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RESEARCH ARTICLE

A Computational Strategy for Validation of Piperidine as Lead from Phytochemical evaluation for Antiglycation activity via AGEs-RAGE Pathway Modulation

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Abstract: This research work shows the potential of piperidine as a lead scaffold for inhibiting glycation, using a computational technique focused on the AGE-RAGE signaling pathway, a critical factor responsible for diabetic complications. Network pharmacology was applied to map overlapping gene targets between piperidine-containing phytochemicals and glycation processes. Protein-Protein Interaction (PPI) networks were subsequently constructed to identify hub genes, while biological relevance was interrogated through Gene Ontology (GO) and KEGG pathway enrichment analyses. To prove these findings, molecular docking simulations (Molegro Virtual Docker v.6.0) were carried out to determine the binding affinity of the compounds against the RAGE protein (PDB ID: 4LP5). The results indicated significant involvement in the AGE-RAGE, type II diabetes mellitus, and insulin resistance pathways, with *MTOR*, *ESR1*, *HSP90AA1*, *NFKB1*, and *STAT3* emerging as central regulatory hubs. Docking results indicated that the phytochemicals possess strong binding affinity for the human RAGE protein, surpassing the affinity of the standard reference, Pyridoxamine. Specifically, consistent hydrogen bonding and steric interactions with key residues suggest a conserved, favorable binding mode within the active site. The results from this work conclude that polyhydroxy piperidine-based phytochemicals can be promising antiglycation agents that operate via multi-pathway modulation and direct RAGE inhibition, supporting the further development of piperidine-based synthetic pharmacophores.

Keywords: Advanced glycation end products; Piperidine; Network pharmacology; Molecular docking simulation; Antiglycation

1. Introduction

In medicinal chemistry, piperidine scaffolds are widely regarded as privileged structures due to their broad therapeutic utility and versatility in drug design. This saturated six-membered nitrogen heterocycle is a core structural motif found in a vast array of natural products and clinically approved pharmaceuticals [1]. Its specific structural characteristics confer advantageous pharmacokinetic properties, including improved solubility, optimized membrane permeability, and high metabolic stability [2]. Moreover, the capacity of the piperidine ring to participate in hydrogen bonding and ionic interactions facilitates the modulation of diverse biochemical pathways [3]. Naturally occurring piperidine derivatives such as piperine, lobeline, coniine, sedamine, and 1-deoxynojirimycin (DNJ) (Figure 1) have exhibited significant antioxidant, anti-inflammatory, neuroprotective, and metabolic regulatory effects, including antiglycation activity [4–7]. Consequently, piperidine-based phytocompounds hold particular promise for addressing pathologies associated with glycation.Glycation is a complex, non-enzymatic cascade initiated by the condensation of reducing sugar carbonyls with free amino groups on biomolecules, yielding unstable Schiff bases. These intermediates undergo Amadori rearrangement to form stable ketoamine adducts, which subsequently degrade via oxidation, cyclization, and dehydration into reactive α-dicarbonyls, such as glyoxal and methylglyoxal. These potent electrophiles cross-link with proteins and lipids to generate Advanced Glycation End-products (AGEs) a heterogeneous class of stable, often fluorescent adducts implicated in the pathogenesis of numerous metabolic and age-related disorders [8]. Given the safety concerns and limited efficacy associated with current antiglycation agents, there is a critical need to identify novel therapeutic candidates, particularly those derived from biologically relevant natural sources. Network pharmacology offers a robust framework for identifying lead compounds among piperidine-based phytochemicals. This methodology combines Gene Ontology, protein-protein interaction networks, and pathway enrichment analyses to prioritize bioactive compounds by shifting from the reductionist "one-drug, one-target" paradigm to a systemic, multi-target approach. This strategy allows for the elucidation of complex mechanisms, the prediction of synergistic interactions, and the minimization of off-

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target effects [9]. Such a holistic approach significantly enhances drug discovery efficiency by identifying key targets for specific pathological conditions. Complementing this, molecular docking simulations are employed to validate the binding affinity and interaction modes of selected derivatives against the Receptor for Advanced Glycation End-products (RAGE), a central mediator of AGE signaling [10]. This dual computational technique allows the rational prioritization of lead molecules based on both mechanistic plausibility and target engagement, establishing a foundation for the future synthesis and biological evaluation of novel piperidine-based antiglycation agents.

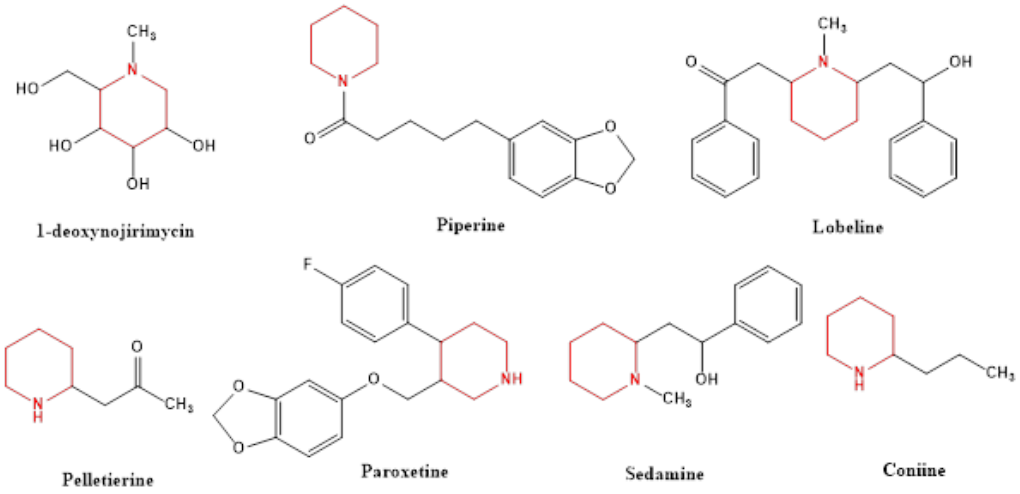

Figure 1. Structural diversity of natural piperidine-based bioactive compounds.

2. Materials and Methods

2.1. Network Pharmacology

Network pharmacology analysis was utilized to identify and visualize the complex interactions between piperidine-containing bioactive compounds and associated biological pathways. This integrative approach combines biological, chemical, and pharmacological data to elucidate the potential mechanism of action and systematically prioritize therapeutic targets.

2.2. Molecular characterization, Pharmacokinetic profiling, and Toxicity prediction of Piperidine-containing Bioactive Compounds

The chemical structure and SMILES of piperidine-containing bioactive compounds were obtained from the PubChem database (http://punchem.ncbi.nim.nih.gov/), summarized in Table 1. Molecular properties were analyzed through the DataWarrior v.6.1.0, while the drug-likeness score was predicted through the MolSoft database (http://molsoft.com/mprop) [11]. The pharmacokinetics profile of bioactive compounds was evaluated by using the SwissADME (https://www.swissadme.ch/index.php) and ADMETLab 3.0 (https://admetlab3.scbdd.com/) [12, 13]. Moreover, the ProTox-II v3.0 database (https://tox.charite.de/protox3/index.php?site=home) was used to assess the toxicological prediction of bioactive compounds [14].

Table 1. SMILES of Piperidine-Containing Bioactive Compounds.

Compound ID	Bioactive Compounds	SMILES
A1	Piperine	O=C(CCCCC1=CC=C(OCO2)C2=C1)N3CCCCC3
A2	Lobeline	O=C(C1=CC=CC=C1)CC2CCCC(CC(O)C3=CC=CC=C3) N2C
A3	Coniine	CCCC1NCCCC1
A4	Sedamine	OC(C1=CC=CC=C1) CC2N(C)CCCC2
A5	Pelletierine	CC(CC1CCCCN1)=O
A6	Paroxetine	FC(C=C1)=CC=C1C2C(COC3=CC(OCO4)=C4C=C3) CNCC2
A7	1-deoxynojirimycin	OC1C(O)C(O)C(CO)N(C)C1

2.3. Target Screening of Piperidine-containing Bioactive Compounds

The SuperPred tool (https://prediction.charite.de/index.php) was employed to predict potential molecular targets for piperidine-containing bioactive compounds [15]. A total of 465 targets were identified based on probability scores (≥65%). Although a higher score (≥90%) indicates stronger predictive confidence, a cutoff of 65% was applied to ensure broader coverage, as predicted results were further validated through molecular docking simulations. The corresponding target name of retrieved using the STRING database (https://string-db.org) for the Homo sapiens organism, by mapping the UniProt ID obtained from the SuperPred database [16].

2.4. Identification of Disease-related target genes

The GeneCard database (http://www.genecard.org/) was employed to retrieve disease-associated genes related to advanced glycation end products. By using the keyword "Glycation". The piperidine-containing bioactive compounds genes were selected based on the Gene Cards Inferred Functionality Score (GIFtS), which prioritizes genes with stronger functional relevance to disease development [17].

2.5. Screening of Common Targets of Disease

The potential targets, "preferred Genes" of the selected bioactive compounds, were obtained from the STRING 12.0 database (https://string-db.org/). The target genes associated with glycation disease and the preferred genes of the bioactive compounds were compared to identify overlapping genes using the Venny 2.1 tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html), which generated the set of common hub genes for further analysis [18].

2.6. Construction of Protein-Protein Interaction (PPI) network and prediction of Hub Genes

The intersecting genes, referred to as common genes or gene hubs, were selected for further network pharmacology analysis. The common genes identified during the previous step were imported into the STRING database, using Homo sapiens as the species and with a minimum interaction score set to medium confidence (≥0.4). Further, the PPI network was analyzed to identify the functional associations and potential hub genes. Subsequently, the PPI network, Drug-target network, and an integrated drug-target-pathway network were analyzed, and visualizations were performed using Cytoscape v3.10.3 [19]. To identify the most significant targets, the CytoHubba plugin in Cytoscape v3.10.3 was employed [20]. The top five hub genes were selected based on node degree values within the PPI network, as higher degree nodes indicate potential biological relevance. Additionally, Additionally, the crucial pathways involved in the therapeutic effects of bioactive compounds were enhanced by customizing node attributes such as color and size based on topological parameters; edge thickness was adjusted according to the interaction confidence score, which supported the critical pathways.

2.7. Gene Ontology (GO) and KEGG Pathway Enrichment Analysis

The functional analysis was performed by using the Gene Ontology (GO) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis via the SRPlot database. The GO functions are categorized into three domains: molecular function (MF), biological process (BP), and cellular components (CC), which describe the cellular environment and functional roles of the target genes. The overlapping target genes were subjected to enrichment analysis, and important parameters, including the top 10 enriched pathways, P-values, enrichment scores, and gene counts, were retrieved from the STRING database. The resulting data were subsequently imported into the SRplot tool (https://www.bioinformatics.com.cn/srplot), used to generate the KEGG enrichment bubble chart as well as GO functional annotation plots [21]. This network pharmacology approach facilitated a comprehensive understanding of the molecular mechanisms and signaling pathways through which bioactive compounds potentially exert therapeutic effects in glycation-related diseases.

2.8. Molecular docking

The two-dimensional (2D) structures of all selected bioactive compounds (A1-7) and standard drugs (Pyridoxamine) as ligands were acquired using ChemDraw 22.2.0 software. Subsequently, the 2D structures were converted to 3D confirmation using ChemDraw 3D 22.2.0, followed by the geometry optimization employing the MM2 force field. This energy minimization step is essential to ensure the ligands accept low energy. After that, the resulting 3D structures were exported into Protein Data Bank (.pdb) files [22]. To increase the reliability of the resultant docking simulations, utilizing the .pdb format for ligand coordinate archives requires precise structure establishment, ensuring appropriate bond connectivity and atomic hybridization states. The Molegro virtual Docker (MVD) v.6.0 software incorporates an algorithm that performs automated hydrogen atom addition and valence state verification upon ligand insertion [23]. A 3D crystal structure of the extracellular domain of human Receptor for Advanced glycation end products was retrieved from the Protein Data Bank (PDB ID: 4LP5) with a resolution of 3.80 Å, accessible via the RCSB portal

URL (http://www.rscb.org/pdb) [24]. The molecular docking simulation was performed by employing MVD v.6.0 software. Before commencing the docking, both the ligands and the target protein structure were prepared. MVD v.6.0 program incorporated an automated cavity detection algorithm, which was utilized to identify potential ligand-binding pockets within a grid of 30×30×30 Å³. The algorithm identified six cavities on the protein surface, among which the most prominent binding site revealed an estimated surface area of 212.48 Å³ and a volume of 72.192 Å², depicted in Figure 2. A spherical restriction with a radius of 15.0 Å, centered at coordinates values X: 667.63, Y: -30.48, and Z: -17.35, encircled this active site during the docking simulations. For the docking simulation, the MolDock scoring function was utilized with a grid resolution of 0.30 Å, and the search algorithm employed was MolDock SE. Each ligand was subjected to ten independent docking runs, with each run consisting of 1500 iterations and a population size of fifty, ensuring exploration of the identified binding site [25].

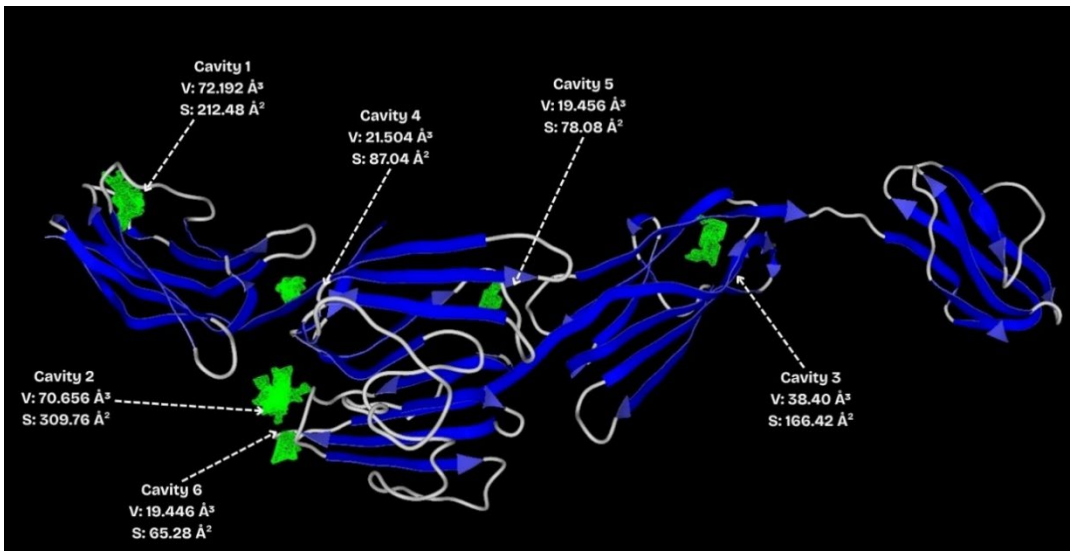

Figure 2. Binding cavities in the human receptor for advanced glycation end products structure (PDB ID: 4LP5).
The protein structure is presented in a blue cartoon model, while cavities are represented in green.

3. Results and Discussion

3.1. Network Pharmacology

Network pharmacology, an emerging discipline that focuses on the complex interactions among diseases, genes, and drug targets, has evolved into an effective research methodology for elucidating their pharmacodynamic mechanisms. In this study, we employed a network pharmacology approach to investigate the mechanism of action and potential targets associated with piperidine-containing bioactive compounds (A1-7) for treating glycation diseases. Using "Glycation" as a keyword, we retrieved 465 genes (69.8%) from the Gene Card database. These were then compared with 129 genes (19.4%) associated with the bioactive compounds, identified through mapping in the STRING 12.0 database. By comparing these gene sets with Venny 2.1, we identified 72 common genes (10.8%) involved in glycation disease, suggesting their potential role in disease modulation (Figure 3a).

The potential interaction among the target genes and the protein-protein interaction (PPI) network was constructed using the STRING 12.0 database. A medium confidence threshold (score ≥ 0.400) was applied, incorporating the 72 common genes identified from the earlier target overlap analysis. The PPI network consisted of 72 nodes representing individual target genes and 495 edges representing protein-protein interactions. The network's remarkable enrichment p-value of < 1.0e-16 and average node degree of 13.8 demonstrated the statistical significance of the genes' observed interconnections. This indicates that each protein in the network interacts with nearly all genes, emphasizing the high degree of interconnectivity (Figure 3b). Moreover, the average local clustering coefficient, which was determined to be 0.0545, indicated a significant degree of local interconnectedness among the target proteins.

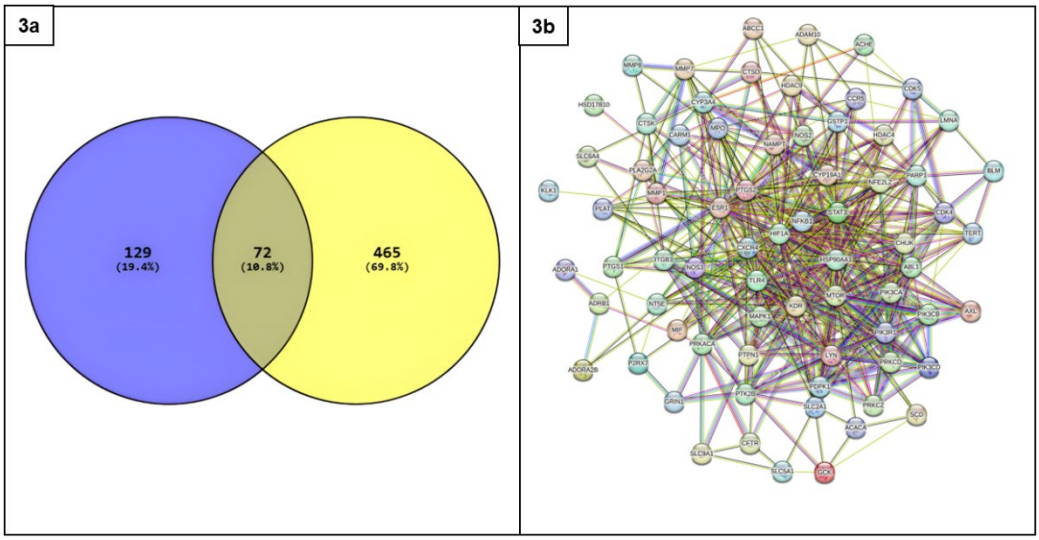

Figure 3. (3a) Venn diagram showing the overlapping genes of piperidine-based bioactive compounds between glycation-associated disease pathways. (3b) Protein–protein interaction (PPI) network of the identified common target genes.

Moreover, a compound-disease-target-pathway for bioactive compounds (A1-7) was constructed and analyzed using Cytoscape 3.6.0 software (Figure 4a). To identify the key molecular targets, hub genes were identified using data from the STRING 12.0 database and processed with the Cytohubba v0.1 tool. The top 10 hub genes were ranked based on the node degree, revealing STAT3 (84), ESR1 (80), HSP90AA1 (78), NFKB1 (76), HIF1A (74), MTOR (70), PTGS2 (68), TLR4 (66), MAPK1 (56), and KDR (54) as those that exhibited the highest degree of interaction (Figure 4b). These gene hubs may play critical roles in the molecular mechanism of underlying glycation disease and could serve as potential therapeutic targets for drug development. The STAT3 protein serves as vital to decreasing glycation-related complications because it modulates metabolic signaling, inflammatory responses, and redox homeostasis. When STAT3 is activated, antioxidant enzymes, including catalase and superoxide dismutase (SOD), are increased, which lowers reactive oxygen species (ROS) and the subsequent generation of AGE (advanced glycation end product) [26]. Additionally, STAT3 controls anti-inflammatory cytokines such as interleukin-10 (IL-10), which inhibits the AGE–RAGE axis-induced pro-inflammatory signaling. Makeover, ESR1 decreases hyperglycemia by enhancing insulin sensitivity and modulating the expression of anti-inflammatory mediators, reducing the quantity of glucose accessible to non-enzymatic glycation [27]. At the molecular level, this controlling function inhibits glycation-associated oxidative and inflammatory damage and prevents the production of advanced glycation end products (AGEs).

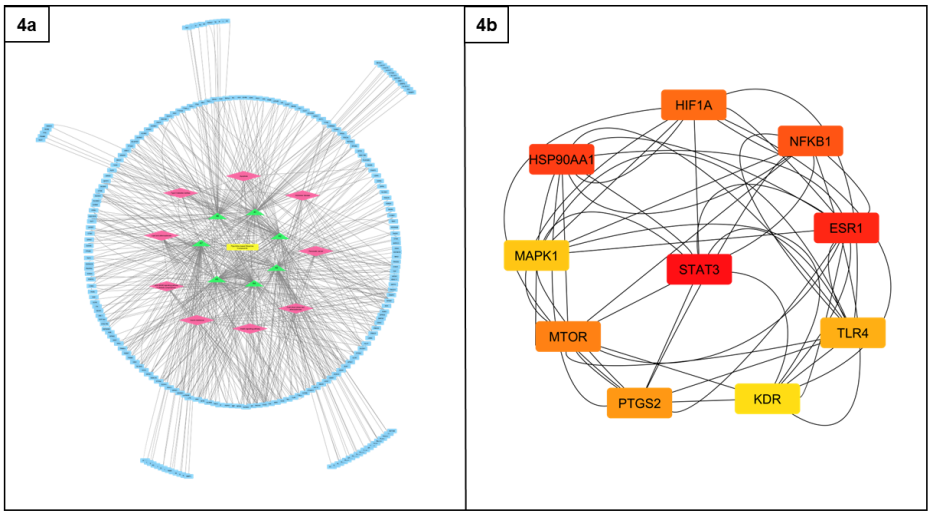

Figure 4. (4a) Compound-disease-target-pathway network of piperidine-based bioactive compounds.
The yellow square represents the piperidine-based lead compound, the green triangle node represents bioactive compounds, and the pink diamond node represents the pathway associated with core targets. (4b) The top 10 hub genes identified using the CytoHubba 0.1 plugin.

The Gene Ontology analysis of piperidine-based bioactive compounds (A1-7) identified a total of 547 biological processes (BP), 83 cellular components (CC), and 78 molecular functions (MF) with an adjusted p-value < 0.05. The top six enriched pathways of GO were selected based on the number of associated genes illustrated in Figure 5a. Among the biological processes, the pathways were primarily associated with the prevention of the glycation process, including biological regulation (GO:0065007), Cellular process (GO:0009987), Regulation of biological process (GO:0050789), Metabolic process (GO:0008152), Primary metabolic process (GO:0044238), and Cellular metabolic process (GO:0044237), etc. The biological regulation pathway identified 71 observed genes out of 12,385 background genes, including CFTR, HSD17B10, MIF, MAPK1, PLAT, NAMPT, GCK, MPO, NFKB1, CTSD, MMP8, NT5E, CDK4, MMP7, ADAM10, SLC6A4, KDR, PIK3CA, SLC9A1, HDAC4, STAT3, CTSK, CCR5, NOS3, AXL, KLK1, ACHE, ADORA2B, TERT, PRKACA, MMP1, CARM1, NOS2, P2RX7, PRKCD, HSP90AA1, PDPK1, BLM, MTOR, PTGS1, PARP1, ADORA1, PTGS2, LMNA, ADRB1, SCD, CHUK, GRIN1, PTPN1, ABL1, TLR4, PIK3CD, PRKCZ, CYP19A1, NFE2L2, PTK2B, GSTP1, ABCC1, PLA2G2A, CXCR4, ESR1, HDAC9, SLC2A1, CDK5, PIK3R1, LYN, HIF1A, ITGB3, ACACA, CYP3A4, and PIK3CB.

The analysis demonstrated a significant strength of 0.20 with a false discovery rate (FDR) of 2.51E-11, indicating a strong association between these genes and cellular processes relevant to the disease mechanism. The biological regulation pathway mitigates glycation primarily by controlling the concentration and reactivity of electrophilic carbonyl compounds, such as reducing sugars and reactive dicarbonyl intermediates, such as methylglyoxal (MGO), which non-enzymatically react with nucleophilic amino groups of proteins, forming Schiff bases and Amadori products. The detoxification of methylglyoxal (MGO), a reactive dicarbonyl that promotes glycation, depends on the glyoxalase system, made up of glyoxalase I and II. Glyoxalase I converts the MGO-glutathione hemithioacetal into S-D-lactoylglutathione, while glyoxalase II hydrolyzes it to D-lactate, regenerating glutathione [28]. This process prevents methylglyoxal from reacting with an amino group of protein (e.g., lysine residues), thereby inhibiting the formation of Schiff bases and Amadori products and the accumulation of AGEs. Additionally, the cellular antioxidants are pivotal in the prevention of the formation of AGEs by neutralizing reactive oxygen species (ROS), which accelerate glycation via the oxidative degradation of dicarbonyl compounds such as MGO, GO, etc. Cellular antioxidants such as glutathione (GSH), vitamin C (ascorbic acid), and the enzyme superoxide dismutase (SOD) mitigate this oxidative stress by scavenging ROS [29]. However, glycation can be prevented by cellular metabolic processes that restrict the accessibility of carbonyl compounds and reactive sugars via regulated pathways. Glucose is rapidly phosphorylated by hexokinase, reducing free glucose available for glycation.

The glyoxalase system detoxifies reactive dicarbonyls like methylglyoxal using glutathione. NADPH levels are also maintained by the pentose phosphate pathway, which supports antioxidant systems that reduce oxidative stress and prevent oxidative processes that contribute to the development of AGE [30]. These metabolic regulators chemically limit the substrates and circumstances required for non-enzymatic glycation. The analysis of the cellular component revealed that the identified biological pathways predominantly occurred in the Membrane-bounded organelle (GO:0043227), Intracellular anatomical structure (GO:0005622), Cytoplasm (GO:0005737), Cell periphery (GO:0071944), Nucleus (GO:0005634), Protein-containing complex (GO:0032991), etc. Notably, six terms are associated with the membrane-bound organelle that displayed the lowest false discovery rate (FDR) of 3.21E-06, indicating their significant involvement in the disease modulation. The analysis identified 68 genes against 13,188 background genes with a strength of 0.15: CFTR, HSD17B10, MIF, MAPK1, PLAT, NAMPT, GCK, MPO, NFKB1, CTSD, MMP8, NT5E, CDK4, MMP7, ADAM10, SLC6A4, KDR, SLC9A1, HDAC4, STAT3, SLC5A1, CTSK, CCR5, NOS3, AXL, KLK1, ACHE, TERT, PRKACA, CARM1, NOS2, P2RX7, PRKCD, HSP90AA1, PDPK1, BLM, MTOR, PTGS1, PARP1, ADORA1, PTGS2, LMNA, ADRB1, SCD, CHUK, GRIN1, PTPN1, ABL1, TLR4, PRKCZ, CYP19A1, NFE2L2, PTK2B, GSTP1, ABCC1, PLA2G2A, CXCR4, ESR1, HDAC9, SLC2A1, CDK5, PIK3R1, LYN, HIF1A, ITGB3, ACACA, CYP3A4, and PIK3CB. The membrane-bound organelles, such as mitochondria, lysosomes, and the endoplasmic reticulum, are susceptible to glycation of amino groups on proteins.

Chemically, glycation can be mitigated by the use of carbonyl trapping agents (e.g., aminoguanidine, pyridoxamine, and carnosine), metal chelators, and antioxidants (N-acetylcysteine, ascorbate, and flavonoids (quercetin and rutin)) that can scavenge reactive oxygen species produced during AGEs formation and inhibit the progression from early glycation products (Schiff base and Amadori product) to AGEs [31]. The oxidative stress and cellular damage result from the cytoplasm's production of reactive oxygen species (ROS), which is greatly increased by high glucose levels. Moreover, cytoplasmic protein glycation produces advanced glycation end-products (AGEs), which impair cellular signaling and enzymatic activity. These AGEs contribute to inflammation and diabetic complications [32]. The glucose levels significantly enhance the production of ROS within the cytoplasm, leading to oxidative stress and subsequent cellular damage. Based on these pathological mechanisms, piperidine-containing bioactive compounds have been identified as potential inhibitors of AGE formation and may serve as therapeutic agents to prevent the progression of diabetic complications.

The GO enrichment analysis of the molecular functions associated with piperidine-containing bioactive compounds (A1-7) revealed significant associations with several biological activities. Notably, enriched GO terms included Catalytic activity (GO:0003824), Protein binding (GO:0005515), Ion binding (GO:0043167), Heterocyclic compound binding (GO:1901363), Organic cyclic compound binding (GO:0097159), and Enzyme binding (GO:0019899), etc. Among these, protein binding showed notable enrichment, modulating 55 genes against a 5522 background of genes, with a strength of 0.028. The identified genes included CFTR, HSD17B10, MIF, MAPK1, PLAT, NAMPT, GCK, MPO, CTSD, MMP8, NT5E, CDK4, MMP7, ADAM10, KDR, PIK3CA,

HDAC4, CTSK, CCR5, NOS3, AXL, KLK1, ACHE, TERT, PRKACA, MMP1, CARM1, NOS2, PRKCD, HSP90AA1, PDPK1, BLM, MTOR, PTGS1, PARP1, PTGS2, SCD, CHUK, PTPN1, ABL1, TLR4, PIK3CD, PRKCZ, CYP19A1, PTK2B, GSTP1, ABCC1, PLA2G2A, HDAC9, CDK5, LYN, ITGB3, ACACA, CYP3A4, and PIK3CB. The α-Glucosidase and α-amylase are key digestive enzymes that are responsible for catalyzing the hydrolysis of dietary polysaccharides into monosaccharides, primarily glucose. Elevated postprandial glucose levels significantly promote the glycation process by increasing the concentration of reducing sugars that can non-enzymatically react with amino groups on proteins, leading to the formation of AGEs [33]. These enzymes' inhibition effectively slows down the process of glucose absorption and the digestion of carbohydrates, which decreases postprandial hyperglycemia and lowers the glycation potential. However, Heterocyclic compounds with amine or hydrazide groups effectively scavenge carbonyls by forming stable adducts with reducing sugars and dicarbonyls, thus preventing glycation [34]. Their antioxidant properties allow them to neutralize ROS, suppressing oxidative glycation pathways. Moreover, act as RAGE antagonists, blocking harmful AGE-induced signaling [35].

The KEGG pathway analysis of piperidine-containing bioactive compounds has revealed that their antiglycation effects are primarily mediated through pathways critical to diabetic complications and metabolic regulation. Among the 164 enriched pathways identified, the top 10 were prioritized based on key parameters: fold enrichment, P-value significance, and associated gene count (Figure 5b). Significantly, the analysis indicated a strong enrichment of pathways directly related to diabetic complications and metabolic dysregulation. Specifically, type II diabetes mellitus, insulin resistance, and the AGE-RAGE signaling pathway in diabetic complications emerged as highly represented, suggesting that the piperidine compounds likely exert their antiglycation effects by modulating pathways central to glucose homeostasis and the progression of diabetic complications. At the molecular level, the KEGG pathway analysis highlighted the noteworthy enrichment represented in Table 2, such as phosphatidylinositol 3-kinase (PIK3CA, PIK3CB, and PIK3CD), nuclear factor kappa B1 (NFKB1), and signal transducer and activator of transcription-3 (STAT3).

The PI3K/AKT signaling module is essential for glucose homeostasis, cellular proliferation, and insulin signaling [36]. Moreover, oxidative stress and glycation can cause dysregulation of the STAT3 transcription factor, which is associated with inflammatory response and cell survival [37]. Additionally, NFKB1 is a vital modulator of inflammatory and immunological responses [38]. The enrichment of the AGE-RAGE signaling pathway in diabetic complications among the KEGG analysis results highlights important targets modulated by piperidine-based bioactive compounds. These findings suggest that the compounds may exert their antiglycation effect by interfering with the biosynthesis of AGEs and inhibiting their binding affinity toward the RAGEs. Piperidine derivatives have distinct chemical features that enable them to act as potent nucleophiles by constructing covalent adducts with electrophilic carbonyl molecules, including reducing sugars and reactive dicarbonyl intermediates, which permit early-stage Maillard reaction intermediates and inhibit the production of AGEs.

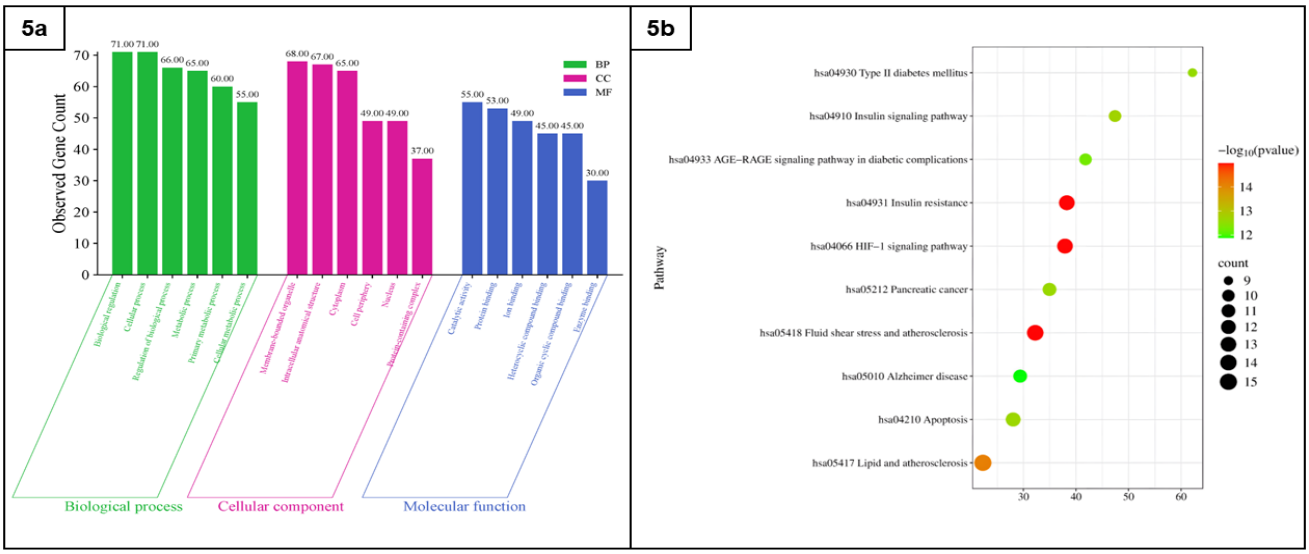

Figure 5. (5a) Gene Ontology (GO) functional enrichment analysis of the identified target genes.
The bar chart shows the number of genes associated with each enriched GO term, with the highest bar indicating the most abundant category. (5b) Top 10 KEGG pathway enrichment results visualized as a bubble chart generated using the SRplot tool. The X-axis denotes the enrichment score, the Y-axis displays pathway names and codes, and the color gradient of the bubbles indicates the significance level of enrichment.

Table 2. KEGG pathway enrichment analysis of piperidine-containing bioactive compounds.

Pathway Code	Pathway	Fold Enrichment	p-value	Gene count	Matching Genes
hsa04930	Type II diabetes mellitus	62.17663043	2.65E-13	9	MTOR, GCK, PIK3CA, PIK3CB, PIK3CD, PIK3R1, STAT3, NFKB1, MAPK1
hsa04910	Insulin signaling pathway	47.43159204	1.92E-13	10	CHUK, MTOR, MPO, NFKB1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1, STAT3
hsa04933	AGE-RAGE signaling pathway in diabetic complications	41.81469298	5.53E-13	10	CDK4, CHUK, MTOR, NFKB1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1, STAT3
hsa04931	Insulin resistance	38.25270062	1.02E-15	13	MTOR, NFKB1, NOS3, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCD, PRKCZ, PTPN1, SLC2A1, STAT3
hsa04066	HIF-1 signaling pathway	37.90175841	1.02E-15	13	MTOR, HIF1A, NFKB1, NOS2, NOS3, PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1, SLC2A1, STAT3, TLR4
hsa05212	Pancreatic cancer	34.95708333	2.39E-13	11	CDK4, NFKB1, NOS3, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCD, PRKCZ, MAPK1, STAT3
hsa05418	Fluid shear stress and atherosclerosis	32.2397343	1.02E-15	14	CDK4, NFKB1, NOS3, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCD, PRKCZ, MAPK1, STAT3
hsa05010	Alzheimer disease	29.37570028	1.34E-12	11	CHUK, GSTP1, HSP90AA1, ITGB3, KDR, NFE2L2, NFKB1, NOS3, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PLAT, PRKCZ
hsa04210	Apoptosis	28.04044118	2.25E-13	12	CHUK, PARP1, CTSD, CTSK, LMNA, NFKB1, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1
hsa05417	Lipid and atherosclerosis	22.27511682	7.55E-15	15	CHUK, HSP90AA1, LYN, MMP1, NFE2L2, NFKB1, NOS3, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1, STAT3, TLR4

3.2. Molecular Docking Simulation

The molecular docking simulations were performed to evaluate the binding affinities and interaction profile of piperidine-based bioactive compounds (A1-7) and pyridoxamine with the human RAGE protein. The molecular docking was carried out utilizing the MVD v.6.0 program, with MolDock SE as the search algorithm. The calculated MolDock score ranged from -71.659 kcal/mol to -124.998 kcal/mol, as represented in Table 3, indicating variable binding affinity across the ligand series. Among all test compounds, compound A7 demonstrated the lowest binding affinity with a MolDock score of -124.998 kcal/mol, the docking pose of A7 and pyridoxamine represented in Figure 6, revealed the formation of four key hydrogen bonds with amino acid residues Ser 211 (A), His 217 (A), and Arg 218 (A), suggesting a stable and specific interaction within the active site. However, the standard drug (pyridoxamine) revealed MolDock scores of -72.2106 kcal/mol. Thus, the polyhydroxy piperidine-containing compound A7 forms a more stable complex with the target than the standard molecules. The H-bond and S-bond interaction of bioactive compounds (A1-7) was evident as they share the same active site as pyridoxamine. Along with that, the ligands and a standard drug have similar

H-bond interaction with comparable amino acid residues Ser 211(A), His 217(A), Arg 218(A) and also share the same steric interaction with Ser 211(A), His 217(A), and His 218(A) residues of the targeted protein (PDB ID: 4LP5). The molecular docking results illustrate significant hydrogen bond and steric interactions, revealing that all bioactive compounds (A1-7), within the same active site, as depicted in Figure 7. Notably, ligands demonstrated comparable binding orientations and interaction patterns within the binding pocket of the RAGE protein. Moreover, the ligands shared steric interactions with the amino, suggesting a structurally and functionally similar binding mode. Overall, finding support for the potential of piperidine derivatives as promising antiglycation agents through effective RAGE inhibition.

Table 3. Molecular docking simulation results of piperidine-containing bioactive compounds.

Compound ID	**MolDock Score (kcal/mol)**	**No. of H-Bonds**	**H-Bond Interaction residues**	**H-Bond Distance No. of H-Bond (Å)**	**No. of S-Bond**	**S-Bond Interaction residues**	**S-Bond Distance No. of H-Bond (Å)**
Pyridoxamine	-72.2106	3	Gly 213(A)	3.01	3	Ser 211(A)	3.23
			His 217(A)	3.10		His 217(A)	2.72
			Arg 218(A)	2.78		Arg 218(A)	2.89
A1	-123.72	2	Arg 218(A)	2.79	3	Ser 211(A)	2.41
			Ala 219(A)	2.81		Pro 212(A)	3.13
						Ala 219(A)	2.86
A2	-116.801	2	Ala 219(A)	3.10	5	Lys 123(A)	3.21
			Ala 221(A)	2.93		His 217(A)	2.88
						Arg 218(A)	2.92
						Lys 220(A)	2.61
						Arg 221(A)	2.80
A3	-73.912	1	His 217(A)	3.19	3	Ser 211(A)	3.04
						Pro 212(A)	2.98
						Pro 215(A)	2.91
A4	-78.181	3	Gly 213(A)	3.02	2	Pro 212(A)	3.09
			His 218(A)	3.01		His 217(A)	2.89
			Pro 215(A)	3.07			
A5	-71.659	1	Arg 218(A)	3.09	1	Pro 212(A)	2.89
A6	-99.777	1	Arg 218(A)	2.60	7	Gly 213(A)	2.14
						Pro 215(A)	3.14
							3.22
						His 217(A)	2.81
						Arg 218(A)	2.26
							2.81
						Ala 219(A)	2.19
A7	-124.998	4	Ser 211(A)	3.30	6	Ser 211(A)	3.06
			His 217(A)	3.00		Pro 212(A)	3.16
				3.19			
			Arg 218(A)	3.08		Pro 215(A)	3.01
						His 217(A)	2.83
						Arg 218(A)	2.63
							2.93

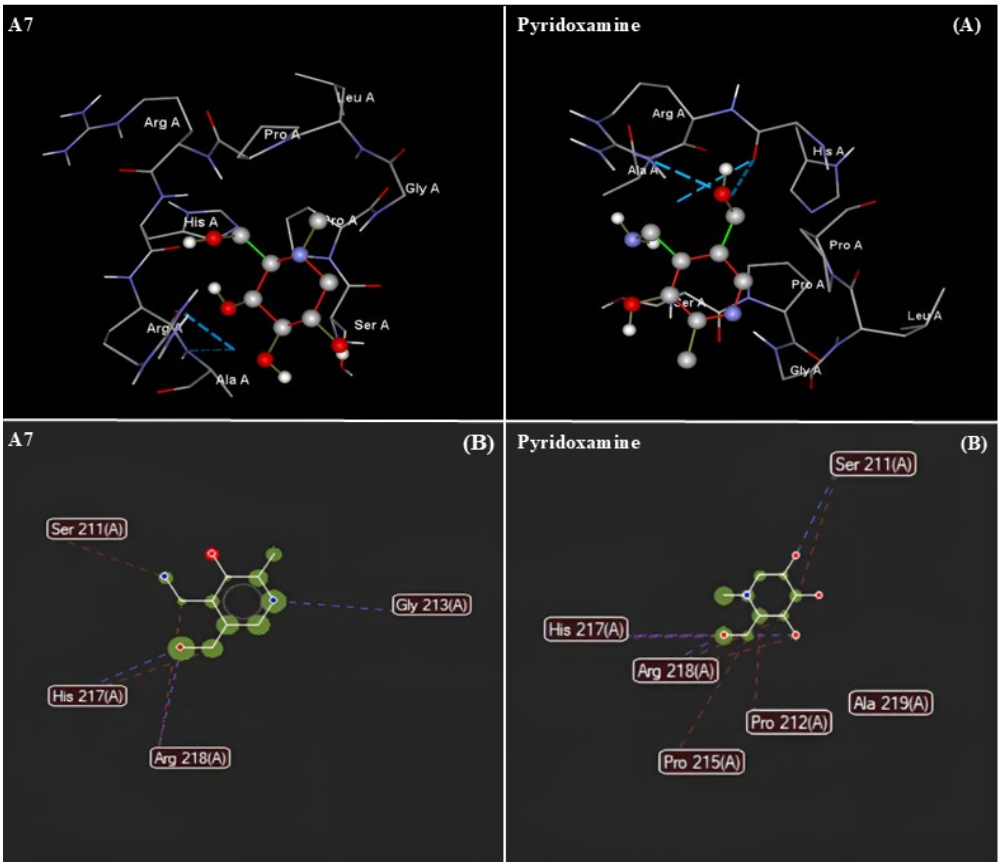

Figure 6. Molecular visualization of protein-ligand complexes interactions between compound A7 and Pyridoxamine with human RAGE protein generated by Molegro Virtual Docker v.6.0. software. (A) The docking view of docked complex amino acids in the active site is presented in thin stick form, while ligands are shown in ball-and-stick form. (B) The blue dotted line represents the hydrogen bond formed between the ligand and the active site of the target protein, whereas the red dotted line represents the steric interaction.

4. Conclusion

Combined network pharmacology and molecular docking simulation confer the antiglycation potential of phytochemicals containing piperidine as a lead structure. The hypoglycemic impact of selected phytochemicals was found to be significant on the basis of common gene analysis, which were associated with glucose metabolism by different metabolic pathways. Out of the different gene modulation pathways, AGE-RAGE, insulin signaling, and type II diabetes pathways were found to be affected dominantly. Further, the RAGE protein, selected on the basis of a network study, was used for molecular docking simulations that demonstrated the strong binding interaction of phytochemicals with the human RAGE protein with reference to the standard pyridoxamine. The consistent hydrogen and steric bond interactions with key amino acid residues indicate a shared and favorable binding orientation among all piperidine-based phytochemicals within the RAGE protein active site. Thus, these collective computational tech niques strongly indicate that the piperidine-based bioactive compounds are promising antiglycation agents, exerting their effects through both multi-target modulation of glycation-related signaling pathways and direct inhibition of RAGE-ligand interactions for managing glycation-associated complications in metabolic disorders such as diabetes mellitus. Finally, it can be concluded that piperidine is a potential lead to design a synthetic pharmacophore that prevents the development of AGE inhibitors, and can be used in the management of glycation-associated disorders.

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