co-crystallized ligand of LOX-1.[45] The binding energies (-7.90 kcal/mol for BI-0115, -6.56 kcal/mol for 6-gingerol, and -6.96 kcal/mol for 6-shogaol) suggest comparable interactions. However, BI-0115 uniquely induces LOX-1 tetramerization,[45] inhibiting oxLDL binding—a mechanism not predictable through docking alone. While 6-gingerol and 6-shogaol show potential as LOX-1 inhibitors, further experimentation and lead optimization are required to confirm their efficacy and clarify their inhibitory mechanisms.

Predicted Interaction of 6-Gingerol and 6-Shogaol with PPAR-γ

Lobeglitazone interacts with PPAR-*γ* through key residues Ile249, Leu255, Arg280, Ile281, Ile341, and Met348 and effectively inhibits Cdk5-mediated phosphorylation at Ser245, enhancing its efficacy compared to rosiglitazone.[46] In contrast, 6-gingerol binds only to Ile341 via π-σ and alkyl/π-alkyl, while 6-shogaol interacts with Ile341 and Met348 via van der Waals forces **(**Figure 5B–C). These differences in interaction patterns were reflected in the lower binding energies of 6-gingerol (-6.82 kcal/mol) and 6-shogaol (-6.73 kcal/mol) compared to lobeglitazone (-9.78 kcal/mol). Consequently, these findings suggest that 6-gingerol and 6-shogaol are less effective than lobeglitazone in targeting PPAR-*γ*.

Predicted Interaction of 6-Gingerol and 6-Shogaol with ACAT1

Nevanimibe interacts with ACAT1 through key residues, including Phe254, Phe258, Phe384, Tyr417, Asn421, and Val424, and inhibits the catalytic residue His460 by steric hindrance, blocking substrate binding.[47] Additional residues, such as Phe382, Trp408, Arg418, and Ser456, also contribute to ACAT1 activity, as point mutation experiments show.[47] The 2D visualizations showed that 6-gingerol forms seven hydrophobic interactions and three hydrogen bonds with ACAT1, while 6-shogaol forms six hydrophobic interactions and three hydrogen bonds **(**Figure 5E–F). Both ligands interacted with Phe384, Tyr417, Asn421, and Val424 via van der Waals and alkyl/π-alkyl interactions. Additionally, 6-gingerol interacted with Phe254 and Phe258 through alkyl/π-alkyl interactions, Ser456 via van der Waals, and His460 via a carbon-hydrogen bond, suggesting better potential to inhibit ACAT1 than 6-shogaol. However, the interaction configurations of both ligands do not support strong binding to the active site, with binding energies up to 3 kcal/mol weaker than Nevanimibe. These results suggest that neither 6-gingerol nor 6-shogaol effectively inhibits ACAT1.

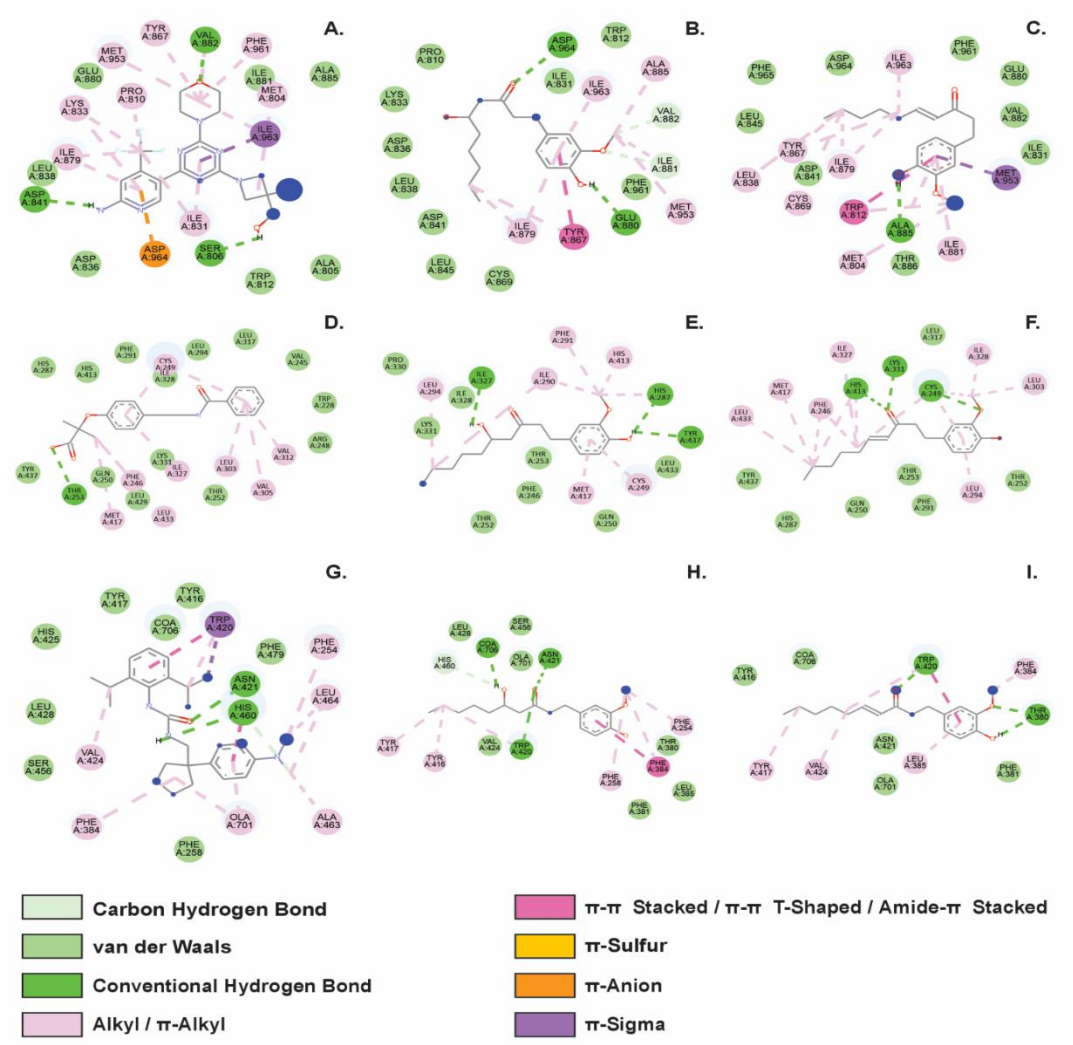

Figure 4: Protein-ligand interaction profiles of 6-gingerol and 6-shogaol with PI3K, PPAR-δ, and LOX-1

Panels A–C depict the molecular docking interactions between the co-crystallized ligand (PIKin3), 6-gingerol, and 6-shogaol, respectively, with PI3K. **Panels D-F** depict the interaction between Bezafibrate, 6-gingerol, and 6-shogaol, respectively, with PPAR-δ. **Panels G-I** depict the interaction between the co-crystallized ligand (BI-0115), 6-gingerol, and 6-shogaol, respectively, with LOX-1. Key interaction types are highlighted, including hydrogen bonds, van der Waals forces, and hydrophobic interactions, illustrating binding affinities and potential inhibitory effects on pathways involved in atherogenesis. Note that many similar interactions are shared between the co-crystallized ligands and 6-gingerol and 6-shogaol. These reflect the potential of both ligands to modulate lipid metabolism and inflammation via PI3K, PPAR-δ, and LOX-1.

6-Gingerol and 6-Shogaol's Predicted Potency in Inhibiting Atherogenesis

The experiment explores the possible mechanisms of preventing the progression of atherogenesis, as reported through several in vivo, in vitro, and randomized trials. Three main proteins were promising targets of 6-gingerol and 6-shogaol: PI3K, LOX-1, and PPAR-*δ*. Experimental studies and the results supported several of these proteins further identified that these proteins may be critical targets mediating the effects of 6-This study explored the mechanisms by which 6-gingerol and 6-shogaol may prevent atherogenesis, identifying PI3K, LOX-1, and PPAR-*δ* as key targets. These findings align with prior research demonstrating the compounds' inhibitory effects on PI3K-related pathways, such as the PI3K/Akt/mTOR pathway in human umbilical vein endothelial cell (HUVEC) cells [48] and the PI3K/Akt pathway in RAW 264.7 cells.[18] While PI3K inhibition has dual effects—reducing inflammation and lipid accumulation but potentially destabilizing plaques through foam cell apoptosis—early intervention may outweigh these risks.[48–52]

LOX-1 interaction with both compounds was supported by their ability to bind crucial residues similar to BI-0115. However, molecular docking cannot simulate LOX-1 tetramerization, which inhibits oxLDL binding. Notably, 6-shogaol demonstrated inhibitory effects on LOX-1 activity in HUVEC cells[53], though no similar studies exist for 6-gingerol. Further lead optimization may uncover their therapeutic potential. PPAR-*δ* showed comparable binding energy and amino acid interactions between the ligands and the co-crystallized ligand, suggesting activation potential. Previous studies reported that 6-gingerol and 6-shogaol increase PPAR-*δ* expression and exert anti-obesity effects,[54] potentially amplifying their efficacy. Conversely, 6-gingerol and 6-shogaol were less likely to bind ACAT1 effectively, with binding energies inferior to Nevanimibe. However, 6-gingerol's carbon-hydrogen bond with His460 suggests a possible inhibitory advantage.[47(p1)] Similarly, neither compound demonstrated strong binding to PPAR-*γ*, although prior studies reported 6-shogaol's ability to activate PPAR-*γ* in microglia, potentially via alternate mechanisms.[55] In summary, PI3K, LOX-1, and PPAR-*δ* emerge as primary targets mediating the effects of 6-gingerol and 6-shogaol on atherosclerosis. These compounds could potentially influence multiple proteins, deviating from the traditional single-target drug model. While molecular docking has limitations, including its inability to account for protein flexibility,[56] water-mediated interactions,[57] or tetramerization effects. These findings highlight the therapeutic promise of these

compounds for further lead optimization. Experimental validation in vitro and in vivo is necessary to confirm these results and advance their application in multi-target therapeutics.

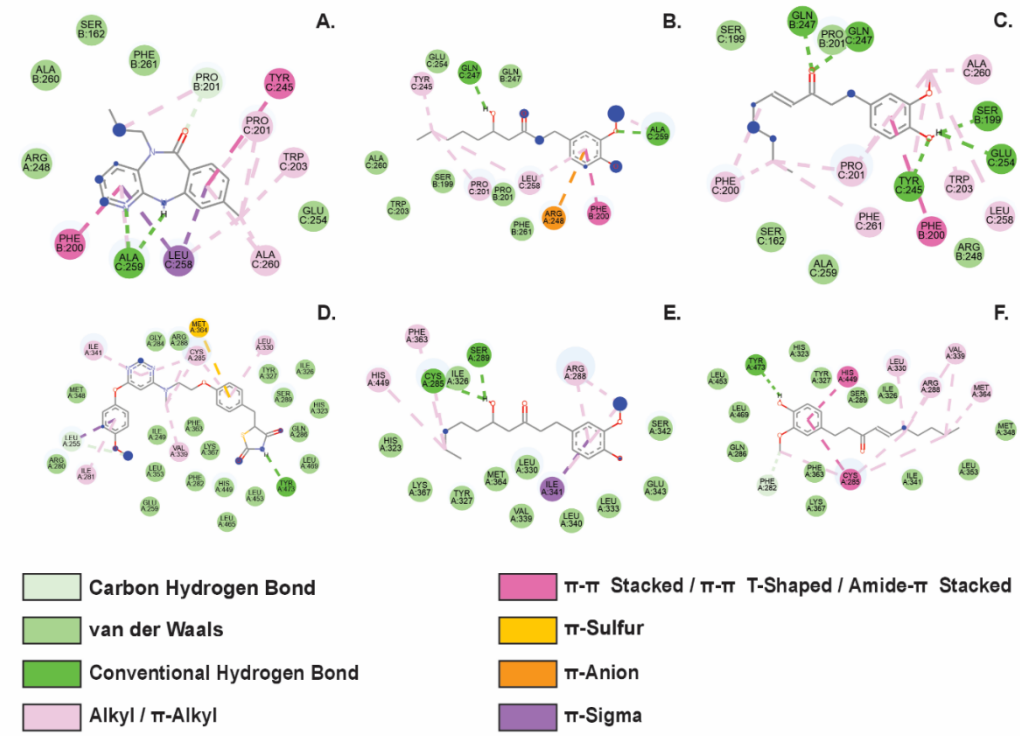

Figure 5: Protein-ligand interaction profiles of 6-gingerol and 6-shogaol with PPAR-γ and ACAT-1

Panels A-C depict the interaction between lobeglitazone, 6-gingerol, and 6-shogaol, respectively, with PPAR-γ. **Panels D-F** depict the interaction between Nevanimibe, 6-gingerol, and 6-shogaol, respectively, with ACAT1. Visualization was done through Discovery Studio. Key interaction types are highlighted, including hydrogen bonds, van der Waals forces, and hydrophobic interactions, illustrating binding affinities and potential inhibitory effects on pathways involved in atherogenesis. Note the limited binding interactions of both 6-gingerol and 6-shogaol with PPAR-γ and ACAT1 in contrast to those in Figure 4.

Conclusion

This study provides insights into the anti-atherosclerotic mechanisms of 6-gingerol and 6-shogaol, highlighting their interactions with key proteins in the lipid and atherosclerosis pathway through network pharmacology and molecular docking. Both compounds demonstrated notable binding energy with PI3K, PPAR-δ, and LOX-1, supporting their potential as modulators of atherogenesis. However, no significant interactions were observed with PPAR-γ and ACAT1. These findings suggest the potential of 6-gingerol and 6-shogaol for further lead optimization and drug development targeting atherosclerosis. Future research should combine molecular dynamics simulations, in vitro assays, and multi-omics data with network pharmacology to validate the effects and identify new targets of 6-gingerol and 6-shogaol in developing natural product-based therapeutics for atherosclerosis.

Conflict of interests

The authors declare no conflict of interests

Author Declaration

The authors declare that the work presented in this article is original and take full responsibility for any claims arising from its content.

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