ORIGINAL ARTICLE

Applying Network Pharmacology and Molecular Docking in the Screening for Molecular Mechanisms of Ampalaya (*Momordica charantia* L.) and Banaba (*Lagerstroemia speciosa* L.) against Type 2 Diabetes Mellitus

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ABSTRACT

Background and Objectives. Type 2 diabetes mellitus (T2DM) is a global health concern affecting more than 400 million people worldwide. Diabetic neuropathy, nephropathy, retinopathy, and cardiovascular complications lead to debilitating effects to patients. To prevent these, the treatment goal is to lower the blood sugar levels and maintain at a normal range which is achieved through conventional treatments like insulin and oral hypoglycemic agents. However, the high cost of these medications implicates patient treatment outcomes. Hence, alternatives are sought for including the use of herbal medicines. *Momordica charantia* (MC) and *Lagerstroemia speciosa* (LS) are common herbal medicines used to manage T2DM. In the Philippines, these herbal preparations are validated for their glucose lowering effects and are commonly found in combination in food supplements. The study aims to screen the possible mechanisms of compounds present in these herbal medicines which can offer possible explanations for their synergistic effects and rationalization of their combination in preparations.

Methods. Network pharmacology was employed to determine pivotal proteins that are targeted by MC and LS compounds. Molecular docking was then done to evaluate the favorability of the binding of these compounds toward their target proteins.

Results. Our results showed that TNF, HSP90AA1, MAPK3, ALDH2, GCK, AKR1B1, TTR and RBP4 are the possible pivotal targets of MC and LS compounds in T2DM.

Conclusion. Terpenoids from MC and decanoic acid from LS are the compounds which showed favorable binding towards pivotal protein targets in T2DM. By binding towards the different key proteins in T2DM, they may exhibit their synergistic effects. However, the results of this study are bound to the limitations of computational methods and experimental validation are needed to verify our findings.

Keywords: molecular docking, network pharmacology, Momordica charantia, Lagerstroemia speciosa, T2DM

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the major global health concerns affecting around 462 million individuals in 2017 and is expected to increase to about seven thousand every 100,000 individual by 2030.[1] In the Philippines, the prevalence of diabetes is about 7.1% in 2019.[2] It is caused by the inability of the body to utilize blood glucose due to insulin resistance or death of the pancreatic β-cells. Complications secondary to the disease include diabetic nephropathy, neuropathy, retinopathy, and other cardiovascular diseases. Moreover, T2DM patients are at a higher risk of having cancers.[3] The goal of the management of the disease is to lower blood glucose levels and maintain these levels at a normal range to delay and prevent these complications. Several treatment options such as insulin injection and oral hypoglycemic agents like metformin have been proven to be effective in managing the disease.[4,5] However, the high cost of these medications implicates public health and treatment outcomes.[6] Hence, the search for alternatives or adjunct treatment options are sought for. Globally, the utilization of herbal medicines, especially in developing countries, is one of the strategies for the management of T2DM because of its low cost, high safety margin, and effectiveness.[7]

Two of the most common herbal medicines are *Momordica charantia* and *Lagerstroemia speciosa*. *Momordica charantia* (MC) is a plant of the Cucurbitaceae family that is known for its pharmacologic properties including anti-inflammatory, antioxidant, antitumor and antidiabetic properties among others.[8] In the Philippines, MC is known as ampalaya and is a validated herbal medicine approved by the Philippine Department of Health with its leaves for blood glucose lowering and antidiabetic effects.[9,10] Studies have shown that the antidiabetic properties of MC can be attributed to cucurbitane terpenoids[11], charantin, polypeptide-p, and vicine[12]. Another herbal medicine developed by the Philippine Department of Science and Technology under the Philippine Council for Health Research and Development is the standardized *Lagerstroemia speciosa* (LC; locally known as banaba) tablets from leaves for lowering blood sugar.[13] Several studies have pointed the antidiabetic mechanisms of LC to the presence of corosolic acid and ellagitannins.[14–17]

MC and LS are commonly combined in herbal preparations such as tea and capsules in the Philippines (verification.fda.gov.ph). Thus, in this work we aimed to identify other possible antidiabetic mechanisms of the individual reported compounds present in the leaves of MC and LS using network pharmacology and molecular docking. These computational methods allow the systematic screening of multiple compounds against multiple targets at the molecular level.[18] In network pharmacology, systems biology, bioinformatics, and computational methods are used to determine the complex relationships between biological systems, diseases and drugs.[19] Usually in combination with network pharmacology is molecular docking which evaluates the favorability of the binding of small molecule ligands toward the protein targets.[20] Our results may offer an explanation on the possible synergistic effects of the extracts of LS and MC against T2DM and may rationalize their combination in preparations.

METHODS

The methods presented here are iterations of our previous work using a different herbal preparation and exploring a different disease. These were adapted from Zhang et al.[21] with some modifications to suit the purposes of our research. All databases and software used in this study are open source which is advantageous in replicating our results.

MC and LS Compounds Identification, Screening and Target Determination

MC and LS compounds were obtained from the Indian Medicinal Plants, Phytochemistry and Therapeutics database [IMPPAT | IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics (imsc.res.in)].[22,23] Only secondary metabolites obtained from leaves and those obtained from unspecified parts of MC and LS were considered. Duplicates were removed and the SMILES (Simplified Molecular Input Line Entry Specification) strings of the compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).[24] The SMILES strings were input in the Swiss ADME database (http://www.swissadme.ch/)[25] to screen compounds for oral bioavailability and drug likeness. Only compounds with good oral bioavailability (-0.7 < XLOGP3 (lipohilicity) < +5.0, 150 g/mol < MW < 500 g/mol, 20Å^2 <TPSA (polarity) <130Å^2, -6 < LogS (ESOL) (insolubility) < 0, 0.25 < Fraction Csp3 (insaturation) <1, 0 < number of rotatable bonds (flexibility) < 9) and those which passed the drug likeness rules were considered for target identification. To determine the targets of the MC and LS compounds, their SMILES strings were input in the Swiss Target Prediction database (http://www.swisstargetprediction.ch/).[26] The file containing the targets were downloaded as CSV file and the targets with probability values greater than zero were collected and standardized as UniProt ID (https://www.uniprot.org/).[27]

T2DM Genes

Genes associated with T2DM were obtained from the MalaCards database (https://www.malacards.org/)[28] using type 2 diabetes mellitus as keywords. This database contains human diseases and their annotations. The gene names were standardized as UniProt ID.

MC and LS Targets in T2DM

Using the FunRich application v.3.1.3 (http://www.funrich.org/)[29], a Venn Diagram was constructed using the standardized gene names to determine the list of genes that are both targeted by MC and LS compounds and are associated

with T2DM. The targets were matched with the MC and LS compounds and a compound target network was constructed using Cytoscape v.3.9.1 (https://cytoscape.org/)[30]. Each node in the network represents MC and LS compounds or their target gene whereas the edges are their interactions.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways Enrichment Analysis

GO and KEGG pathways enrichment analyses were conducted using the DAVID (Database for Annotation, Visualization and Integrated Discovery) Bioinformatics Database (https://david.ncifcrf.gov/tools.jsp).[31,32] This database contains a comprehensive set of functional annotation tools to interpret the biological meaning of large gene sets. The UniProt ID (identified as UniProt Accession by the database) of the T2DM targets of MC and LS were input in the database and the GO analysis based on biological processes (BP), cellular components (CC), and molecular functions (MF). Moreover, KEGG pathway enrichment analysis was also conducted to determine which pathways the targets are most likely to be involved in. The results of each analysis were ranked according to *p* values (p-value ≤0.05) and visualized using R studio (https://www.rstudio.com/).

Protein-protein Interaction Analysis

The UniProt ID of the MC and LS targets in T2DM were input in the STRING database (https://string-db.org/).[33,34] This database contains known and predicted protein-protein interactions. Here, we used *Homo sapiens* as the filter organism, confidence score of 0.700 (high confidence), and false discovery rate stringency of 5% (medium stringency). The created protein-protein interaction network was extracted and visualized using Cytoscape. Each node represents the protein target whereas the edges represent their interactions. Using the Cytoscape Analyzer, the centrality measures (degree centrality, betweenness centrality, and closeness centrality) were obtained and the targets were ranked according to these measures. The top three nodes for each centrality measure were obtained. Molecular Complex Detection (MCODE) plug-in in Cytoscape was also used to determine which nodes within the protein-protein interaction network were highly connected. The parameters were set as default with the degree cut-off = 2, node score cut-off = 0.2, k-score = 2, and maximum depth = 100. Only one cluster resulted from the analysis.

Molecular Docking

Protein selection and preparation

After protein-protein interaction analysis, the genes were ranked based on the measures of centrality (degree, betweenness, closeness). Genes ranked with the highest measures of centrality were identified together with the proteins that they code. The 3-D crystal structures of the proteins were obtained from the protein data bank (www.rcsb.org).[35] The PDB file format of these proteins were downloaded and processed in Swiss PDB Viewer v4.1.0[36] and Autodock Tools v1.5.6[37]. The Swiss PDB Viewer was used to repair missing atoms in the structure. The standard protocol by Forli et al.[38] was followed using Autodock Tools. Briefly, the water molecules and heteroatoms were removed from the structure. Then, polar hydrogens were added, and the nonpolar hydrogens were merged. Gasteiger charges were then added, and the macromolecule was saved as PDBQT file.

Ligand preparation

Ligand structures were obtained from PubChem.[24] The 3D structures were downloaded as SDF files and were then converted into MOL2 files using OpenBabel v2.3.1.[39] The structures were processed using Autodock Tools.[37] Following the protocol by Forli et al.[38], the nonpolar hydrogens were merged, polar hydrogens were added and the Kollmann charges were calculated. The structures were then saved as PDBQT file, and the energy minimized using MMFF94 forcefield.

Search space validation

For crystal structures of proteins with co-crystallized ligands, the ligands were redocked in the protein ensuring that the redocked pose has an RMSD value of <2.0 Å. Redocking was done ten times to ensure that the results are consistent. As for the proteins 1qrz and 1huc which do not have co-crystallized ligands, CASTp3.0 webserver (http://sts.bioe.uic.edu/castp) was utilized to determine the ligand-binding pocket used for the docking procedure.[40]

Docking Procedure

All molecular docking procedure was conducted using Autodock Vina.[41] The default parameters were used, with the number of modes = 10, energy range = 3, exhaustiveness = 8. The xyz coordinates as well as the grid size (in Å) were adjusted to ensure that the ligands and that the amino acids reported to be involved in protein-ligand interactions fall within the set search space. Protein-ligand interactions were visualized using PyMol v.2.5.4[42] and Discovery Studio Visualizer[43].

RESULTS

Target Identification and Compound-Target Network Construction

In the data analyses, we used the gene names instead of the protein names for consistency to create the compound-target network. It should be noted that it is the protein coded by these genes that are targeted by the MC and LS compounds.

There were 303 MC and 43 LS compounds documented in the IMPPAT database. However, only 227 compounds passed the drug likeness and oral bioavailability filters. These compounds have a total of 462 target genes which code for

specific target proteins. The 512 T2DM related genes were obtained from the MalaCards database.

From the list of genes, we created a Venn diagram (Figure 1) using the FunRich application. Not all genes were recognized by the application and were excluded hence decreasing the total number of genes mapped. The intersection corresponds to the genes that code for proteins potentially targeted by LC and MS compounds. These 89 genes were matched with the corresponding compounds from LC and MS that target them, and a compound-target network was constructed using Cytoscape as shown in Figure 2. Each green node represents the LS and MC compounds whereas each violet node represents the target gene. The lines (edges) represent a nondirectional interaction. From the constructed network, we can note that MC and LS compounds have multiple targets.

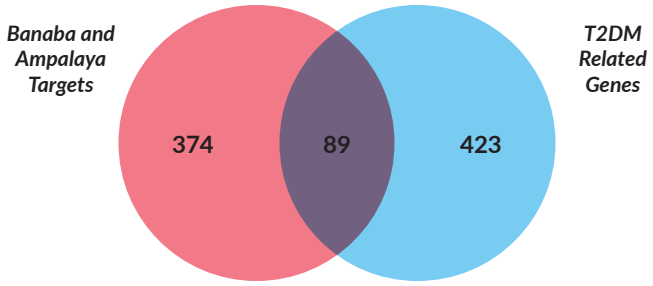

Figure 1. Venn diagram showing the 374 targets of *Momordica charantia* and *Lagerstroemia speciosa* reported compounds *(red)* and the 423 T2DM-related genes *(blue)*. The intersection shows that 89 T2DM related genes are possible targets of the MC and LS compounds.

Protein-Protein Interaction, GO and KEGG Pathway Enrichment Analysis

We also constructed the protein-protein interaction (PPI) network to determine important proteins in T2DM that can be targeted by the MC and LS compounds. The PPI network (Figure 3) was constructed from the 89 targets using the STRING database. The MCODE plug-in of Cytoscape was also utilized to determine protein clusters. The cluster with the highest score was shown in Figure 3B. The results of the ranking of each node based on centrality measures is summarized in Table 1. The top genes that are pivotal to T2DM targeted by LS and MC compounds include TNF, HSP90AA1, GCK, AKR1B1, ADLH2, RBP4 and TTR. The centrality gives an estimate of how important a particular node is and is commonly measured using the degree, the betweenness and the closeness centrality. The degree gives a rough estimate of the centrality but is only a local measure and does not take into consideration the rest of the network. Hence, betweenness, and closeness centralities were also included in the ranking of the pivotal

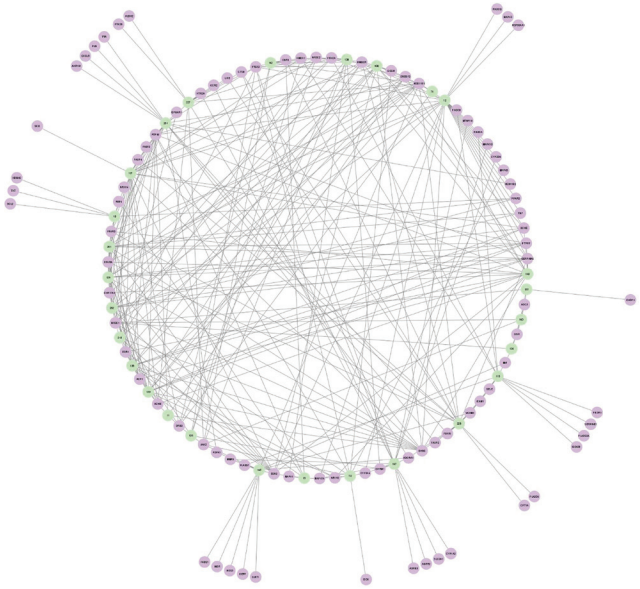

Figure 2. The compound-target network of MC and LS compounds *(green)* and T2DM genes *(violet)*. Each node represents compounds from MC and LS or T2DM-related genes. The lines represent the nondirectional interaction between each node. The network shows that each compound can possibly target multiple T2DM genes.

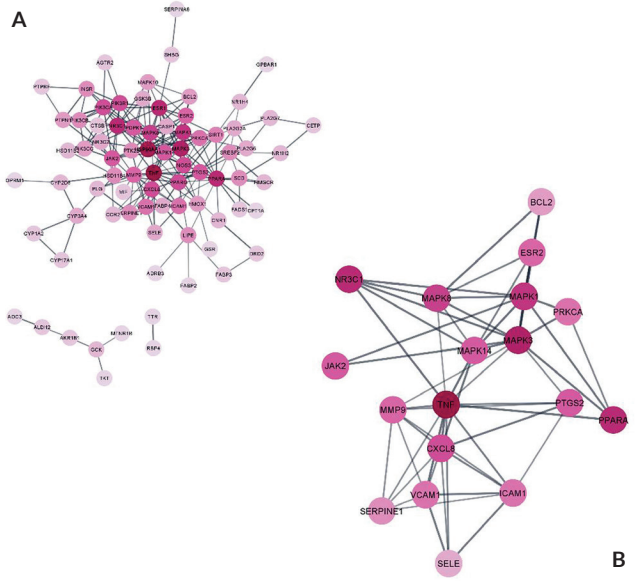

Figure 3. Protein-protein interaction network of the T2DM genes coding for proteins that are targeted by MC and LS compounds. On the left **(A)** is the protein-protein interaction network of the 89 genes whereas on the right **(B)** is the cluster of proteins obtained after MCODE plug-in of cytoscape was applied. The darker the color, the higher is the degree centrality.

proteins. The closeness centrality measures the average of all the shortest distance of the other nodes from a given node whereas the betweenness centrality measures the number of shortest paths passing through a node. The higher the value of these centrality measures, the more important the node is.[44,45]

Gene ontology and KEGG pathway enrichment analyses were done to determine the pathways, cellular components, molecular functions, and biological processes associated with the pivotal proteins. The bubble plots showing the results of the gene ontology and KEGG pathway enrichment analyses are shown in Figure 4. The genes are highly associated with the positive regulation of nitric oxide biosynthetic process, positive regulation of ERK1 and ERK2 cascade as well as in response to insulin. In terms of cellular components, the gene sets are highly associated with the caveola, plasma membrane and phosphatidylinositol 3-kinase complex. For molecular functions, they are highly associated with steroid binding, RNA polymerase II transcription factor

Table 1. Ranking of the Different Genes based on Degree, Betweenness, and Closeness Centrality

Node	Degree Centrality Rank	Node	Betweenness Centrality Rank	Node	Closeness Centrality Rank
TNF	1	***GCK***	1	***RBP4***	1
HSP90AA1	2	***AKR1B1***	2	***TTR***	1
MAPK3	3	***ALDH2***	3	***GCK***	3
NR3C1	4	***PPARG***	4	***AKR1B1***	3
PPARA	4	***PPARA***	5	***ALDH2***	5
ESR1	4	***TNF***	6	***HSP90AA1***	6
MAPK1	7	***HSD11B1***	7	***MAPK3***	6
MAPK8	8	***NR3C1***	8	***PPARG***	8
NOS3	9	***HSP90AA1***	9	***TNF***	9
CXCL8	9	***MAPK3***	10	***MAPK1***	10
PPARG	9	***LIPE***	11	***PPARA***	11
PIK3R1	9	***ESR1***	12	***NR3C1***	11
PIK3CA	9	***JAK2***	13	***ESR1***	13

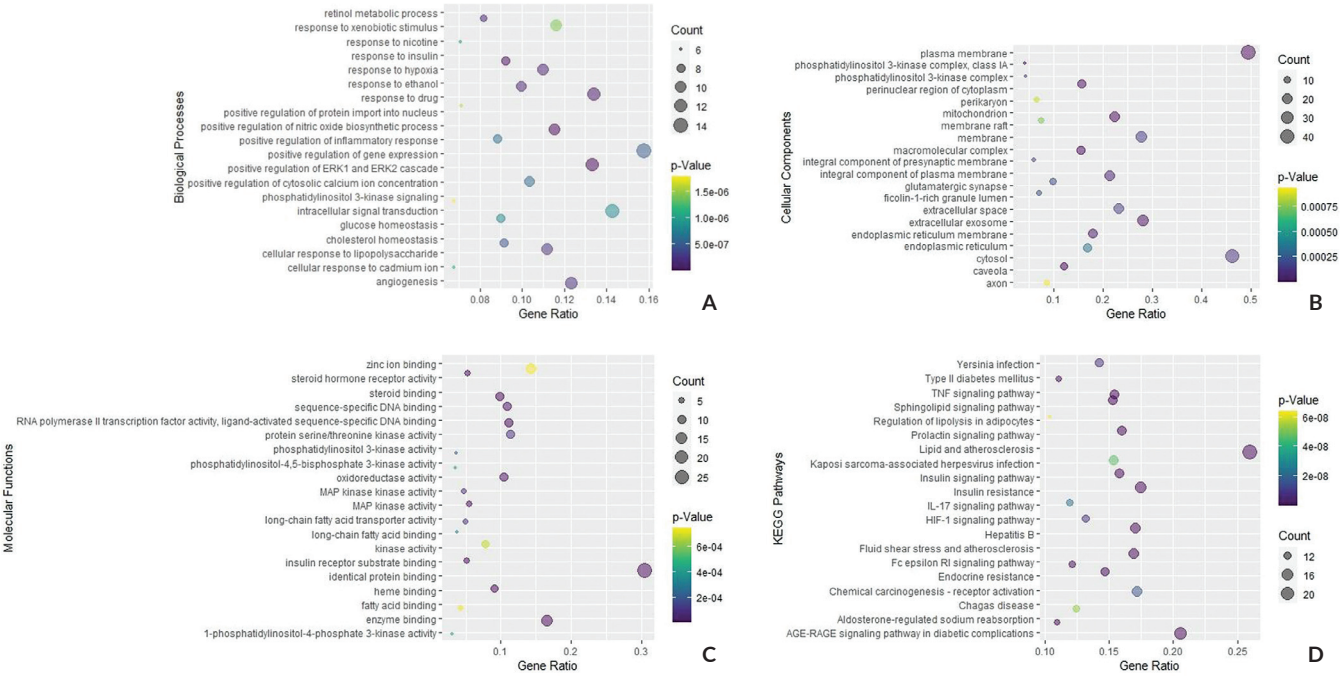

Figure 4. Bubble plot of the top 20 biological processes **(A)**, cellular processes **(B)**, molecular functions **(C)**, and KEGG pathways **(D)** associated with the genes in T2DM that are targeted by *Momordica charantia* L. and *Lagestroemia speciosa* L. compounds. The size each bubble corresponds to the number of genes whereas the intensity of the color corresponds to the *p*-value. The lower the *p*-value, the greater is the association.

Table 2. Protein-ligand Interactions between the Pivotal Proteins Involved in T2DM and the Compounds Found in *Momordica charantia* L. and *Lagerstroemia speciosa* L.

Protein	PDB ID	Compound	Binding Energy (kcal/mol)	Hydrogen Bond Interactions	Hydrophobic Interactions	Other Interactions
Human Transthyretin	1BM7	FLF501*	-4.9	Thr119	Ala108, Leu110, Lys15, Val121, Thr119	Ala108 (halogen)
		decanoic Acid	-3.8	-	Ala108, Leu17, Lys15, Val121	-
Human hsp90	1OSF	KOS280*	-9.7	Asp54, Asp93, Asn106, Lys58, Lys112, Phe138, Thr184	Asp102	-
		momordicine II (aglycone)	-7.1	Asp93, Lys112, Thr184	Leu48, Phe138, Val186	-
Human Serum Retinol Binding Protein	1RBP	retinol*	-9.8	Gln98	Ala43, Ala55, Ala57, Leu37, Met73, Met88, Tyr90	-
		retinol	-9.7	Gln98	Ala43, Ala55, Ala57, Leu37, Met73, Met88, Tyr90	-
		thiamine	-7.0	Leu35	Ala43, Ala57, Phe137	-
Tumor necrosis factor-alpha	2AZ5	3071*	-8.9	-	Tyr59	Gly121 (halogen)
		momordicine II (aglycone)	-9.3	Gly122, Ile58, Leu120, Tyr151	Tyr59, Tyr119	-
		triterpenoids	-8.6	Ser60, Tyr151	-	-
Human glucokinase	3FR0	AJB501*	-9.4	Arg63, Tyr215	Ile159, Ile211, Pro66, Tyr214, Val62, Val455	-
		nerolidol	-7.4	Arg63	Ala456, Ile211, Met210, Met235, Pro66, Val62, Val452, Val455	-
Human ERK1	4QTB	38Z*	-14.0	Ala52, Asp123, Lys71	Ala52, Ala69, Arg84, Asp128, Asp184, Ile48, Ile73, Ile101, Leu173, Thr85, Thr127, Tyr53, Tyr81	Lys131, Met125 (unfavorable donor-donor)
		momordicine II (aglycone)	-7.5	Ser170	Ala69, Leu124, Leu173, Met125, Val56	-
Aldehyde Dehydrogenase 2	5L13	6ZE*	-11.5	Cys302, Cys303	Cys301, Met124, Met174, Phe170, Phe292, Phe29, Phe459, Phe465, Val120, Trp177	-
		4-octenoic acid methyl ester	-6.3	Asn169, Cys302	Asn169, Cys301, Phe170, Phe296, Phe459	-
Aldose Reductase	2R24	LDT	-9.8	His110, Trp111, Tyr48	Cys303, Leu300, Trp20, Trp111, Val47, Cys80	Ala299 (halogen), Val47 (halogen)
		decanoic acid	-6.2	Lys21	Lys262, His110, Tyr209	Ser210 (unfavorable acceptor-acceptor)

**reference compounds*

activity, and enzyme binding. The results showed that the most enriched pathways include the AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis, and insulin resistance.

Molecular Docking Analysis

Molecular docking using Autodock Vina was conducted to determine the favorability of the binding of the LS and MC compounds towards the pivotal proteins, and the results are summarized in Table 2. The 3D and 2D visualization maps are shown in Figures 5-12. The binding energy is measured in kcal/mol and the more negative the values are, the more favorable the predicted binding of the ligand towards the protein will be.

DISCUSSION

The effect of MC and LS in T2DM has been widely studied. MC suppresses mitogen-activated protein kinases (MAPKs) and NF-κB in pancreatic cells, promotes glucose and fatty acid catabolism, stimulates fatty acid absorption, induces insulin production, improves insulin resistance, activates activated protein kinases (AMPK) and inhibits fructose-1,6-bisphosphate and glucose-6-phosphatase.[46] The antidiabetic activity of LC has been attributed to corosolic acid which increases insulin secretion and stimulate glucose uptake in cells. Moreover, ellagitannins including lagerstroemin, flosin B and reginin A were reported to be present in LC and were shown to promote glucose uptake in adipose cells.[15,16] Through network pharmacology and molecular docking, we

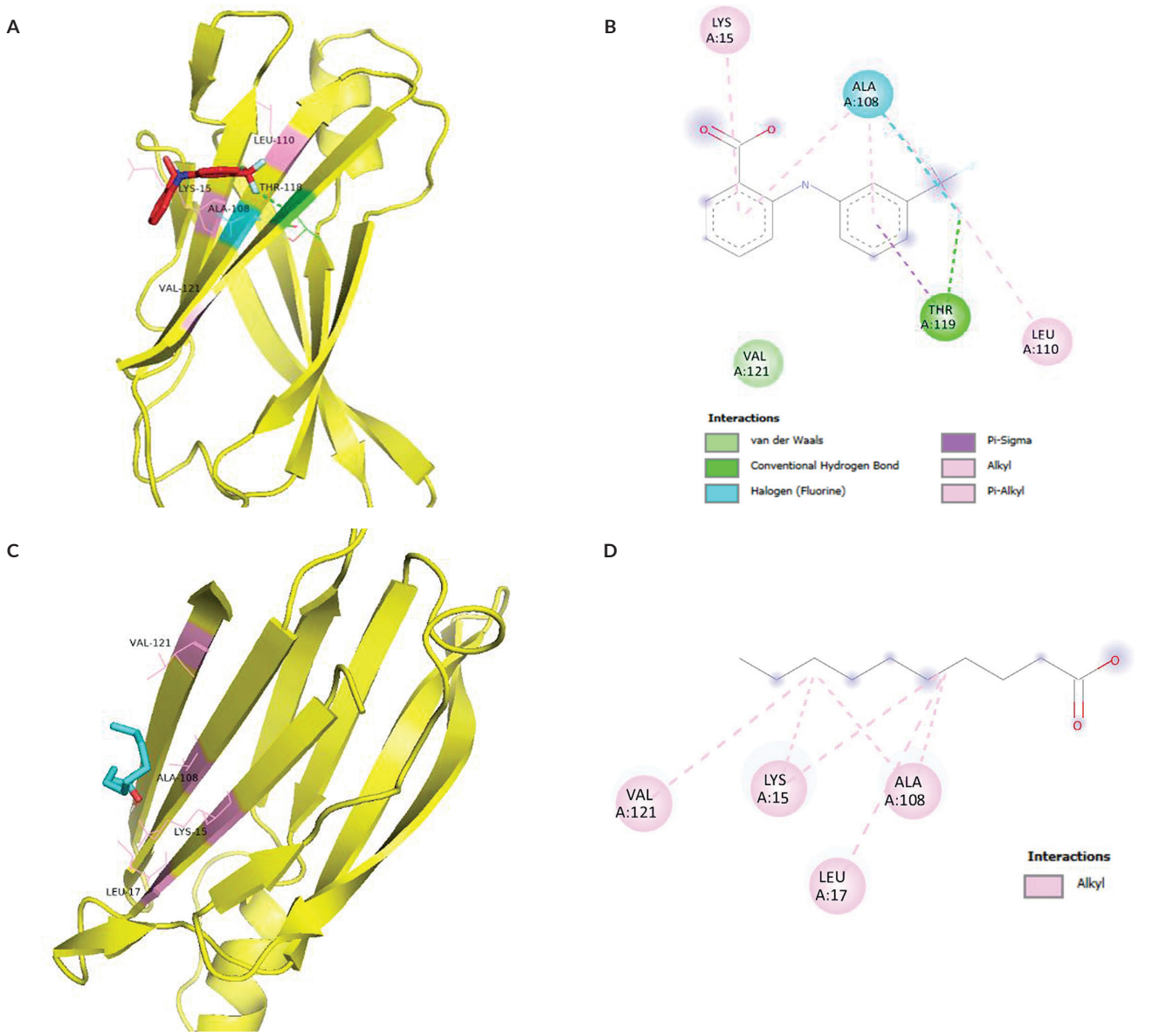

Figure 5. Visualization of the protein-ligand interactions between human transthyretin (pdb ID: 1bm7) and the co-crystallized ligand FLF501 **(A and B)** and the compound decanoic acid from *Lagerstroemia speciosa* **(C and D)**. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

present other possible mechanisms of the compounds present in these plants at the molecular level against T2DM.

Plasma transthyretin (also known as prealbumin and TTR) is a protein synthesized in the liver, choroid plexus, and in the pancreatic islets. Abnormal plasma levels of transthyretin are associated with several pathologic conditions including malnutrition, inflammation, Alzheimer's disease, and diabetes. In T2DM, transthyretin levels are increased and that plasma transthyretin levels exceeding 290 mg/L were found to be associated with an increased risk of having the disease as well as with an impaired glucose regulation. Transthyretin works by influencing the secretion of insulin and glucagon.[47,48] Moreover, it stabilizes the retinol-binding protein 4 (RBP4) at a higher steady-state concentration in circulation which eventually lead to insulin resistance. However, Jayaweera and colleagues showed that transthyretin can slow down the formation of islet amyloid polypeptide (IAPP). These are protein fibrils which, under pathologic conditions as in T2DM, form aggregates and accumulate in the pancreas thereby destroying pancreatic β-cells. The inhibitory effects of transthyretin on IAPP-amyloid formation is maintained at a low pH and is impaired by transthyretin-stabilizing

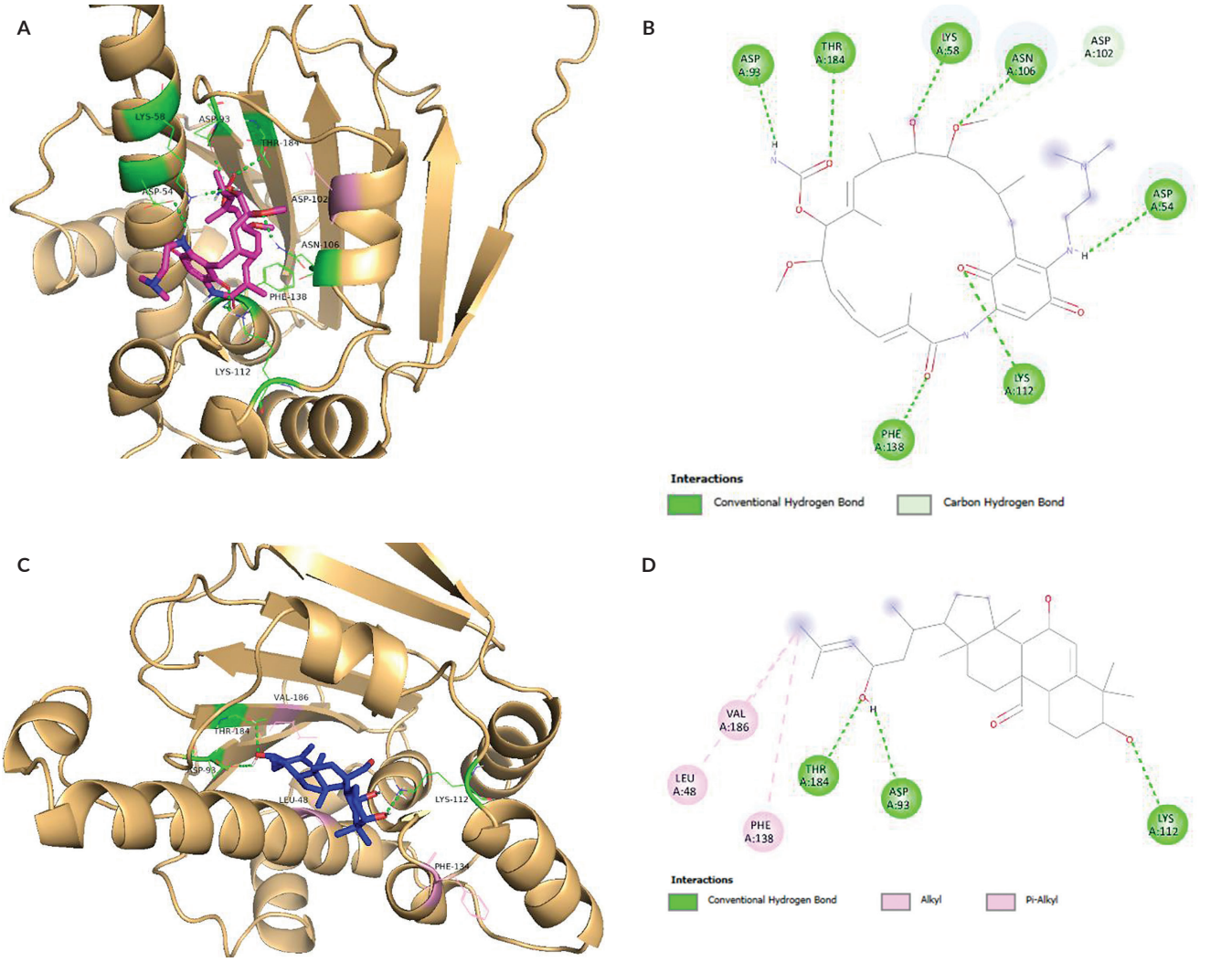

Figure 6. Visualization of the protein-ligand interactions between the protein human HSP90 (pdb ID: 1OSF) and the co-crystallized ligand KOS280 **(A and B)** and the momordicine II aglycone **(C and D)** from *Momordica charantia*. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

ligands.[49] This area of study still needs to be explored because of contradicting results. In our work, decanoic acid, which is found in LS has been shown to favorably bind with TTR as shown in Table 2 and Figure 5 involving primarily hydrophobic interactions. The protein-ligand interactions involved amino acid residues that are similar to those of TTR and FLF501, an investigatory compound which inhibit TTR. This suggests that decanoic acid can potentially target TTR and may affect T2DM conditions. Decanoic acid has been shown to modulate the effects of PPAR-γ and improve glucose sensitivity and lipid profiles in animal models[50] but its activity against TTR remains unexplored.

Heat shock proteins are stress proteins which under physiological conditions protect the body against stressful conditions including heavy metal exposure, oxidative stress, nutritional deficiency, inflammation, and cardiovascular injury among others. Moreover, they are involved in correct protein folding, anti-apoptotic, pro-angiogenic and antioxidant activities. In diabetic individuals, the levels of HSPs are significantly lowered due to the activation of protein kinase C via the NF-κB pathway leading to increased inflammation. Hence, one of the treatment strategies for diabetes is to increase the expression of HSPs which also ultimately help in the prevention of β-cell apoptosis. One of the HSPs is HSP90 which binds to misfolded β-amyloid proteins and prevents further protein aggregation. Paradoxically, since HSP90 interact with HSF-1 (heat-shock factor-1), inhibiting HSP90 cause an increase in HSP expression.[51,52] Additionally, HSP90 maintains and helps in the function of GSK-3 (glycogen synthase kinase-3), which have detrimental

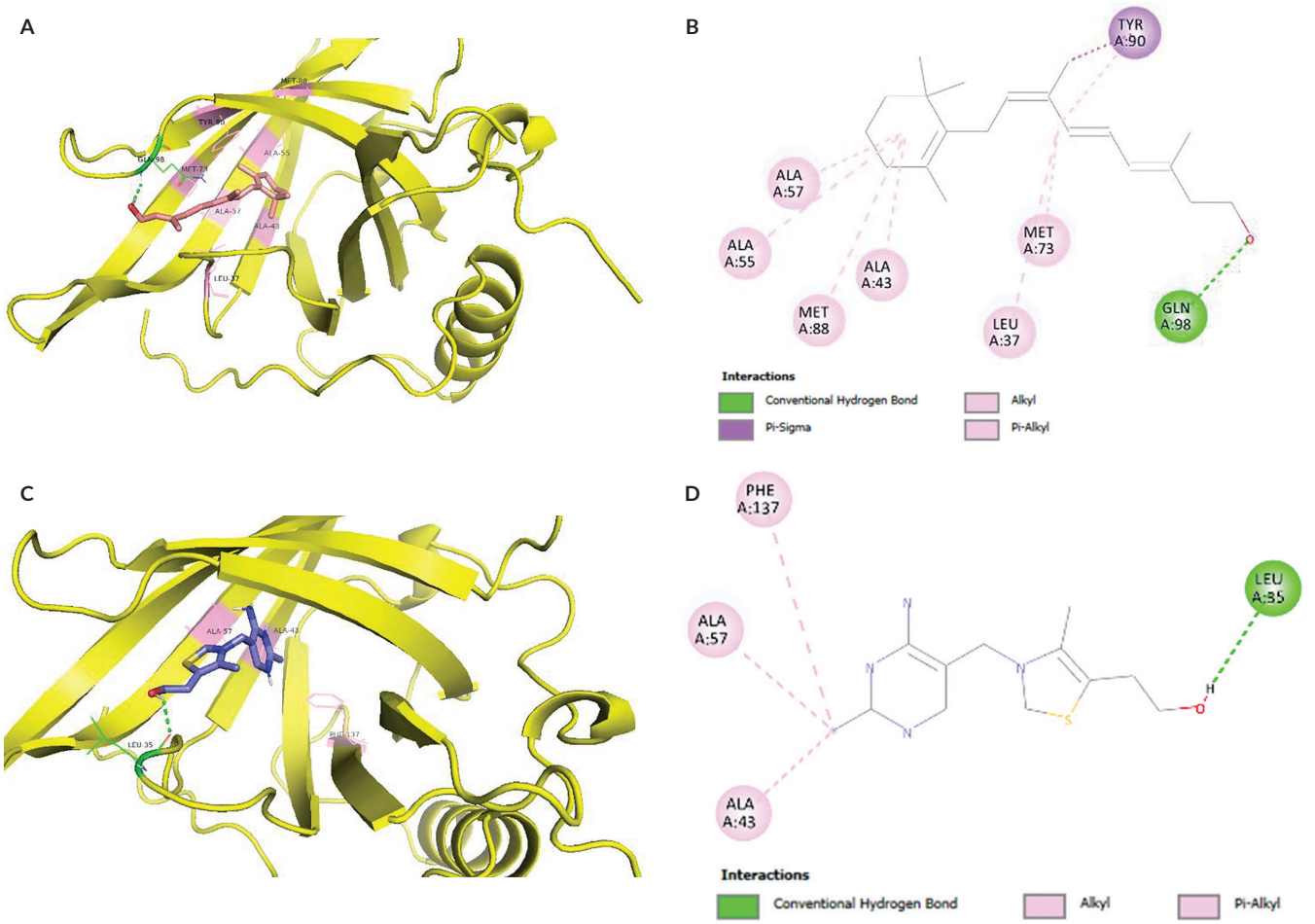

Figure 7. Visualization of the protein-ligand interactions between the protein human serum human retinol binding protein (pdb ID: 1RBP) and the co-crystallized ligand retinol **(A and B)** and the compound thiamine **(C and D)** from *Momordica charantia*. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions). Note that retinol is also found to be present in *Momordica charantia* and the protein-ligand interactions are also represented in **(A)** and **(B)**.

effects in T2DM. Post-translational modifications such as acetylation and phosphorylation as well as formation of large complexes with other HSPs play a crucial role in determining the effect of HSP90 activity towards T2DM.[29] In our current work, the aglycone portion of momordicine II, a compound found in MC, have resulted in favorable binding towards HSP90 (-7.1 kcal/mol) as shown in Table 2. Moreover, the amino acids involved in the protein-ligand interactions between KOS280 (investigational HSP90 inhibitor) and HSP90, especially the hydrogen bonding interactions, are also present in the protein-ligand interactions between the aglycone of momordicine II and HSP90 (Figure 6). These suggest that the aglycone of momordicine II can potentially target the protein HSP90. The saponin rich extract of MC which include the compound momordicine II has been shown to increase insulin secretion in MIN6 β-cells but the exact biological mechanism was not mentioned.[53] In a review conducted by Liew and colleagues, several studies have shown that terpenoids, the group of compounds to which momordicine II belong, possess HSP90 inhibitory activities in cancer cell lines.[54]

Retinol-binding protein-4 (RBP4) is a transporter protein which carries vitamin A in the circulation and has been implicated in numerous diseases such as T2DM. In fact, it serves as a biomarker for insulin resistance and metabolic disorders. When its levels are increased, insulin resistance was observed in mice models. RBP4 is also associated with peroxisome proliferator-activated receptor, impaired glucose transport in adipocytes, reduced regulation of inflammation as well as in the downregulation of GLUT4 in adipocytes. Hence, antagonizing RBP4 as well as preventing the formation of RBP-4-TTR complex is one of interests in research for possible targeting of T2DM.[55] Here, retinol, the natural ligand of RBP4, was found to be present in MC as

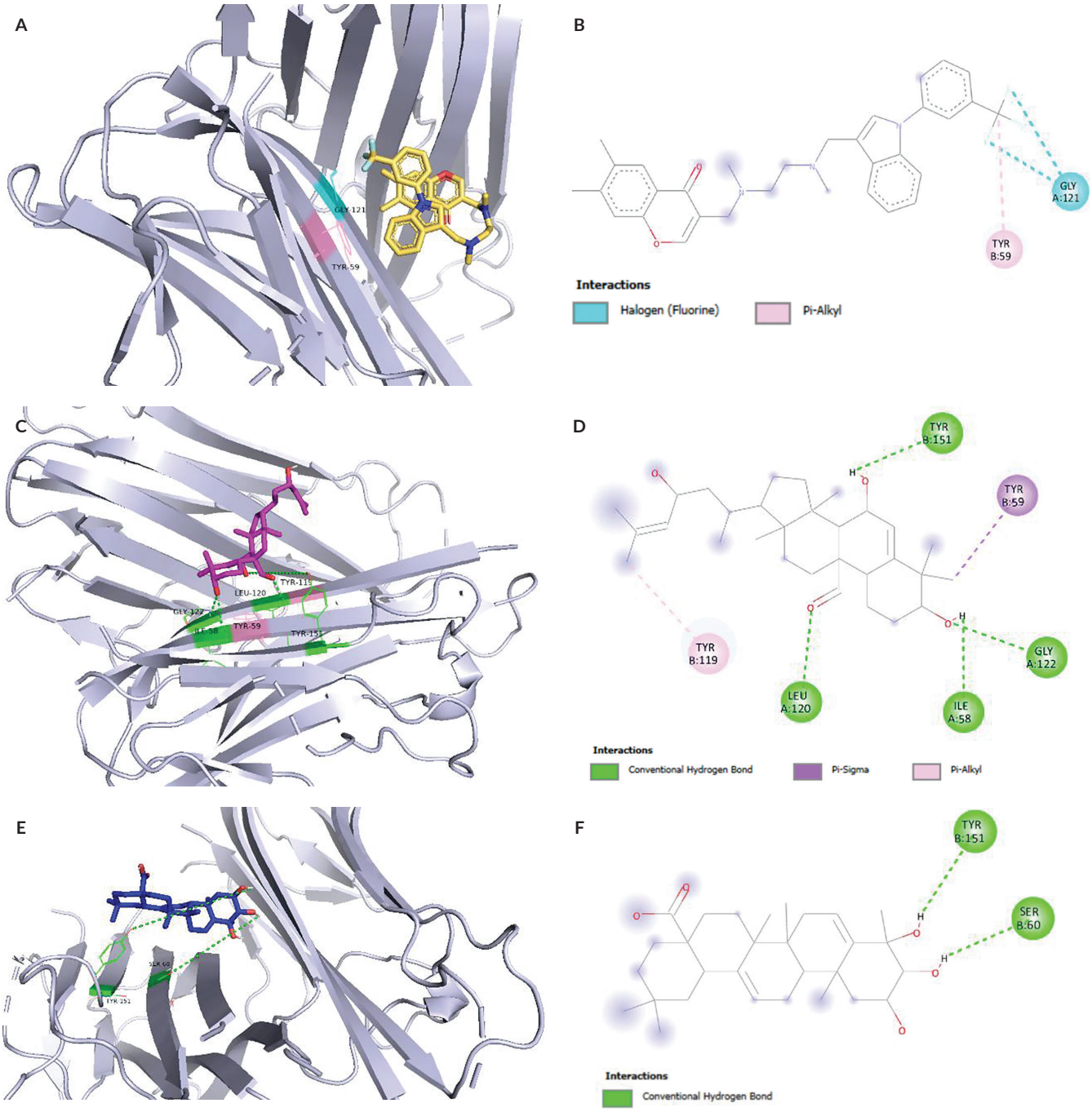

Figure 8. Visualization of the protein-ligand interactions between the protein TNF-α (pdb ID: 2az5) and the co-crystallized ligand 3071 **(A and B)**. Shown also are the interactions between TNF-α and the compounds momordicine II aglycone **(C and D)** and triterpenoids **(E and F)** from *Momordica charantia*. Shown in **(B)**, **(D)** and **(F)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)**, **(C)** and **(E)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

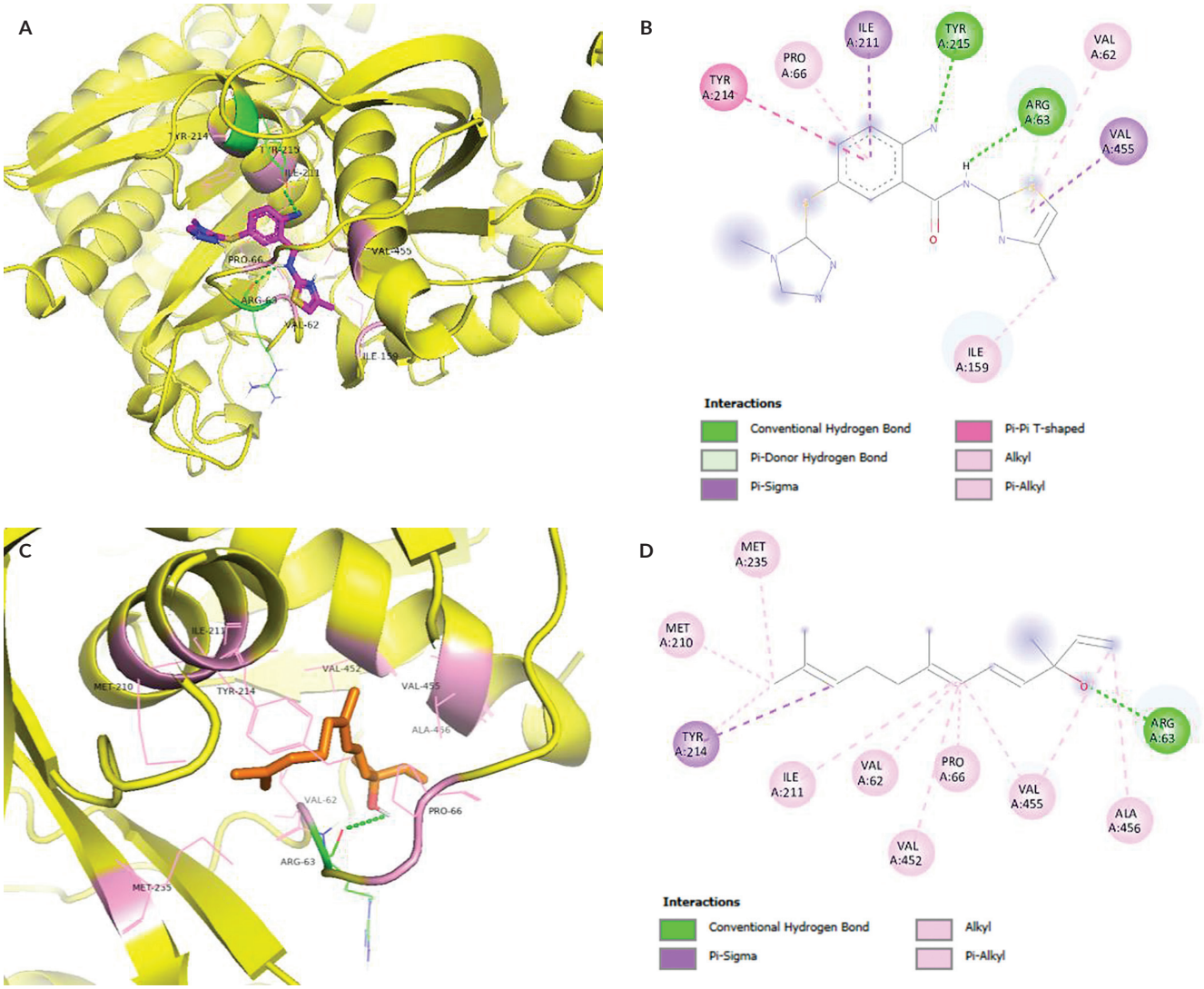

Figure 9. Visualization of the protein-ligand interactions between the protein human glucokinase (pdb ID: 3fr0) and the co-crystallized ligand AJB501 **(A and B)** and the compound nerolidol **(C and D)** from *Momordica charantia*. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

shown in Table 2 and Figures 7A and 7B. However, studies on the effects of retinol in T2DM are inconsistent and need further investigation. For example, consumption of retinol in humans has been associated with lower diabetes-related retinopathies[56] and that in animal models, administration of retinyl palmitate has slightly improved GLUT4 expression and has protected β-cells from oxidative stress[57]. In contrast, serum vitamin levels were higher in human subjects with impaired glucose tolerance than their normal counterparts.[58] Another vitamin found in MC which binds favorably to RBP4 is thiamine as shown in Table 2 and Figures 7C and 7D. Thiamine has been implicated in T2DM and it was reported that T2DM patients have reduced levels of thiamine. Thiamine is involved in insulin synthesis and secretion[59] and reversal of hyperglycemia-induced endothelial dysfunction[60] among others[61]. However, its effect on RBP4 is yet to be elucidated.

Tumor necrosis factor-α (TNF-α) is another protein that can be possibly targeted by MC and LS. As seen in Table 2, the momordicine II aglycone from MC and the triterpenoids from LS have favorable binding affinity towards TNF-α. The 2-D and 3-D representation of the docking results are presented in Figure 8. TNF-α is one of the key mediators of inflammatory response and insulin resistance in T2DM. The induction of pro-inflammatory cascade by TNF-α ultimately results in cellular apoptosis. In T2DM, TNF-α downregulates of the expression of GLUT4 and promotes serine phosphorylation of insulin receptor substrate-1 resulting in

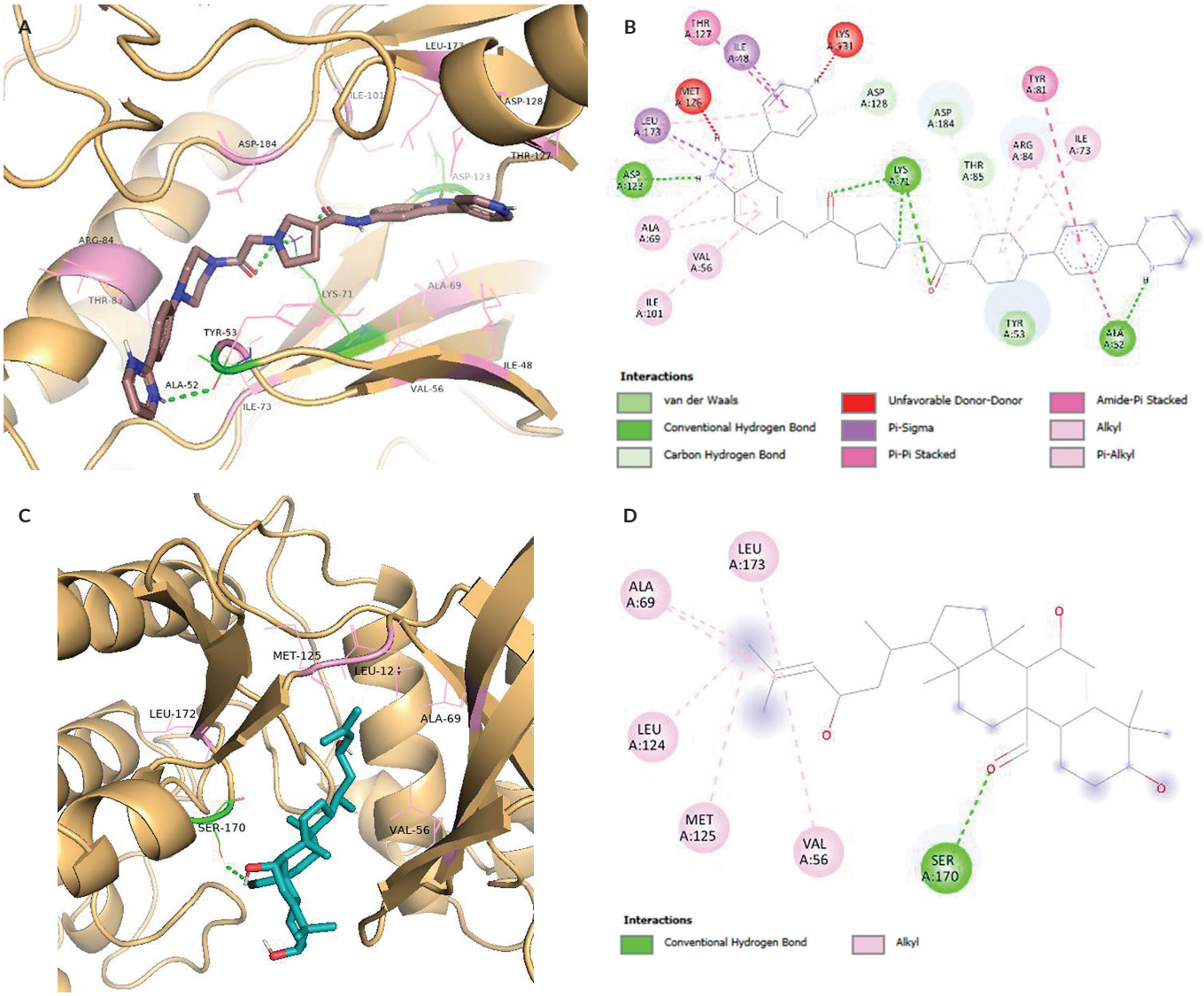

Figure 10. Visualization of the protein-ligand interactions between the protein human ERK1 (pdb ID: 4qtb) and the co-crystallized ligand 38Z **(A and B)** and the compound momordicine II aglycone **(C and D)** from *Momordica charantia*. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

insulin resistance.[62] Hence, targeting TNF-α gains traction in diabetes research.[63] In an in silico study created by Kim and colleagues, saponins from Vietnamese ginseng, which share similar structural features with that of triterpenoids and the aglycone of momordicine II, have been shown to bind favorably to TNF-α.[64] Moreover, terpenoids have been shown to inhibit the secretion of pro-inflammatory cytokines including TNF.[65,66] Taken together, these compounds found in MC and LS can be experimentally validated with respect to their ability to potentially inhibit TNF-α.

The pancreas, liver, hypothalamus, and gastrointestinal tract harbor most of the glucokinase in the body. The enzyme is involved in the rate limiting step of glucose metabolism which is phosphorylation to glucose-6-phosphate. Its activity, however, is not affected by the phosphorylated product. In the β-cells, it functions in the release of insulin depending on glucose concentration whereas in the liver, it converts glucose to glycogen. Glucokinase in the hypothalamus prevents hypoglycemia caused by overactivation of glucokinase in the liver and pancreas leading to decrease in the release of adrenaline, noradrenaline, and glucagon. In animal models, it was observed that the absence of glucokinase leads to an increased in fasting blood glucose level, hyperglycemia, and impaired glucose tolerance. Overexpression of the enzyme, on the other hand resulted in improved glycolysis and glycogen synthesis. However, this overexpression eventually led to

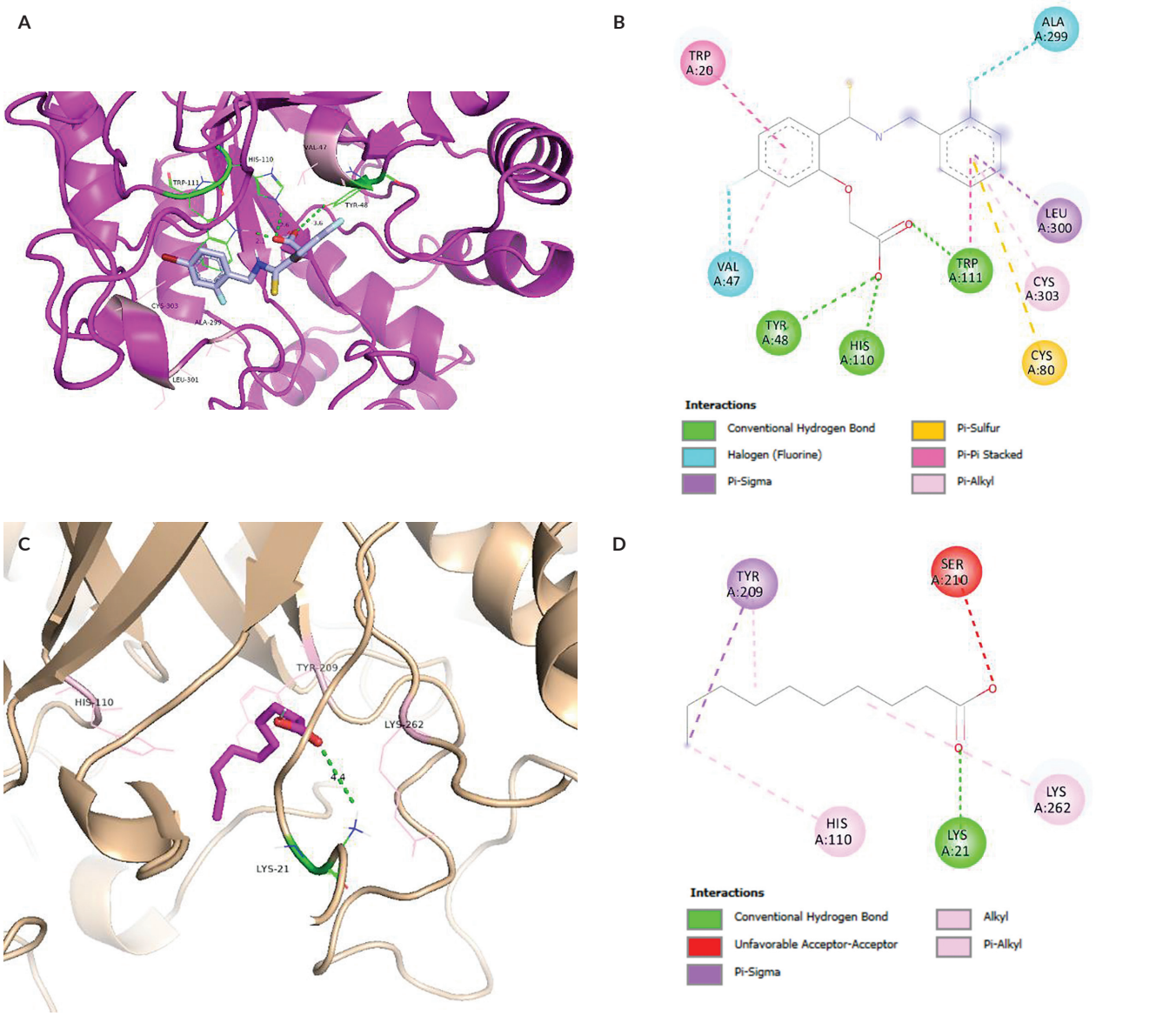

Figure 11. Visualization of the protein-ligand interactions between the protein aldose reductase (pdb ID: 2r24) and the co-crystallized ligand LDT **(A and B)** and the compound decanoic acid **(C and D)** from *Lagerstroemia speciosa*. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

increased weight, glucose intolerance, and reduced insulin sensitivity. At present, several glucokinase agonists are being developed and are being tested in the clinical trial stages. Glucokinase agonists bind to the receptor binding site through hydrophobic and hydrogen bonding interactions. The amino acid residues involved in the protein-ligand interactions are THR65 in hydrogen bonding, and TYR214 and VAL455 in hydrophobic interactions, among others.[67] Most of these interactions are also present between nerolidol from MC and the glucokinase protein model as shown in Table 2 and Figure 9. Nerolidol belong to the group of terpenoids, and these compounds are known to possess antidiabetic activity.[68,69] In type 2 diabetic rats, nerolidol has been shown to reduce elevated glucose levels, glycosylated hemoglobin, and has improved body weight and insulin level. Moreover, it was able to reduce oxidative stress markers and enhanced and activated glucose transporter-4 in skeletal muscle cells thereby improving insulin transport.[70] Hence, the antidiabetic properties of nerolidol can be further explored and that our results suggest that glucokinase can be a potential target for validation.

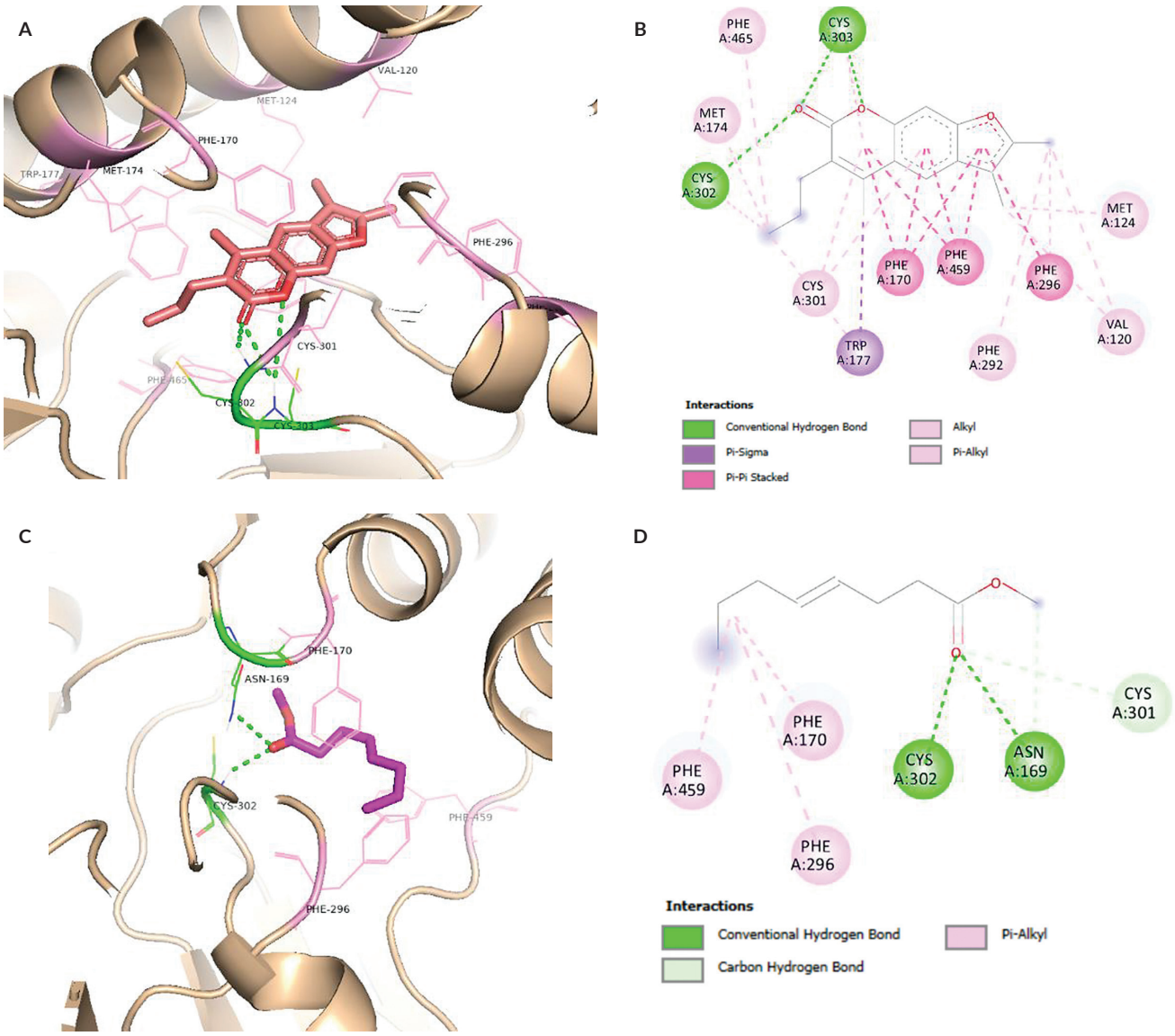

Figure 12. Visualization of the protein-ligand interactions between the protein aldehyde dehydrogenase 2 (pdb ID: 5l13) and the co-crystallized ligand 6ZE **(A and B)** and the compound 4-octenoic acid methyl ester **(C and D)** from *Momordica charantia*. Shown in **(B)** and **(D)** are the 2D interaction maps indicating the type of intermolecular forces involved in the interactions. The 3D interactions are shown in **(A)** and **(C)**. The same color schemes as indicated in the 2D interaction maps are used in the 3D interaction images (e.g., *green* for hydrogen bonding and *light pink* for hydrophobic interactions).

Human ERK1 belongs to the mitogen-activated protein kinases (MAPK). It is activated by glucose and agents which stimulate insulin secretion in the pancreatic β-cells. ERK1/2 are required for the gene transcription of insulin induced by glucose and in the inhibition of events due to prolonged exposure to high glucose.[71] Its activity is increased during adipocyte hypertrophy and that blocking the ERK pathway enhanced lipolysis activity. Moreover, oral administration of ERK inhibitors improved insulin sensitivity and glucose tolerance in mouse models.[72] However, in another study, activation of the ERK/12 signal transduction pathway is associated with insulin secretion in β-cells and that inhibition of the said pathway may lead to β-cell apoptosis and may cause diabetes.[73] In the current study, the aglycone of momordicine II is found to bind favorably to ERK1 as shown in Table 2. The amino acid residues involved in the protein-ligand interactions are different when compared to the investigational inhibitor 38Z, as shown in Figure 10, suggesting that the aglycone binds in a different manner and may or may not elicit a contrasting effect. In a study involving bovine aortic endothelial cells, it was found that MC extracts increase the phosphorylation of ERK1/2[74] hence increasing its activity. In contrast, MC extract inhibited ERK1/2 in gemcitabine resistant pancreatic cancer cells.[75] As previously

mentioned, momordicine II is a terpenoid. Terpenoids have varying effects on ERK1/2. For example, the diterpenoid andrographolide and the triterpenoid cucurbitacin D increase ERK1/2 whereas the triterpenoids fucoxanthin and lucidenic acid have the opposite effects.[76] These contradicting effects may warrant further studies and future experiments may explore the activity of momordicine II in the ERK1/2 pathway which is one of the pivotal proteins identified to be involved in T2DM.

Aldehyde dehydrogenase 2 (ALDH2) is implicated in the cardiovascular disease secondary to T2DM. ALDH2 is involved in the prevention of apoptosis of cardiac cells through detoxification of acetaldehyde and in the reduction of reactive oxygen species thereby eliciting protective roles. Additionally, diabetic patients with mutations in the ALDH2 gene have shown higher macrovascular complication.[77,78] Much of the research in ALDH2 focuses on its role in anticancer treatment since most of cancer cells recalcitrant to treatment overexpress this enzyme.[79] Consequently, because people with T2DM are at a higher risk of developing cancer[80], targeting this enzyme in T2DM is challenging and needs precautions. Here, 4-octenoic acid methyl ester from MC have favorable binding affinity towards ALDH2. As shown in Table 2 and Figure 11, the amino acid residues involved in its binding towards the receptor is different from that of 6ZE, an investigatory inhibitor, suggesting a different mode of binding which may potentially give a different action. This may be a starting point for further investigation since targeting the ADLH2 enzyme imposes several complications as previously mentioned.

Aldose reductase (A) is another enzyme which is pivotal to T2DM that can be possibly targeted by MC and LS compounds. It catalyzes the conversion of aldehydes to alcohol and is implicated in diabetes complications by converting glucose to sorbitol. Moreover, overexpression of AR has been shown to increase oxidative stress and that AR inhibitors reverse these effects.[81] Additionally, human AR expression in mouse models has increased diabetes and ischemia whereas inhibition offers cardio protection from ischemia-reperfusion injury.[82] As documented, Lys-77, Tyr48, His110 and Asp43 are key amino residues that are essential for the catalytic activity of AR.[83] In the present study, decanoic acid from LS has a calculated negative binding energy suggesting a favorable binding towards AR. As shown in Table 2 and Figure 12, only His110 is involved in the protein-ligand interaction between decanoic acid and AR. This suggests that decanoic acid may potentially give a different effect and binding mode. To the best of our knowledge, no reports have been documented on the activity of decanoic acid towards AR and warrants further investigation.

The results presented herewith give insights on the other possible mechanisms of MC and LS compounds against T2DM. However, we are bound by the limitations of rigid molecular docking and future studies involving in vitro and in vivo experiments are needed to validate our findings.

CONCLUSION

By interacting with the different pivotal proteins associated with T2DM such as the human transthyretin, retinol binding protein, TNF-α, aldehyde dehydrogenase 2, aldose reductase, glucokinase, heat shock protein 90, and human ERK1, compounds from *Momordica charantia* and *Lagerstroemia speciosa* may exhibit their antidiabetic effects. Moreover, since their compounds bind favorably on various proteins, they have a potential to exhibit synergistic effects and may rationalize the combination of these herbal medicines in different preparations. However, the results of the current work are bound by the limitations of computational methods. Experimental validations are needed to verify the results. Moreover, the stability of the binding of the compounds towards their target proteins as well as the agonistic or antagonistic activities were not assessed in this study.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

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