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Original article

Berberine modulates cardiovascular diseases as a multitarget-mediated alkaloid with insights into its downstream signals using *in silico* prospective screening approaches

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ABSTRACT

Atherosclerosis is potentially correlated with several cardiac disorders that are greatly associated with cellular oxidative stress generation, inflammation, endothelial cells dysfunction, and many cardiovascular complications. Berberine is a natural isoquinoline alkaloid compound that widely modulates pathogenesis of atherosclerosis through its different curative potentials. This *in silico* screening study was designed to confirm the potent restorative properties of berberine chloride as a multitarget-mediated alkaloid against the CVDs and their complications through screening, identifying, visualizing, and evaluating its binding models, affinities, and interactions toward several CVDs-related targets as direct and/or indirect-mediated signals via inhibiting cellular ER stress and apoptotic signals and activating autophagy pathway. The drug-likeness properties of berberine were predicted using the computational QSAR/ADMET and Lipinski's RO5 analyses as well as *in silico* molecular docking simulations. The potent berberine-binding modes, residues-interaction patterns, and free energies of binding scores towards several CVDs-related targets were estimated using molecular docking tools. Furthermore, the pharmacokinetic properties and toxicological features of berberine were clearly determined. According to this *in silico* virtual screening study, berberine chloride could restore cardiac function and improve pathogenic features of atherosclerotic CVDs through alleviating ER stress and apoptotic signals, activating autophagy, improving insulin sensitivity, decreasing hyperglycemia and dyslipidemia, increasing intracellular RCT signaling, attenuating oxidative stress and vascular inflammation, and upregulating cellular antioxidant defenses in many cardiovascular tissues. In this *in silico* study, berberine chloride greatly modulated several potent CVDs-related targets, including SIGMAR1, GRP78, CASP3, BECN1, PIK3C3, SQSTM1/p62, LC3B, GLUT3, INSR, LDLR, LXRα, PPARγ, IL1β, IFNγ, iNOS, COX-2, MCP-1, IL10, GPx1, and SOD3.

1. Introduction

Hypercholesterolemia is mainly linked with cardiovascular diseases (CVDs), stroke, peripheral vascular inflammatory disorders, and diabetes (Ooi et al., 2018, Alia et al., 2021). In 2019, CVDs are clearly considered as a major health complication that causes 17.9 million deaths worldwide. By 2030, the expected deaths of CVDs will increase to 24 million people per year worldwide (Moghaddam et al., 2022).

Abbreviations: ABCA1, ATP-binding cassette transporterA1; ADMET, Absorption, distribution, metabolism, excretion, and toxicity; AKT1, Serine/threonine-protein kinase; AMPK, AMP-activated protein kinase; BBB, Blood-brain barrier; CASP3, Apoptosis-related cysteine peptidase; CNS, Central nervous system; CVDs, Cardiovascular diseases; CYP7A1, Cholesterol 7α-hydroxylase; ER, Endoplasmic reticulum; GPx1, Glutathione peroxidase1; HDL, High-density lipoprotein; HMGCR, 3-hydroxy-3methyl-glutaryl CoA reductase; IFNγ, Interferon γ; IL10, Interleukin 10; IL1β, Interleukin 1β; INSR, Insulin receptor; LDL-c, Low density lipoprotein-cholesterol; LDLR, Low-density lipoprotein receptor; LXR, Liver X receptor; MAMs, Mitochondrial-associated ER membranes; MAPK1, Mitogen-activated protein kinase 1; MCP-1/CCL2, Monocyte chemoattractant protein1/chemokine ligand2; MMP9, Matrix metallopeptidase 9; NFE2L2/Nrf2, Nuclear factor erythroid 2-related factor2; NOS2/iNOS, Inducible nitric oxide synthase; NR1H3/LXRα, Liver X receptor α; PCSK9, Proprotein convertase subtilisin/kexin type 9; PPARs, Peroxisome proliferator-activated receptors; PTGS2/COX-2, Prostaglandin-endoperoxide synthase 2/cyclooxygenase2; QSARs, Quantitative structure-activity relationships; RCT, reverse cholesterol transportation; RO5, Rule of five; SIGMAR1, Sigma-1 receptor; SLC2A4/GLUT4, Solute carrier family2 member4/glucose transporter4; SOD3, Superoxide dismutase3; SR-BI, Scavenger receptor B type 1; TNFα, Tumor necrosis factor α.

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Atherosclerosis, a chronic vascular inflammatory disorder, is clearly introduced as steroid plaques that result from a deposition of low density lipoprotein-cholesterol (LDL-c) on the walls of coronary arteries due to hypercholesterolemia. Furthermore, Atherosclerosis greatly develops to chestpain, myocardial infarction, ischemia, fibrosis, and several cardiac problems (Ma et al., 2021). Oxidative stress generation and inflammatory responses are greatly associated with the pathogenesis of CVDs through attenuating antioxidant defenses and accelerating LDLs oxidation (ox-LDLs), macrophage foam cells formation, monocytes adherence and migration, endothelial cells dysfunction, atherosclerotic plaques/lesions formation, plaques rupture, vascular remodeling, and atherothrombosis as well as infiltration of vascular smooth muscle cells to arterial intima (Ooi et al., 2018, Cao et al., 2021, Huwait et al., 2021, Ghatani et al., 2022). Targeting oxidative stress is an effective response to attenuate several cardiac diseases. Oxidative stress generation regulates myocardial injury, ischemia, and vascular remodeling through accelerating cardiac toxicity, cardiomyopathy, and death of cardiomyocytes. Apoptosis is a caspase3-dependent programmed cell death that causes cell shrinkage, chromatin condensation, DNA fragmentation, and formation of apoptotic bodies (Martinet and De Meyer, 2009, Shen et al., 2022). Natural products widely introduce important structural and chemical characteristics, which consider as a source of essential bioactive therapeutic-based compounds. Natural products greatly improve oxidative stress-mediated diseases such as cancer, CVDs, and neurodegenerative disorders (Ooi et al., 2018).

Berberine is an isoquinoline alkaloid compound isolated from Traditional Chinese herb *Coptis chinensis*, which introduces a broad spectrum of biological therapeutic activities to regulate the pathogenesis of CVDs. Berberine is clearly considered as an effective traditional Chinese herbal that manages several disorders such as bacterial infection, diabetes, hypertension, obesity, hyperglycemia, dyslipidemia, and atherosclerosis (Ma et al., 2021). Recent findings reported that berberine improves glucose and lipids metabolism and restores monocytes adhesion and migration, LDL-c oxidation, macrophage foam cells formation, endothelial cells dysfunction, myocardial infarction, and an accumulation of atherosclerotic lesions through alleviating oxidative stress and vascular inflammation (Xie et al., 2020, Cao et al., 2021). Berberine also inhibits formation of atherosclerosis plaques, attenuates cardiac injury, blocks pro-inflammatory cytokines expression, and reduces cardiomyocytes apoptotic signals through modulating autophagy, promoting cell proliferation, and inhibiting ER stress response and cell apoptosis (Liao et al., 2018, Cao et al., 2021).

Identification of the potential target proteins for selected known bioactive compounds clearly represents a critical step for drug design and development. Molecular docking tool is used to optimize known drugs and identify novel binders through predicting their binding modes and affinities toward their potential targets (Galati et al., 2021). In silico modeling and simulation analysis are widely used to identify, evaluate, visualize, and develop the activities of drugs-mediated downstream signaling pathways (Ayaz et al., 2020). Furthermore, molecular docking is commonly used as a computational-based drug designing tool to identify and visualize the binding models, affinities, non-covalent interactions, cavities, and grooves between drugs and their targets as well as to predict the biochemical downstream signaling of these target-ligand complexes (Azmi et al., 2021). The drug-likeness properties of berberine were predicted using computational QSAR/ADMET prediction and Lipinski's RO5 filter analyses and *in silico* molecular docking simulations. This *in silico* virtual screening study was designed to confirm the potent restorative therapeutic properties of berberine as a multitarget-mediated alkaloid against the features of CVDs and their complications (**Supplementary Fig. S1**) through visualizing and evaluating its binding models, affinities, and interactions towards several CVDs-related targets as direct and/or indirect-mediated signals via inhibiting cellular endoplasmic reticulum (ER) stress response and apoptotic signal and activating autophagic flux signaling pathway.

2. Computational methods

2.1. Biology proteins mapping simulation

The STITCH5.0 and STRINGv11.5 enrichment database websites were used at http://stitch.embl.de and http://string-db.org/ servers to construct the chemical-protein (CPI)- and protein–protein (PPI) interaction clustering networks that visualized and determined the relationships and correlations between berberine and/or several CVDs-associated overlapping targets, respectively (Szklarczyk et al., 2016, Szklarczyk et al., 2018).

2.2. In silico QSAR prediction of physicochemical properties, pharmacokinetic profiles, drug-likeness scores, and toxicity potentials of berberine

The first step was to access the PubChem server (https://pubchem.ncbi.nlm.nih.gov/) to get the canonical SMILES of berberine chloride. The second stage was to estimate its absorption, distribution, metabolism, excretion, and toxicity (ADMET) potentials. In the present *in silico* work, berberine was subjected to predict its physicochemical and molecular properties by using different ADMET predictive web tools, such as Molinspiration Cheminformatics software version 2022.08 (http://www.molinspiration.com), Cosmos, SwissADME (http://www.swissadme.ch/), ProTox-II (https://tox-new.charite.de/), and admetSAR2 (http://lmmd.ecust.edu.cn/admetsar2) servers. Physicochemical properties, lipophilicity indices, water solubility, pharmacokinetics (ADMET potentials) features, biological drug-likeness scores, and medicinal chemistry of berberine were determined.

2.3. In silico virtual screening analysis for several berberine-related CVDs targets

2.3.1. Preparation and optimization of berberine and its target proteins

Molecular docking is an important tool that indicates the conformation and orientation of small molecules (ligands) into the binding sites of their target proteins (macromolecules). Searching algorithms introduce poses (binding modes) that are ranked according to their scoring functions (Pantsar and Poso, 2018, Torres et al., 2019). For leads preparation, the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the canonical SMILES of berberine chloride (MF:$C_{20}H_{18}ClNO_4$, MW:371.8 g/mol, CID:12456). The ACD labs ChemSketch program version 12.0 was used to generate, clean, and optimize the chemical structure of berberine chloride that was saved as a MDL MOL-file format (**Supplementary** Fig. S5**a**). Furthermore, the OpenBabel GUI v2.3.2 software was used to minimize energy of berberine chloride that was converted from MDL MOL- to PDB-file format (.pdb) (http://openbabel.org/wiki/Main_Page).

For proteins preparation, the x-ray crystallographical structures of several CVDs-related target proteins were as the following: Homo sapiens SIGMAR1 (membrane protein, PDB ID:5HK1, 2.51 Å) (Schmidt et al., 2016); GRP78 (70 kDa heat shock protein 5/BIP) ATPase domain (chaperone, PDB ID:5EVZ, 1.85 Å) (Hughes et al., 2016); Caspase-3 (apopain/cpp32, hydrolase, PDB ID:1GFW, 2.80 Å) (Lee et al., 2000); BECN1 evolutionarily conserved domain (membrane protein, PDB ID:4DDP, 1.55 Å) (Huang et al., 2012); PIK3C3 (transferase, PDB ID:3LS8, 2.25 Å); p62/SQSTM1 ZZ domain (signaling protein, PDB ID:5YP7, 1.42 Å) (Kwon et al., 2018); mutNLIR_LC3B (protein binding, PDB ID:5XAE, 2.00 Å) (Kwon et al., 2017); GLUT3 (transport protein, PDB ID:7SPS, 2.30 Å) (Wang et al., 2022); INSR tyrosine kinase (transferase, PDB ID:5E1S, 2.26 Å) (Sanderson et al., 2015); LDL Receptor YWTD-EGF Domain Pair (lipid transport, PDB ID:1IJQ, 1.50 Å) (Jeon et al., 2001); LXRα LBD (nuclear receptor/transcription factor, PDB ID:3IPS, 2.26 Å) (Fradera et al., 2010); PPARγ LBD (nuclear receptor/transcription factor, PDB ID:2ZNO, 2.40 Å) (Oyama et al., 2009); IL1β (cytokine, PDB ID:1HIB, 2.40 Å) (Camacho et al., 1993); IFNγ (immune

system, PDB ID:1FG9, 2.90 Å) (Thiel et al., 2000); iNOS (oxidoreductase, PDB ID:1NSI, 2.55 Å) (Li et al., 1999); COX-2 (oxidoreductase, PDB ID:5KIR, 2.70 Å) (Orlando and Malkowski, 2016); MCP-1 (chemoattractant, PDB ID:1DOK, 1.85 Å) (Lubkowski et al., 1997); IL10 (cytokine, PDB ID:1ILK, 1.80 Å) (Zdanov et al., 1995); GPx1 (oxidoreductase, PDB ID:2F8A, 1.50 Å); SOD3 (Cu-Zn oxidoreductase, PDB ID:2JLP, 1.70 Å) (Antonyuk et al., 2009). These CVDs-related targets were retrieved from the RCSB-PDB database (http://www.rcsb.org/) as PDB-file formats. The PDB structures have various missing information's on specific connectivity, along with the bond orders and formal changes. For energy minimization, the target proteins were processed with empirical force fields using Swiss-PDBViewer v4.1.0 program to generate the protein structure's stable conformations. Finally, the quality of the modeled proteins was confirmed by using the Ramachandran plot study through PROCHECK/PDBsum database server (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) (Laskowski et al., 1993). For the selected CVDs-related targets, the Ramachandran plots and their statistics were performed (**Supplementary Figs. S2 and S3**). For the predicted models of the CVDs-related targets, the Ramachandran plot statistics suggest that 93.8, 93.7, 83.6, 90.6, 93.9, 86.8, 95.3, 96.1, 93.1, 83.1, 93.6, 87.7, 80.8, 74.6, 87.4, 88.3, 93.2, 95.1, 91.1, and 91.7 % of the SIGMAR1, GRP78, CASP3, BECN1, PIK3C3, p62, LC3B, GLUT3, INSR, LDLR, LXRα, PPARγ, IL1β, IFNγ, iNOS, COX-2, MCP-1, IL10, GPX1, and SOD3 residues, respectively are presented in the most favoured regions (**Supplementary Fig. S3**).

2.3.2. Molecular docking simulation analysis (pre-processing and optimizing)

Molecular docking was performed using AutoDock v4.2.6 software (Scripps Research Institute, San Diego, CA, USA) that used to generate the estimated free energy of binding (kcal/mol) and effective concentration (EC50) of berberine towards their CVDs-related target proteins. For optimization, the structure of ligand (berberine chloride) was detected and chosen torsion roots, which were saved as a PDBQT-file format (.pdbqt). For proteins optimization, water molecules, heteroatoms, and complex moieties were removed, and polar hydrogen atoms and Kollman and gasteiger charges were added and saved as PDBQT-file formats (.pdbqt).

2.3.3. Protein grid maps generation and running of AutoGrid4 and AutoDock4

For definition of the binding sites, the grid boxes were centered on macromolecules with 0.375 Å spacing, center grid box values X = -5.173, Y = 34.515, and Z = -30.241, and npts 200 × 200 × 200 as a number of points through X-, Y-, and Z-dimensions. For the best docking conformation, Lamarckian Genetic Algorithm (GA) was applied in the target-drug interactions, and 10 GA runs were performed with the following factors: 150 as a size of population, 250,000 as a number of energy evaluations, and 27,000 as a number of generations. The ten conformations were clustered using a root-mean square deviation (RMSD) tolerance (rmstol) of 2.00 Å. The lowest energy conformation (binding mode) was saved as a pdb-file format. If the estimated free energy of binding is < -5 kcal/mol, it represents that the target protein has certain binding affinity toward its ligand (Gurung et al., 2016, Gaillard, 2018, Li et al., 2021, Li et al., 2023). For the ligand-interacted complexes, the elevated negative values of the binding energy are positively associated with their binding modes and docking characteristics. For the best binding modes, the estimated free energy of binding was the lowest negative values (Kumar et al., 2019).

2.3.4. Visualization of target-berberine docking complexes and determination of their intermolecular bindings and interactions

For all CVDs-related target complexes, the most favorable binding mode/pose was selected according to the lowest estimated Gibbs free energy of binding (ΔG; kcal/mol) (**Supplementary Fig. S4**). The lowest values of free energy of binding mean the highest binding affinities/scores/modes. The intermolecular interaction between the amino acid residues of the CVDs targets and berberine chloride was analyzed by visualizing the docking modes using BIOVIA Drug Discovery Studio Visualizer software. For the most favorable conformation/clustering, the free energy change (ΔG) was defined as the sum of free energy changes as van der Waals forces (vdW), electrostatic interactions (Elec), hydrogen bonding events (Hb), desolvation activity (Desolv), and torsional energetics (Kesamaru et al., 2019).

3. Results

3.1. Chemical-protein (CPI)- and protein–protein interaction (PPI) networks analysis

In the living organisms, the interactions between proteins and chemical compounds are considered as an integral part of the biological processing. STITCH5.0, a free available online server tool is used to determine and demonstrate the binding affinities and interactions between specific chemicals and/or their target proteins in an interaction network (Szklarczyk et al., 2016). In the berberine confidence view, the stronger associations were demonstrated by thicker lines/links/edges. The thickness of edges described the strength of data support (Fig. 1a**)**. The chemical-protein interactions (CPI) showed as green links, while protein–protein interactions (PPI) demonstrated as grey links. The large colored nodes represented query proteins with known 3D structures (first shell of interactors) such as AKT1, CASP3, MAPK1, LDLR, PCSK9, PTGS2/COX-2, SLC2A4/GLUT4, and MMP9 (Fig. 1a**)**. The characteristics of the berberine confidence view were as the following: 0.150 as a minimum interaction score, 20 as a maximum number of interactors/nodes, 133 as a number of edges, and 0.884 as a clustering coefficient value. According to the functional enrichment analysis (biological process/gene ontology), the berberine-associated cellular macromolecular component complexes network was showed as red-colored nodes (Fig. 1b).

The PPI network is considered as a group of target proteins that associate to regulate their cellular components, biological signaling, and molecular activities. In the PPI network, each node demonstrates protein of interest, while the number of edges between nodes demonstrates protein–protein relationships (Szklarczyk et al., 2018). In this *in silico* study, the PPI network demonstrated core potential targets of the berberine-correlated CVDs (Fig. 2a). In the full STRING PPI network, the edges indicated both direct (physical) and indirect (functional) protein associations between the selected berberine-related targets such as AKT1, CASP3, MAPK1, LDLR, PCSK9, PTGS2/COX-2, and SLC2A3/GLUT3 and other CVDs-related target proteins, including BECN1, NFE2L2/Nrf2, NR1H3/LXRα, INSR, HSPA5/GRP78/BiP, NOS2/iNOS, IL1β, IFNγ, PRKAA1/AMPKα1, PRKAA2/AMPKα2, ABCA1, ABCG1, IL10, SOD3, SIRT1, HMGCR, SIGMAR1, PPARγ, SQSTM1/p62, PIK3C3, CCL2/MCP-1, and MAP1LC3B (Fig. 2a). The filled colored nodes represented query proteins with known 3D structures (first shell of interactors). The characteristics of the PPI-related CVDs confidence view were as the following: 0.400 as a minimum interaction score, 29 as a number of interactors/nodes, 175 as a number of edges, and 0.710 as a clustering coefficient value. According to the functional enrichment analyses (biological process/gene ontology), each cellular macromolecular component complexes network was clearly colored as shown in Fig. 2b.

3.2. In silico predictive QSARs model of berberine

The biological drug-likeness scores and ADMET properties were predicted for berberine by using Cosmos, SwissADME, admetSAR2, and Molinspiration Web Servers. As a physicochemical characteristic, the topological polar surface area (TPSA) is calculated from the surfaces of drugs that are occupied by OH and NH bonds. TPSA is closely correlated with the H-bonding potentials of drugs and introduced as a good

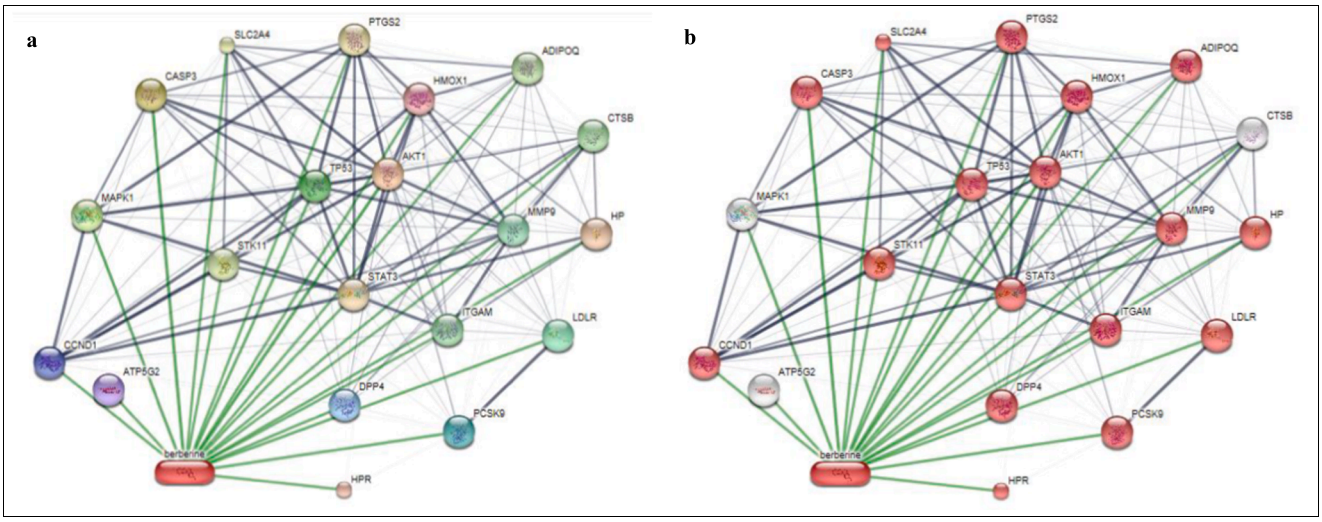

Fig. 1. **(a)** The STITCHv.5.0 berberine-correlated target proteins association network was performed. **(b)** The STITCHv.5.0 CPI network represented the berberine-associated cellular macromolecular component complexes network as red-colored nodes (http://stitch.embl.de/).

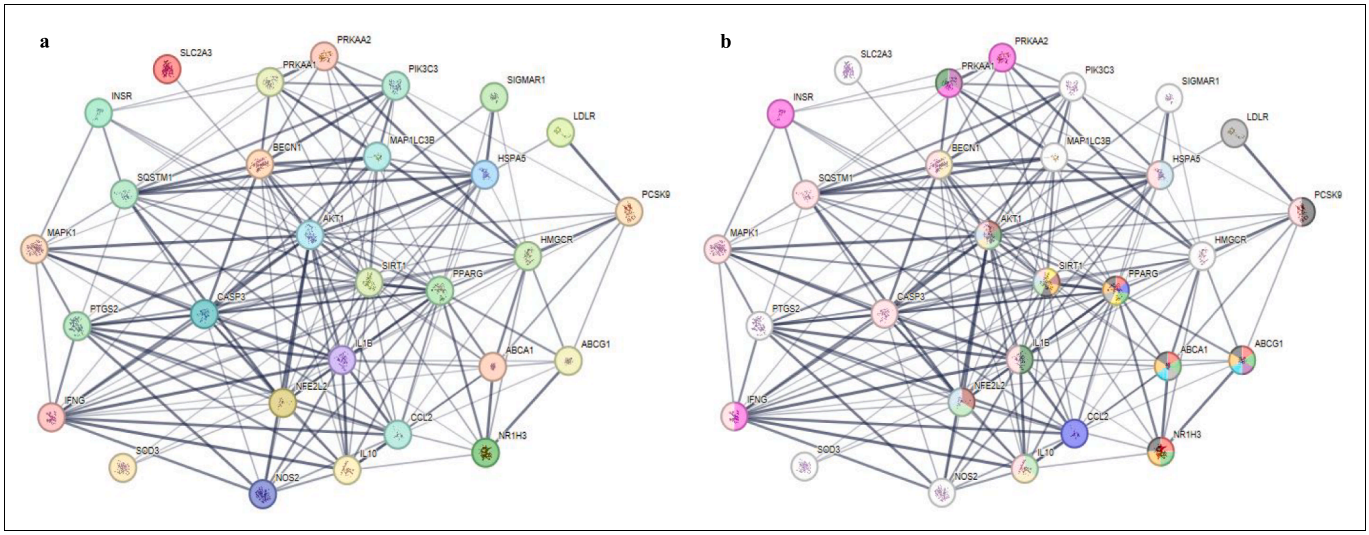

Fig. 2. **(a)** The STRINGv11.5 PPI network demonstrated core potential targets of the berberine-correlated pathogenesis of CVDs. **(b)** According to the functional enrichment analyses, the PPI networks of the different cellular macromolecular component complexes were clearly colored as the following signaling: cholesterol storage negative regulation (red), vascular endothelial cells proliferation negative regulation (blue), negative regulation of macrophages-derived foam cells differentiation (green), positive regulation of adipose tissues development (yellow), positive regulation of cholesterol biosynthetic process (purple), positive regulation of glycolytic process (pink), negative regulation of oxidative stress-induced intrinsic apoptosis (dark red) or cell death (light green), cellular response to LDL particles (grey), negative regulation of lipid catabolic process (dark green), reverse cholesterol transport (magenta), regulation of lipid storage (orange), regulation of cholesterol transport (dark grey), negative regulation of autophagy (light brown), proteasome-mediated ubiquitin-dependent protein catabolism (light blue), and apoptotic process (light pink/rose) (https://string-db.org/).

indicator for the drug bioavailability. Lead molecules with TPSA values ≥ 140 Å^2 represent poor intestinal absorption, and TPSA values < 90 Å^2 are needed to penetrate BBB and act on receptors in the CNS (Srivastava, 2021, Anandan et al., 2022, Shah et al., 2022), which clearly presented as a good physicochemical characteristic of berberine. Recent studies reported that the drug oral bioavailability is positively correlated with the rotatable bond count ≤ 10 and TPSA ≤ 140 Å^2 (Ayaz et al., 2020, Anandan et al., 2022), which clearly observed and confirmed in this *in silico* study of berberine. As physicochemical characteristics, rotatable bond count and TPSA value of berberine were 2 and 40.8\AA^2 (20 \AA^2< TPSA < 130\AA^2), respectively. For the lipophilicity indices, the values of log $P_{o/w}< 5$ represent an increasing in the affinities of drugs towards the aqueous phase, while the values of log $P_{o/w}> 5$ introduce limitations in the drug penetration and its absorption through the biomembranes (Srivastava, 2021). **Supplementary Table S1 and Fig. S5**b demonstrated characteristics of the SwissADME graphical bioavailability radar chart of berberine that introduced its safe and favorable size, lipophilicity, polarity, insolubility, insaturation, and flexibility (http://www.swissadme.ch/). Furthermore, **Supplementary Fig. S5c and d** showed the 3D geometrical mapping form for lipophilicity (miLogP) and polarity (TPSA) of berberine (www.molinspiration.com).

The values of log *Kp* > 2.5 represent low skin permeability of the proposed drugs (Vijayakumar et al., 2022). The skin permeation log *Kp* (cm/s) value of berberine was − 5.43. As pharmacokinetic properties, berberine has moderate water solubility and human oral bioavailability and high human GI absorption, plasma protein binding, glucocorticoids and androgens receptor binding, skin, and Caco-2 permeability

potentials as well as highly penetrates BBB and acts on receptors in the CNS (Supplementary Table S1). According to the Lipinski's RO5, the molecular weight (MW) < 500, the calculated octanol/water partition coefficient (log*P*) < 5, the number of H-bond donors (HBD) (number of OH and NH bonds) < 5, the number of H-bond acceptors (HBA) (number of N and O atoms) < 10, and the value of TPSA ~ 40\AA^2 represent that the proposed drugs will orally become active and good bioavailable (Lipinski, 2004, Srivastava, 2021, Shah et al., 2022), which clearly observed as physicochemical characteristics of berberine. Physicochemical properties, lipophilicity indices, and drug-likeness scores of berberine such as MW, HBD, HBA, TPSA, log *P*, and violations of Lipinski's RO5 were determined to evaluate its biologically active score (Supplementary Table S1).

As toxicological features, berberine activities are negatively correlated with Ames mutagenesis, carcinogenicity, eye corrosion and irritation, hepatotoxicity, nephrotoxicity, toxicity of PPARγ or Nrf2 signaling pathways, and skin sensitisation (Supplementary Table S1).

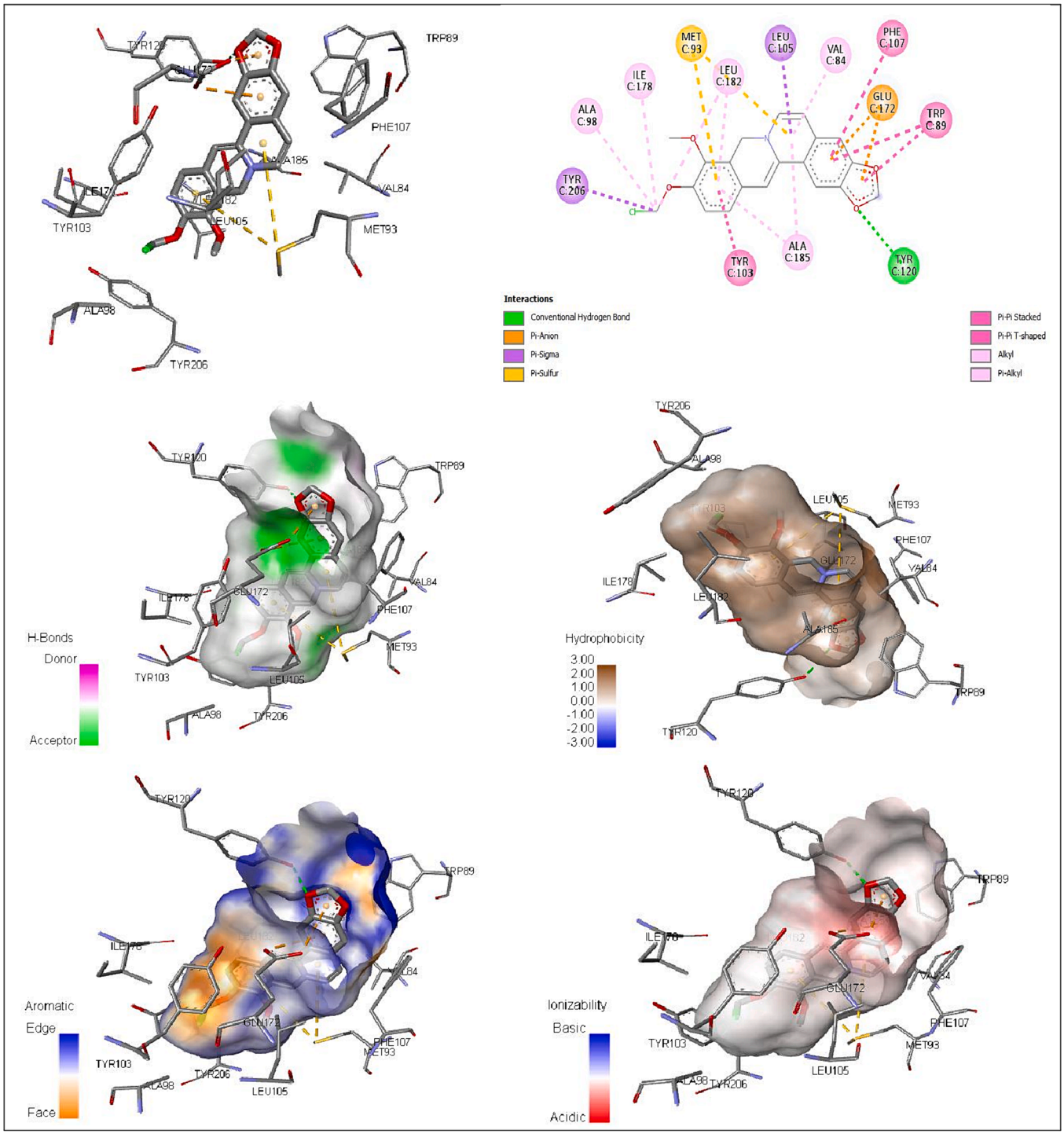

Fig. 3. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the SIGMAR1-berberine interacted form show active key residues of SIGMAR1 using BIOVIA drug discovery studio visualizer software.

3.3. *In silico structure-based virtual screening modelling of selected CVDs-related targets (berberine targets)*

Figs. 3-10 **and Supplementary Figs. S6 and S7** clearly demonstrated diversity of the interactions between berberine and active amino acid residues of its selected CVDs-related targets as H-bonding (conventional; carbon; π-Donor), electrostatic (π-Cation; π-Anion; π-Sulfur), and hydrophobic (π-π Stacked; π-π T-Shaped; Alkyl; π-Alkyl; π-σ; Amide-π Stacked) interactions. According to the scoring functions, the strength of the binding affinities/modes is greatly associated with the intermolecular interactions between the ligands and their target proteins as classical and/or non-classical H-bonds, electrostatic, and hydrophobic interactions (Pantsar and Poso, 2018), which confirmed this *in silico* findings and results. Figs. 3-10 also showed the structure-based (SB)-pharmacophore modelling characteristics (HBA, HBD, hydrophobicity, aromaticity, and ionizability) for certain CVDs targets-berberine complexes such as SIGMAR1, GRP78, CASP3, BECN1, SQSTM1/p62, LDLR, LXRα, IFNγ, and MCP-1. **Supplementary Table S2** demonstrated the binding scores (kcal/mol), binding affinities (Ki), and the stability (RMSD-tolerance) of the CVDs targets-berberine complexes. The RMSD cutoff values < 2.00 Å represent the favoured and stable ligand-target conformations (Alamri et al., 2021), which clearly observed in the rmstol values of the berberine-CVDs targets clustering (**Supplementary Table S2**). The properties of the H-bonding interactions between berberine and its selected CVDs-related target proteins as H-bond interaction order, HBD, HBA, and H-bond distance (Å) were widely reported in **Supplementary Table S3**. The H-bonding potentially enhances the binding affinities/modes/specificities of ligands towards their targets, which greatly participates with drug design development to target certain chemical and biological processes, molecular recognition, and biological activities (Anandan et al., 2022).

4. Discussion

Bioinformatics analysis is a powerful biological area that associates the computational-based methods to evaluate the biological processing and provide some accurate predictions for several true *in vitro* and *in vivo* studies and clinical trials (Moradi et al., 2022). Molecular docking is a computational approach used to identify, visualize, determine, and analyze the suitable non-bounded intermolecular interactions of a lead molecule as an activator and/or inhibitor with a specific protein of interest in a complex form (Azmi et al., 2021). Molecular docking analysis

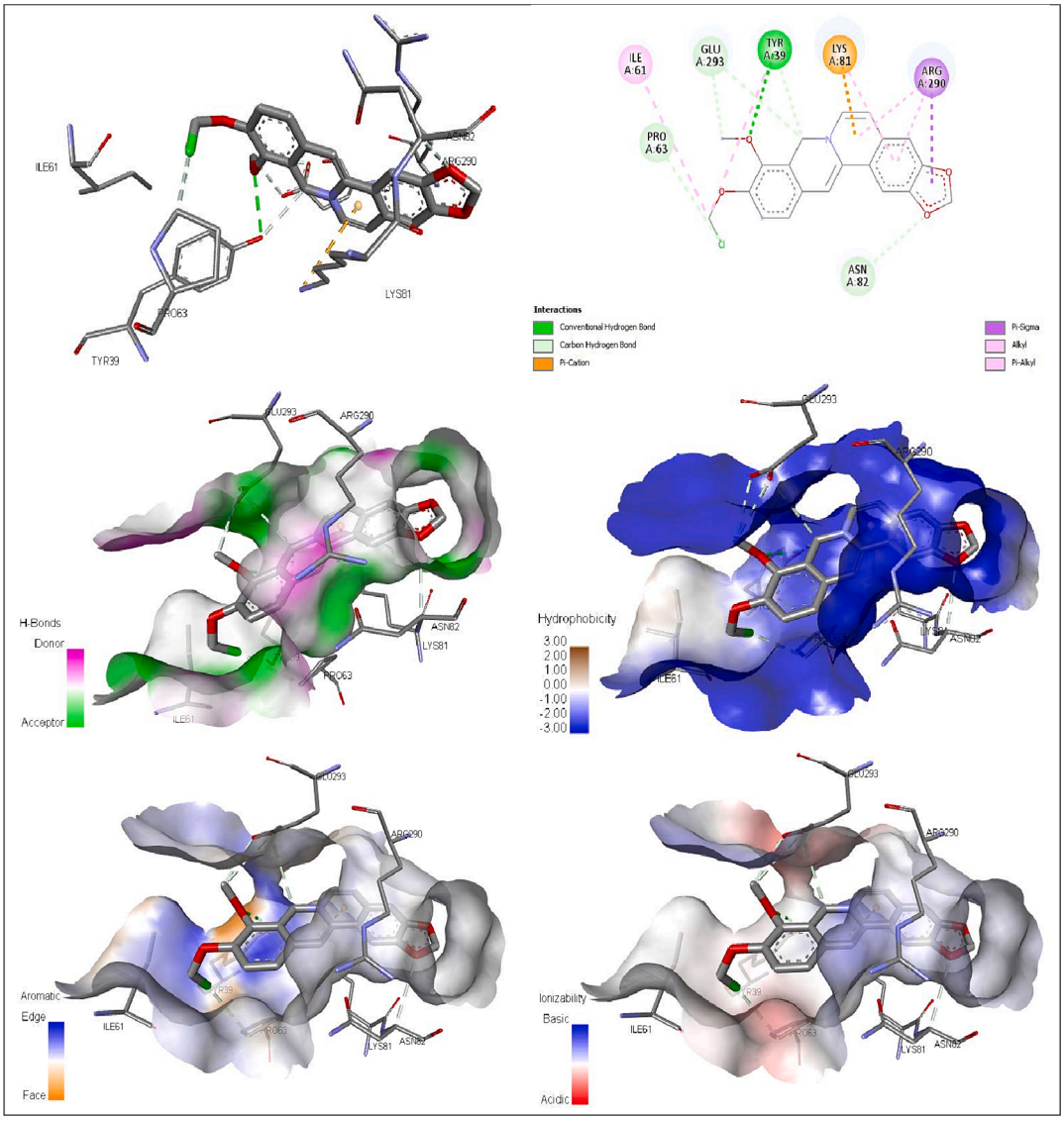

Fig. 4. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the GRP78-berberine interacted form show active key residues of GRP78 using BIOVIA drug discovery studio visualizer software.

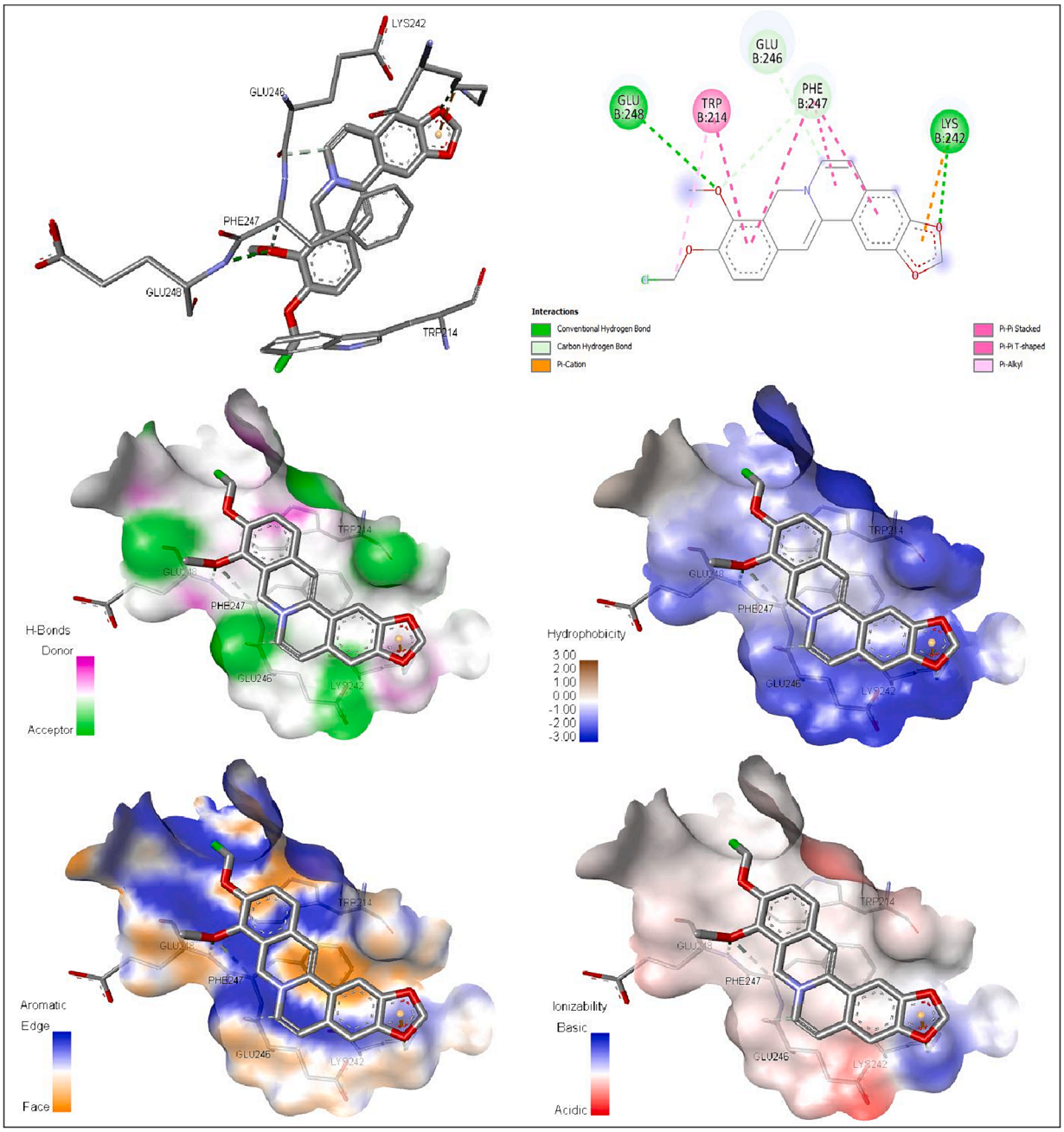

Fig. 5. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the CASP3-berberine interacted form show active key residues of CASP3 using BIOVIA drug discovery studio visualizer software.

determines and evaluates the conformation and orientation of ligands into the binding sites of their targets. Searching algorithms generate conformations that are ranked according to their scoring functions (Pantsar and Poso, 2018, Torres et al., 2019). Furthermore, molecular docking predicts the biological activities, affinities, and modes of leads towards specific targets, signals, and mechanisms (Azmi et al., 2021). In this investigation, the *in silico* virtual screening analysis was carried out to visualize and analyze the docking modes/scores of berberine chloride towards many selected CVDs-related targets, including SIGMAR1, GRP78, CASP3, BECN1, PIK3C3, SQSTM1/p62, LC3B, GLUT3, INSR, LDLR, LXRα, PPARγ, IL1β, IFNγ, iNOS, COX-2, MCP-1, IL10, GPx1, and SOD3. This *in silico* study also demonstrated the binding modes, affinities, scores, and intermolecular interactions between selected targets-mediated pathogenesis of CVDs and berberine chloride, which potentiated the biological curative activities of berberine as an anti-diabetic, anti-obesity, hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-apoptotic agent.

Atherosclerotic CVDs are resulted from deposition and retention of atherosclerotic plaques, monocytes adhesion and migration, and macrophage foam cells formation in the walls of coronary arteries that restrict blood flow and cause tissue hypoxia. Atherosclerotic CVDs is also characterized by a low-grade, persistent, and chronic inflammatory features (Xie et al., 2020, Wang et al., 2023). Sigma-1 receptor (SIGMAR1) is a chaperone transmembrane receptor presented at the mitochondrial-associated ER membranes. Recent findings confirm the beneficial properties of SIGMAR1 activation on the cellular ER stress response, autophagic flux signaling, reactive oxygen species clearance, antioxidant defenses, and the stability of structure and function of the ER, mitochondria, and numerous intracellular survival pathways (Zhemkov et al., 2021). Previous study reported that the receptor

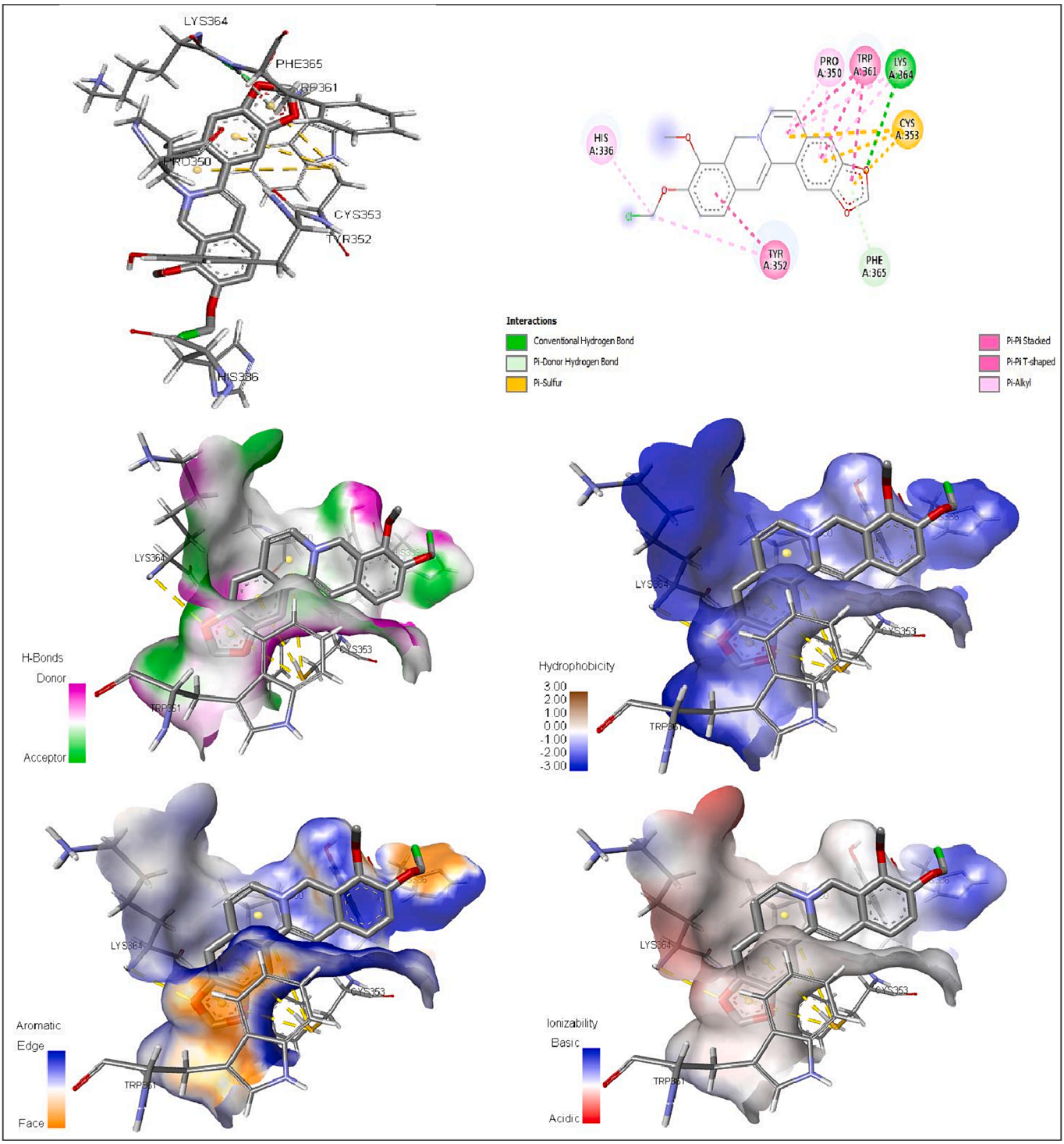

Fig. 6. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the BECN1-berberine interacted form show active key residues of BECN1 using BIOVIA drug discovery studio visualizer software.

Glu172 residue introduces charge-charge interactions with SIGMAR1 ligands, and greatly represents an essential residue to bind the co-crystallized ligands, which clearly observed in this study as two π-Anion electrostatic interactions with berberine chloride. As a SB-pharmacophore model, the SIGMAR1 binding pocket is highly hydrophobic, which clearly observed in our findings (Schmidt et al., 2016). As binding pocket residues, Val84, Trp89, Met93, Leu95, Leu105, Phe107, Ile124, Trp164, Leu182, and Tyr103 residues introduce diversity in the hydrophobic interactions with SIGMAR1 ligands, which clearly confirmed using berberine chloride as a SIGMAR1 ligand (Schmidt et al., 2016).

Dysfunction of autophagy and disturbance of carbohydrates, lipids, and proteins homeostasis are greatly correlated with the liver diseases, cancer, metabolic disorders, neurodegeneration, and the pathogenesis of atherosclerotic CVDs. The modulation of autophagy, a clearance process, has become one curative pharmacological mechanism to target the pathogenesis of CVDs (Mei et al., 2015). To maintain cellular homeostasis, Autophagy is a bulk-catabolic process that degrades their

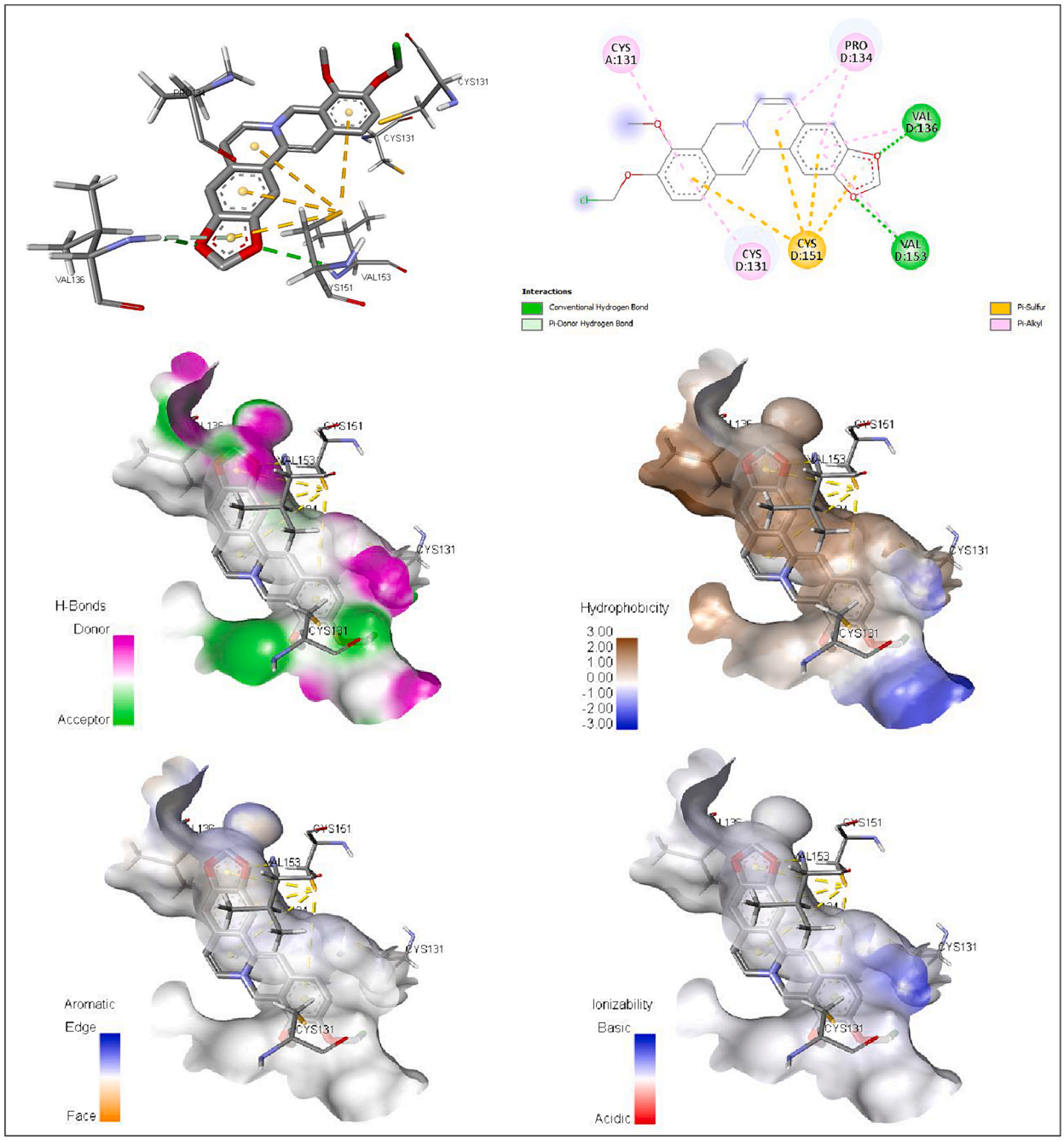

Fig. 7. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the SQSTM1/p62-berberine interacted form show active key residues of SQSTM1/p62 using BIOVIA drug discovery studio visualizer software.

engulfed and misfolded proteins and damaged dysfunctional organelles and recycles sugars, fatty acids, and amino acids as building blocks (Marzetti et al., 2013). Tissue homeostasis represents a strict balance between the cell proliferation, differentiation, and the cell death. Apoptosis is a programmed cell death mechanism that allows abnormal dysfunctional cells to commitsuicide. The apoptotic process is regulated as intrinsic mitochondrial-mediated- and extrinsic death receptor-mediated signaling (Ramos et al., 2019). Chronic ER stress response greatly stimulates pro-inflammatory and pro-apoptotic programs, which impairs cellular calcium and redox homeostasis and triggers processes of cellular damage and death (Aishwarya et al., 2021).

Berberine is a natural isoquinoline alkaloid isolated from Traditional Chinese herbs such as *Coptis chinensis* and *Berberis vulgaris*. Berberine is identified as a safe and curative agent to treat diabetes, hyperlipidemia, and pathogenesis of CVDs through upregulating cellular glucose and lipids metabolism, attenuating oxidative stress generation and inflammation, and inhibiting apoptotic signals (Younis et al., 2022, Wang et al., 2023). ER stress response potentiates myocardial cells apoptosis, cardiac remodeling, and heart failure. Inhibition of myocardial cells apoptosis significantly reduces cardiac remodeling and improves cardiac function. In the apoptosis processing, caspase-3 is considered as an effective downstream modulator. After myocardial infarction, the relative expression of caspase-3 in the myocardial tissues of heart failure highly increases. After myocardial infarction, berberine inhibits apoptosis of myocardial cells, reduces cardiac remodeling, improves cardiac function, and prevents heart failure through inhibiting ER stress

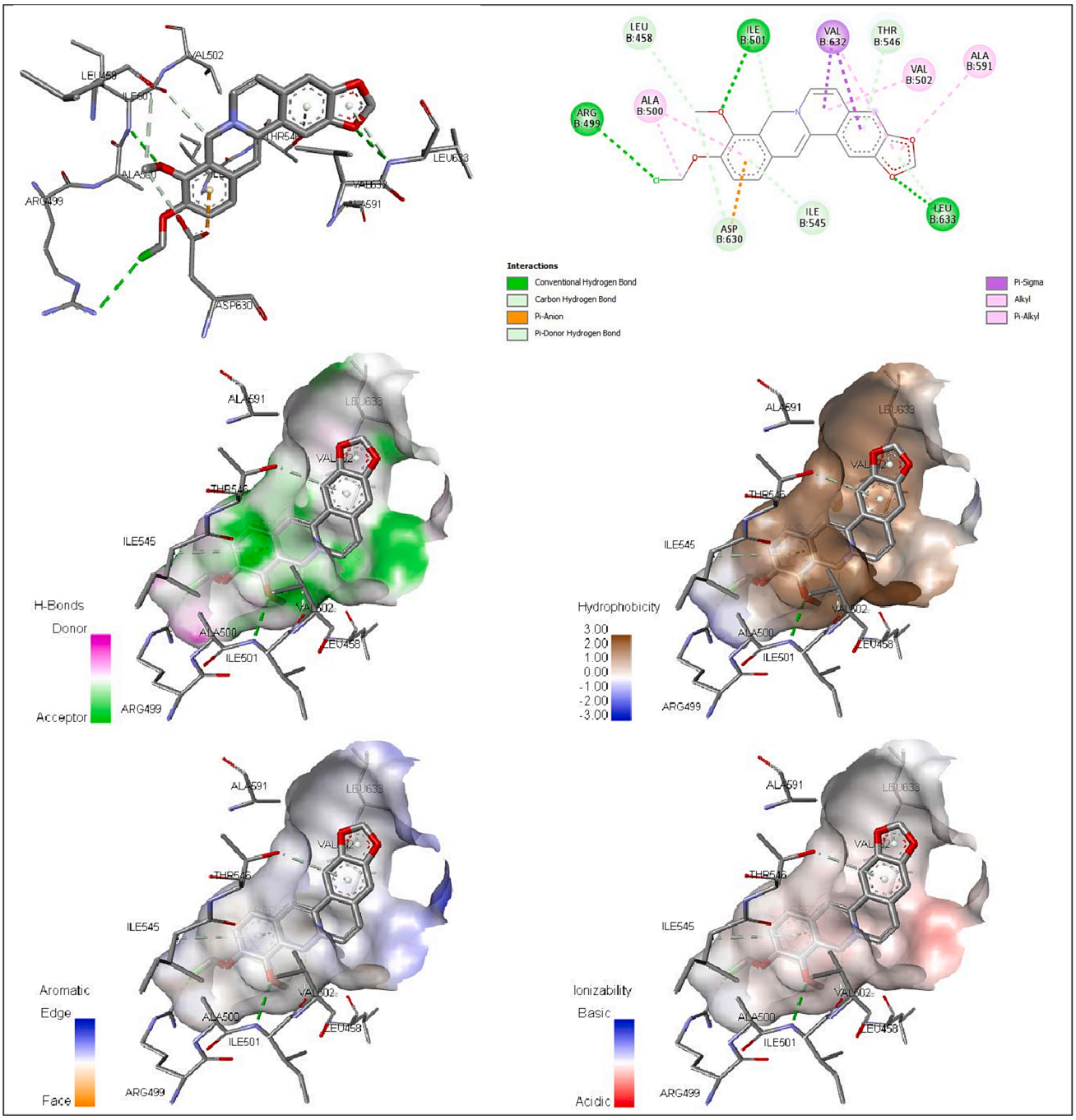

Fig. 8. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the LDLR-berberine interacted form show active key residues of LDLR using BIOVIA drug discovery studio visualizer software.

response. After myocardial infarction, berberine administration also elevates expression of anti-apoptotic Bcl-2/Bax ratio and Bcl-2 and reduces expression of pro-apoptotic Bax, CHOP, caspase-3, caspase-12, and ER stress response GRP78 (Liao et al., 2018), which clearly observed in this *in silico* screening investigation. In this *in silico* study, the $GRP78_{ATPase}$ binding pocket residues included Tyr:39A (conventional HB, carbon HB, and π-Alkyl hydrophobic) and Glu:293A (carbon HB) residues that introduced diversity in the non-covalent interactions with berberine chloride, which clearly reported as key interacted residues with other $GRP78_{ATPase}$ ligands (Hughes et al., 2016).

AMP-activated protein kinase (AMPK) is one of the most potent therapeutic targets of CVDs, which presents in the most mammalian tissues such as cardiovascular organs. In response to different cellular stress stimuli, AMPK is activated as a key regulator of energy homeostasis. Under stress response, AMPK activation downregulates biosynthetic and upregulates catabolic pathways. AMPK activators induce autophagy to maintain mitochondrial homeostasis, which improve cellular insulin sensitivity and glucose uptake and induce fatty acids and glucose oxidation (Moghaddam et al., 2022). Autophagy is a bulk self-degradation process that maintains cellular homeostasis, removes abnormal aggregates and organelles, and enhances cell survival under different stress conditions. Autophagy is widely regulated by a series of autophagy-related (ATG) genes/proteins such as ULK1/2, PIK3C3, BECN1, LC3B, p62, ATG9, and LAMP2A (Jamar et al., 2021, Park et al., 2021). Under oxidative stress responses, sequestosome-1 (SQSTM1/p62) as an autophagy adaptor protein potentially upregulates

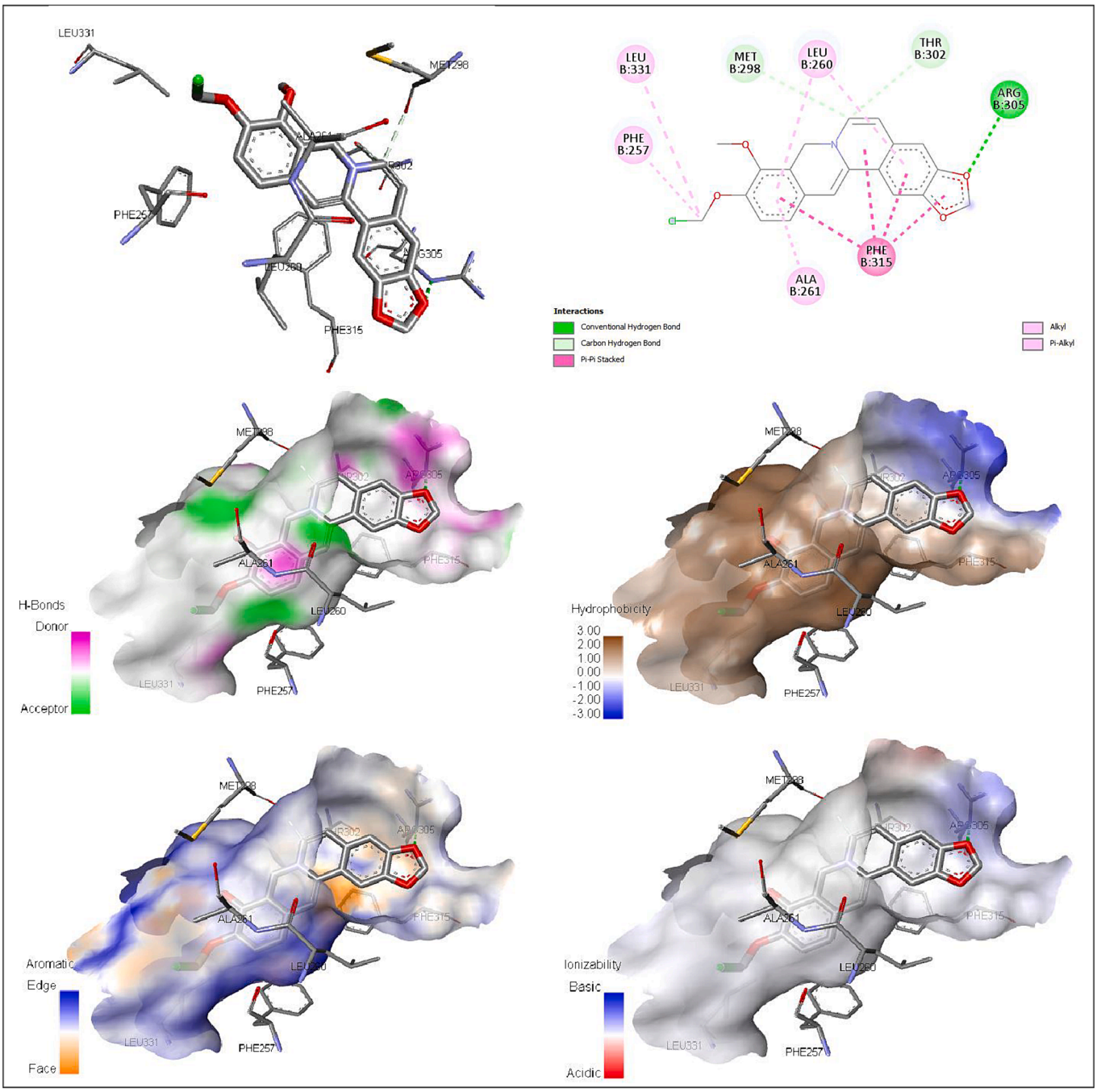

Fig. 9. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the LXRα-berberine interacted form show active key residues of LXRα using BIOVIA drug discovery studio visualizer software.

autophagic flux signaling process, reduces inflammatory modulators, eliminates oxidants and free radicals, and activates antioxidant defenses (Ooi et al., 2018). In response to cellular ox-LDL particles overloading, autophagy is upregulated in the endothelial cells or smooth muscle cells to promote cell survival (Mei et al., 2015).

Atherosclerosis is introduced as a chronic inflammation of artery walls, which results from an accumulation of LDL-c in the form of arterial lesions and plaques. Atherosclerotic plaques cause heart attacks and ischemic stroke. AMPK activators also inhibit the activities of several metabolic enzymes that accelerate the biosynthesis of cholesterol and fatty acids in skeletal muscles such as 3-hydroxy-3methyl-glutaryl CoA reductase (HMGCR). AMPK activators also greatly regulate lipids metabolism in adipose tissues (Moghaddam et al., 2022). Sirtuin1 (SIRT1) is a class III histone deacetylase potentially associated with regulation of several cellular processes including inflammation, apoptosis, oxidative stress, and antioxidant defenses to maintain cellular homeostasis. Hyperglycemia, systemic insulin resistance, and dyslipidemia potentially reduce SIRT1 downstream signals. AMPK introduces a positive feedback loop to activate SIRT1 that decreases pathogenesis of oxidative stress-induced cardiac injury and fibrosis and apoptosis of cardiomyocytes through improving the mitochondrial fatty acids β-oxidation and regulating the activity of PPARγ and p53 (Dubey et al., 2021), which widely demonstrated in the STRINGv11.5 PPI networks of the selected CVDs-related targets (Fig. 2a and b). AMPK also regulates apoptosis and autophagy of smooth muscle cells, endothelial cells, and macrophages. As an antioxidant modulator, AMPK activation reduces

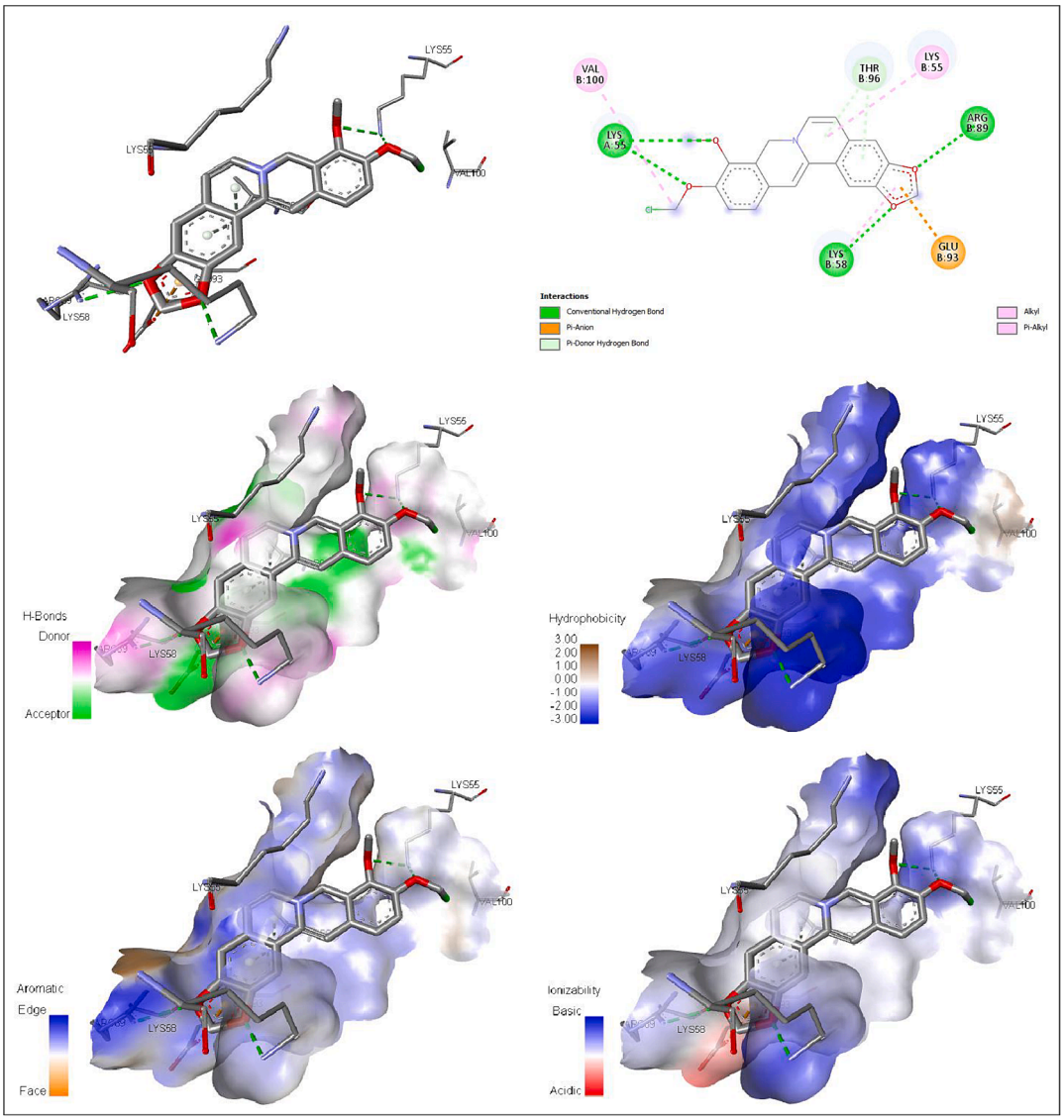

Fig. 10. The three- and two-dimensional docked structures as well as the SB-pharmacophore modelling characteristics (H-bonds donor/acceptor, hydrophobicity, aromaticity, and ionizability) of the IFNγ-berberine interacted form show active key residues of IFNγ using BIOVIA drug discovery studio visualizer software.

progression of atherosclerosis through inhibiting macrophage cells proliferation, attenuating excess ER stress-induced cardiac hypertrophy and cardiomyocytes death, and upregulating cardiomyocytes autophagy signaling (Wang et al., 2017). Dysfunction of cardiac autophagy is positively correlated with the pathogenesis of diabetic cardiomyopathy, heart failure, hypertrophic cardiomyopathy, dilated cardiomyopathy, and cardiac aging (Mei et al., 2015, Wu et al., 2021). In diabetic mice, downregulation of the AMPK activities potentially reduces autophagy flux signaling in cardiomyocytes, accelerates endothelial cells dysfunction and apoptosis, and develops vascular oxidative stress generation and inflammation (Marzetti et al., 2013). In patients with heart failure, a shift from AMPKα2 to AMPKα1 isoform is observed in the hearts. The overexpression of AMPKα2 enhances mitophagy to prevent heart failure in mice (Wu et al., 2021).

Several findings reported that berberine has cardioprotective characteristics, which restores pathogenesis of several cardiac problems and reduces ER stress-induced endothelial dysfunction as a natural AMPK/autophagy signaling activator. Berberine suppresses doxorubicin-induced cardiac injury and fibrosis via increasing AMPK response, restoring mitochondrial dysfunction, and reducing apoptotic signaling. Furthermore, berberine attenuates vascular inflammation and oxidative stress generation and reduces pathogenesis of atherosclerosis via upregulating AMPK signals. Moreover, berberine suppresses formation of the macrophage foam cells through activating the AMPK/SIRT1/PPARγ signaling processing, which clearly showed in the STITCHv.5.0 CPI- and the STRINGv11.5 PPI networks of the berberine-correlated targets and the selected CVDs-related targets, respectively (Figs. 1 and 2). In cardiomyopathic diabetic rats, berberine attenuates cardiac hypertrophy through activating the AMPK/AKT signals, inducing insulin sensitivity, promoting glucose uptake and metabolism, and restoring autophagic flux signaling (Jia et al., 2017, Zeng et al., 2019). In this *in silico* work, the STRINGv11.5 PPI networks of the selected CVDs-related targets clearly demonstrated and potentiated the curative properties of berberine towards the pathogenesis of CVDs (Fig. 2a and b).

Huang et al. study reported that Trp361 residue, an aromatic amino acid residue, is considered as a part from the aromatic finger (Phe359, Phe360, and Tyr361) of $BECN1_{ECD}$ that potentially regulates the function of $BECN1_{ECD}$ domain (initiating the omegasomes and autophagosomes formation) through its stacking hydrophobic interactions, which clearly observed in our findings as π-π Stacked and π-π T-Shaped hydrophobic interactions with berberine chloride (Huang et al., 2012). The ZZ-domain of p62 includes key residues such as Cys128, Cys131, and

Cys151 that upregulate its action towards autophagic flux signaling (Kwon et al., 2018). In this study, the key residues of the p62 ZZ-domain introduced π-Alkyl hydrophobic and π-Sulfur interactions with berberine chloride (p62 ligand). In the INSR catalytic sites, the ligands non-covalently bind to the ATP-binding pocket and form diversity in the interactions to the INSR backbone as Met1079 residue in the kinase region (Sanderson et al., 2015), which introduced in this study as Alkyl and π-Alkyl hydrophobic interactions with berberine chloride to improve the cellular energy homeostasis.

Imbalance the cholesterol biosynthesis develops hypercholesterolemia that potentially upregulates through the action of HMGCR. This enzyme is suppressed either by the high amounts of cholesterol through feedback inhibition, or hormones-induced HMGCR dephosphorylation, or by degradation of LDL-c via upregulating action of its hepatic receptors (LDLR) (Azmi et al., 2021). According to previous preclinical findings, recent *in silico* outcomes confirmed that natural alkaloids introduce anti-diabetic properties and control hyperglycemic and dyslipidemic features through inhibiting the action of HMGCR (Azmi et al., 2021). Peroxisome proliferator-activated receptors (PPARs) are considered as critical lipid sensors that play important roles in lipid homeostasis and fatty acids and glucose metabolism. PPARα is primarily expressed in brown adipose tissues, liver, heart, kidney, and skeletal muscles. It is considered as a master modulator of lipids metabolism through regulating fatty acids transportation and their mitochondrial β-oxidation. PPARγ is mainly expressed in white adipose tissues, macrophages, and vascular smooth muscles, which potentially regulates adipogenesis, lipid storage, and carbohydrates metabolism (Oyama et al., 2009, Lin et al., 2022). PPARγ is greatly used as a powerful therapeutic target in the metabolic diseases such as diabetes and CVDs (Sellami et al., 2022). Glucose homeostasis and lipids metabolism are highly regulated by ligand-activated transcription factors such as PPARs that are considered as nuclear hormone receptors. In patients with diabetes, PPARγ activation greatly improves insulin and glucose indices, increases cellular insulin sensitivity, restores lipid metabolism and adipocytes differentiation, activates antioxidant defenses, and reduces oxidative stress generation and inflammatory responses, which effects on the functions of skeletal muscles, adipocytes, and liver (Prasad et al., 2022). In the LDL receptor YWTD-EGF domain pair, receptors with YWTD (Tyr–Trp–Thr–Asp) modules/repeats/domains regulate cellular lipoproteins uptake, clearance of plasma protein complexes, and development of the extracellular signals. In each YWTD motif, the conserved Asp residues play critical roles in defining and stabilizing the propeller structure of LDLR (Jeon et al., 2001). Along with a variety of other bindings, berberine chloride introduced two carbon HB and one π-Anion electrostatic interactions with the LDLR Thr:546B and Asp:630B key residues, respectively that stabilized the structure and function of LDLR, upregulated its downstream signaling, and improved lipids homeostasis. In the LBD pocket of PPARγ, berberine chloride formed diversity of interactions with active Ser:289B, Cys:285B, Ile:326B, Met:329B, Leu:333B, Tyr:222B, Ser:332B, Leu:228B, Leu:330B, Ala:292B, and Arg:288B key residues, which introduced as a potent PPARγ modulator, upregulated reverse cholesterol transportation (RCT) signaling, improved lipids homeostasis, reduced formation of the macrophage-derived foam cells, and alleviated oxidative stress generation and vascular inflammation. Previous PPARγ agonists confirmed our observations (Oyama et al., 2009).

For cellular lipids homeostasis, RCT signaling pathway is an essential process used to remove excess cholesterol from peripheral tissues by high-density lipoprotein (HDL) particles for excretion through the liver and small intestine. Ineffective RCT signaling pathway develops hyperlipidemia, promotes monocytes adhesion and migration, accelerates macrophage foam cells formation, and increases oxidative stress generation and inflammation (Zheng et al., 2022). In this *in silico* study, we targeted selected RCT-related modulators to demonstrate the potent cardioprotective properties of berberine chloride against hyperlipidemia-mediated pathogenesis of atherosclerotic CVDs. The liver X receptor (LXR) expresses into two isoforms as LXRα and LXRβ that play an important role as reverse cholesterol transporters (Fradera et al., 2010). LXRs greatly improve lipids homeostasis, upregulate cellular cholesterol metabolism and its efflux signaling, alleviate cellular cholesterol overloading, and attenuate cellular lipids uptake/buildup in macrophages (foam cells formation). LXRs activation can also inhibit inflammatory responses. LXRs represent a promising therapeutic target for treatment of CVDs, dyslipidemia, and cancer (Sellami et al., 2022). Activation of LXR potentially enhances the expression of its downstream RCT-related genes such as ATP-binding cassette transporter (ABC) A1, ABCG1, and cholesterol 7α-hydroxylase (CYP7A1) (Zheng et al., 2022). Autophagy, a self-eating mechanism, protects against atherogenesis and vascular inflammation through upregulating lipids metabolism (lipophagy), attenuating ox-LDL particles formation, accelerating free cholesterol efflux (RCT) signaling, reducing macrophage foam cells formation, and alleviating stabilization of atherosclerotic plaques. In macrophages, disturbance of autophagy develops advanced plaques and increases apoptosis and oxidative stress (Martinet and De Meyer, 2009, Mei et al., 2015). In ApoE$^{-/-}$ mice fed with HFD, the administration with berberine upregulated RCT signaling pathway, enhanced cholesterol efflux transporters, reduced progression of the hepatic steatotic and atherosclerotic characteristics, attenuated infiltration of the macrophages, and inhibited oxidative stress generation. In ApoE$^{-/-}$ mice fed with HFD and HepG2 cells, the administration with berberine also improved the relative expression levels of RCT-mediated lipids metabolism genes and proteins, which enhanced the levels of hepatic LDLR, ABCA1, ABCG1, and SR-B1 and decreased the level of hepatic PCSK9 through upregulating and activating the MAPK/ ERK1/2 signaling pathway (Ma et al., 2021). Berberine greatly upregulates LXRα/ABCA1-dependent cholesterol efflux signaling and reduces macrophage foam cells formation through activating AMPK/SIRT1 and autophagy signals, decreasing expression of pro-inflammatory modulators (TNFα,IL1β, IL6, and iNOS), and increasing expression of anti-inflammatory mediators (IL10) (Wang et al., 2023). In the LBD pocket of LXRα nuclear receptor, berberine chloride formed diversity of interactions with active Leu:331B, Phe:257B, Met:298B, Leu:260B, Thr:302B, Arg:305B, Phe:315B, and Ala:261B key residues, which introduced as a potent LXRα regulator, upregulated RCT signaling, improved lipids homeostasis, reduced formation of the macrophage-derived foam cells, and alleviated oxidative stress generation and vascular inflammation. Previous LXRα agonists confirmed our findings (Fradera et al., 2010).

Atherosclerotic CVDs are resulted from permeation and accumulation of ox-LDLs into the arterial intima. Modified LDLs (ox-LDLs) accelerates formation of endothelial lesions, atherosclerotic plaques burden, and macrophage foam cells (Wu et al., 2022). Transcription factor Nrf2 and its downstream targets as reduced GSH, GPx, GST, GR, SOD, CAT, NQO1, and HO-1 serve as potent master regulators to upregulate the cellular antioxidant defenses. These antioxidant modulators improve vascular endothelial cells dysfunction, foam cells and atherosclerotic lesions formation, pathogenesis of CVDs, and apoptosis through upregulating the cellular cholesterol uptake receptors and its efflux transporters signaling and alleviating the levels of vascular oxidative stress and pro-inflammatory modulators such as LPO, IL6, IL1β, TNFα, IFNγ, iNOS, and MCP-1 (Ooi et al., 2018, Wu et al., 2022). As proatherogenic factors, TNFα, IL6, MCP-1/CCL2, and MMP9 potentially accelerate monocytes migration and adhesion, induce endothelial cells dysfunction, accelerate foam cells formation, disturb macrophages cholesterol efflux signaling, and develop pathogenesis features of CVDs (Huwait et al., 2021, Wang et al., 2023).

Berberine attenuates cardiac fibrosis and endothelial cells dysfunction through improving lipids metabolism and inhibiting inflammatory responses via suppressing MMP2/9 expression. In atherosclerosis, the inflammatory MAPK and PI3K-Akt signaling pathways play an important role in the pathogenesis of foam cells formation. Berberine alleviates cardiac injury, attenuates an accumulation of atherosclerotic lesions, blocks pro-inflammatory cytokines expression (IL6 and TNFα),

and reduces cardiomyocytes apoptotic signals through suppressing the inflammatory p38α MAPK and PI3K-Akt signaling pathways (Xie et al., 2020, Cao et al., 2021). Berberine restores oxidative stress and vascular inflammation-mediated atherosclerosis through upregulating AMPK processing. Berberine also inhibits formation of atherosclerosis plaques through modulating autophagy, cellular proliferation, and apoptosis. As a berberine-related target, PCSK9 is considered as a cholesterol homeostasis regulatory molecule that controls LDLR levels on the surface of hepatocytes. Under abnormal conditions, PCSK9 activation as a pro-inflammatory marker accelerates cardiovascular inflammation, develops dyslipidemia, progresses pathogenesis of CVDs, increases degradation of hepatic LDLR, and inhibits cellular LDL-c particles uptake transportation and their renal clearance (Cao et al., 2021, Ma et al., 2021). Several studies demonstrated that berberine reduces inflammation (PCSK9) and restores disturbance in the glucose and lipids metabolism to protect endothelial function and improve glucose and lipid profiles. *In vitro* findings reported that berberine reduces PCSK9 expression, improves dyslipidemic features, and suppresses macrophage foam cells formation (Cao et al., 2021), which clearly observed in this *in silico* investigations.

In the P form of MCP-1, an additional stabilization is provided by molecular interactions (H-bonds) between OH groups of active Tyr13 key residues from two MCP-1 monomers (A and B chains) (Lubkowski et al., 1997). In the present study, berberine as a potent MCP-1 antagonist disturbed this stabilization, molecular binding, and the MCP-1 active downstream signaling, which formed two conventional H-bonds (Lys:35A and Asn:14B), two π-Donor H-bonds (Tyr:13A), one π-π T-Shaped hydrophobic interaction (Tyr:13B), and several π-Alkyl hydrophobic interactions (Pro:37A and Pro:37B) with the active MCP-1 key residues. Berberine also induced stabilization of the hydrophobic residues core of the IL10 domain structure and upregulated its downstream signals through forming carbon H-bond (Leu:23A), π-Sulfur electrostatic (Met:77A), and several hydrophobic interactions (Arg:27A, Leu:26A, Leu:94A, Phe:30A, Tyr:72A, Ala:80A, and Val:76A) with the active IL10 key residues, which confirmed previous findings (Zdanov et al., 1995). As a cytokine synthesis inhibitory factor, IL10 attenuates inflammatory features through inhibiting synthesis of a number of pro-inflammatory cytokines, including IFNγ, IL2, IL3, IL6, IL8, IL12, and TNFα (Zdanov et al., 1995), which confirmed prediction of this *in silico* study. Furthermore, berberine potentiated the activity of SOD3 against oxidative stress generation and inflammation through forming non-covalent intermolecular interactions as carbon H-bonds (Val:191D and Gln:46D), π-Donor H-bond (Val:191C), and several hydrophobic interactions (Ala:55B, Cys:190C, Leu:103C, Ala:51C, Val:191D, and Leu:103D) with the active SOD3 key residues, which confirmed previous findings (Antonyuk et al., 2009).

In this study, the lower scoring functions of the berberine-correlated CVDs targets represented the strength of the target-ligand binding activities/affinities, stability of the complex binding conformation, and diversity of the non-bounded intermolecular interactions (**Supplementary Table S2**), which confirmed recent findings (Hossain et al., 2023). The number of H-bonds is greatly correlated with the complex binding mode efficiency, which represents the strongest non-covalent intermolecular interactions (C et al., 2022). The binding affinity (Ki) represents the half-maximum activity of a selected ligand towards specific protein of interest, which is used to predict and evaluate the critical role of ligands as activators and/or inhibitors in upregulation and/or down-regulation the biological functions of specific proteins of interest. Compounds with Ki values < 100 μM are considered as potent leads whereas Ki values > 100 μM are non-potent leads (C et al., 2022), which clearly showed in this *in silico* results (**Supplementary Table S2**).

5. Conclusion

In this *in silico* virtual screening study, SIGMAR1 activation was introduced as a potent berberine target that mediated pathogenesis of atherosclerotic CVDs through reducing ER stress response, inhibiting cellular damage and death, upregulating autophagy flux signaling, improving insulin sensitivity, restoring glucose and lipids metabolism, activating antioxidant defenses, and attenuating oxidative stress generation and vascular inflammation. This study explained that berberine, a SIGMAR1 stimulator, was evaluated as a potent upstream regulator for ER stress response and autophagic flux signaling, which introduced potent cardioprotective properties. Under berberine stimulation, SIGMAR1 activation upregulated downstream ER stress sensor modulators, activated autophagy, improved cellular energy homeostasis, accelerated RCT signaling, restored lipids metabolism, protein quality control, and mitochondrial function, and induced cell survival as well as attenuated cellular injury, damage, and death.

6. Future implications

In a future study, the *in vitro* and *in vivo* studies will be performed to further validate the potent curative properties of berberine against the pathogenesis of atherosclerotic CVDs. Therefore, berberine can be considered as a candidate for more pre-clinical and clinical trials as the future of clinical CVDs therapy.

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CRediT authorship contribution statement

Sanaa Almowallad: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology, Project administration, Software. **Rehab Al-Massabi:** Data curation, Visualization, Investigation, Validation, Formal analysis, Methodology, Project administration, Software.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2024.103977.

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